OL-01 Innovative Technology for Sequence Analysis of Intact Proteins on a Chromatographic Time-Scale

Donald F. Hunt

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This lecture will focus on data generated with a new ion source that facilitates simultaneous generation of positively charged sample ions by electrospray ionization and negative charged reagent ions for both electron transfer dissociation (ETD) and ion-ion proton transfer (IIPT) reactions on Orbitrap mass spectrometers. Chemical derivatization of intact proteins to enhance charge and direct fragmentation will also be discussed. Implementation of multiple C-trap fills, peak parking, and ion ejection strategies to enhance sequence coverage of intact proteins will also be described. Use of IIPT/ETD facilitates near complete sequence coverage on many intact proteins and is ideally suited for locating multiple posttranslational modifications on the same protein molecule.

OL-02 New Gene Expression Regulation World Based on Transcriptome Network

Yoshihide Hayashizaki

RIKEN Preventive Medical Innovation Program (PMI), Japan

The development of next-generation sequencers has brought not only high-throughput sequencing but also new possibilities for various kinds of analysis methods of genetic information. Now, a next-generation sequencer is not just a machine to detect nucleotide bases. It has evolved into an analyzer for rapid conversion of analogue information extracted from biological molecules into genome-wide digital data. Our center organized FANTOM (Functional Annotation of Mammalian Genome), an international consortium, which is leading transcriptome research in the world. Using the next-generation sequencers, we developed a unique technique, CAGE (Cap Analysis of Gene Expression), which enabled us to conduct comprehensive large-scale promoter analysis (Omics research); for example, the analysis of transcription starting sites. In the FANTOM4 project we analyzed transcriptional regulatory networks during a process of cell differentiation based on actual measurement value using the 'Basin Network' concept. This was achieved using the CAGE technology; an analysis of 3D relationships among the regions of DNA using Chip-seq and various bioinformatics techniques. This network analysis method enabled us to make a complete catalog of networks that regulate distinct cellular states. The aim of our current project, FANTOM5, is to gain a full understanding of transcriptional regulation in a human system by generating transcriptional regulatory networks that define every human cell type. We have succeeded in creating cells with a specific function without going through the iPS cell. This is pioneering the way for various medical applications. We have found approximately 100,000 new promoters which are involved in 'Basin Network' in each cellular state. We also identified 2,113 bidirectional promoters from 42,887 human transcriptional units (TUs). Bidirectional promoters were characterized as 'Enhancer' and shown to be highly tissue specific. Another major discovery is that ncRNAs constitute the majority of the RNA population as shown by our analyses. Through FANTOM activities we found that huge amounts of ncRNAs play important roles in the gene expression regulatory network at all stages including DNA, transcription, RNA, translation and protein. These discoveries replace the current central dogma of molecular biology by a new one.

PL01-01 New Technologies for MS-Based Proteomics and their Application in Biology and Biomedicine

Matthias Mann

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Mass spectrometry-based proteomics, particularly in a quantitative and high resolution format, has become a very powerful technology to study gene expression at it 'end point' - the level of proteins. The technology of high resolution mass spectrometry (MS)-based proteomics has progressed tremendously over the last ten years[1]. Our group has worked intensively on improving and streamlining the shot-gun proteomic workflow, especially in the areas of sample preparation, chromatography, mass spectrometry and computational analysis. Together, proteomic advances now allow near complete quantification of the yeast proteome in just a few hours[2] - a task that previously took weeks of measurement time[3].

This new capability relies on 'single-run' or 'single-shot' proteomics, in which we couple a relatively long HPLC column to a high resolution, bench-top mass spectrometer with very high sequencing speed[4]. Single-run proteomics occupies a middle ground between deep shot-gun proteomics and targeted proteomics. This third way of doing bottom-up proteomics has several attractive features. It uses minimal amounts of sample and allows measurement of many states of the proteome while remaining unbiased in the sense that no subgroup of the proteome needs to be selected for analysis. As such, single-run proteomics is applicable to focused biological studies as well as for systems biology. In our laboratory, this approach is already used routinely for yeast. In this talk I will present the current technological state of the approach and its application to yeast systems biology. Furthermore, I provide an update on how far its realization has progressed to much more complex mammalian system, exemplified here by human cancer cell lines.

MS-based proteomics can also analyze post-translational modifications on a very large scale - for example, more than 50,000 phosphorylation sites can readily be detected in a cell line. Our laboratory extensively uses quantitative proteomics data not only for protein expression measurement but also for the detection of specific protein interactions. In this format, protein quantification (by SILAC labeling or in a label-free format) is applied to distinguish background binders from true binders. By quantification of binders to bait molecules vs. a control bait, the need for stringent washes is reduced and transient binders can still be detected. We will describe application of our generic workflow to interactions with specific DNA elements in the genome (such as GWAS derived SNPs or QTLs), RNA structures, post-translational modifications as well as to stimulus dependent interactions in signaling pathways.

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PL01-02 Defining the Human Tissue-Specific Proteomes Based on Transcriptomics and the Human Protein Atlas

Mathias Uhlen

Science for Life Laboratory, Royal Institute of Technology (KTH), Sweden

Central questions in human biology relate to how cells, tissues and organs differ in the expression of genes and proteins and what consequences the global expression pattern has for the phenotype of various cells with different functions in the body. Here, we have made an attempt to define the human tissue-specific proteome based on parallel transcriptomics analysis (RNA-seq) of dissected samples from most major organs and tissues in the human body. This allowed us to identify, on a genome-wide level, tissue-specific expression of transcripts for all putative protein-coding genes (n=20,050) in these organs and tissues. This data has been integrated with Human Protein Atlas (www.proteinatlas.org) data with the aim is to generate a first draft on a whole-proteome level of protein localizations in human cells, tissues and organs, including various disease-related tissues. The current version 11.0 of the Human Protein Atlas contains data from more than 18,000 validated antibodies targeting 15,000 genes corresponding to 75% of the protein-encoded genes in humans. The portal contains more than 13 million high-resolution images generated by immunohistochemistry and confocal microscopy. Integration of the antibody-based profiling and the transcriptome analysis has allowed us to build a human body map across all major human organs and tissues to allow the systematic exploration of the protein-coding genome, including an attempt to define the tissue-specific and "house-keeping" proteomes of humans.

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PL02-01 Proteomics and Neurobiology

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A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large-scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale quantitative analysis of protein data from experiments. We' ve been applying mass spectrometry based methods to the study of brain function and disease. Application of these methods to the study of Alzheimer' s mouse models, brain development, schizophrenia, cortical barrel cortex by sensory deprivation during synaptogenesis, and the identification of trans-synaptic ligand receptor interactions will be described.

PL03-01 SWATH-MS: Principles and Applications to Quantitative Biology

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A range of proteomics technologies has been highly successful in supporting projects in molecular and cell biology and in biochemistry. For most of these projects proteomics has focused on the identification and quantification of proteins and their modifications in a small number of samples. For some fields of research, exemplified by clinical (e.g. biomarker) or systems biology studies, it is essential to accurately quantify specific sets of proteins across large numbers of samples at a high degree of reproducibility and high sample throughput.
To address these needs we recently introduced SWATH-MS. Like other Data Independent Acquisition (DIA) methods, SWATH-MS essentially converts all physical specimens in a sample. In the case of proteomics, the proteolytic peptides of a protein extract convert into a digital file that can be perpetually interrogated for the presence and quantity of any protein that is in the detection range of the system. In the process, high mass accuracy fragment ion maps are acquired for all sample analytes within a user-defined retention time and mass range window, by repeatedly cycling through consecutive precursor isolation swaths. The thus generated complete and permanent fragment ion records are then, in a second step, queried for the presence and quantity of specific peptides, using spectral libraries as prior information. Essentially, SWATH-MS combines DIA and high throughput targeted data analysis.
In the presentation we will discuss with specific applications how the favorable performance characteristics of the SWATH-MS technique translate into new biological knowledge. Examples include the accurate quantification of proteins across numerous samples to identify pQTL' s and to measure the dynamics of signaling systems and of protein interaction networks, as well as the use of a chromatographic retention time dimension to improve the assignment of protein phosphorylation sites in phosphoproteomic studies.

PL03-02 neXtProt: The Human Protein Knowledge Platform in the Context of HPP

Amos Bairoch

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neXtProt (www.nextprot.org) is a web-based protein knowledge platform developed within the SIB -Swiss Institute of Bioinformatics to support research on human proteins. As such, its role is analogous to that of Model Organism Databases (MODs) for model species. The core data set in neXtProt is the whole corpus of manually curated annotations extracted from UniProtKB/Swiss-Prot5 for human proteins. This set is continuously being complemented with a wide range of quality-filtered data from high throughput studies. Special attention is given to the quality of the data integrated in order to avoid flooding the system with noisy data.
neXtProt actively supports the efforts of HUPO Human Proteomics Project (HPP). In this context we are integrating into neXtProt data originating from proteomics experiments, including peptides and PTMs identification. We are also distributing regularily updated tables that allow to track the progress of HPP chromosome per chromosome. To allow our users to make the best use of the information that is available in the platform we have developed exports options (PEFF, XML) as well as programmatic access (API). We are currently developing a new search interface that will allow precise querying and retrieval of any type of information stored in the platform.
There is a very long road in front of us and the challenge of integration the mass of heterogeneous knowledge on human proteins is a distant perspective and we hope you will enjoy working with neXtProt and will help us making it evolve by telling us of your specific needs.

PL04-01 Unleashing the Power of Proteomics to Develop Blood Based Cancer Markers

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There remains an urgent need for simple non-invasive tests to detect cancer, to personalize treatment and to monitor for disease regression or progression. Over the past decade, the depth of analysis currently possible using proteomics has increased substantially, allowing the identification of well over 2,000 proteins in plasma, reaching protein concentrations well below the ng/ml. We have applied an integrated strategy for the discovery of blood based cancer biomarkers using rigorous experimental design applied to biospecimens that minimize bias and that are highly relevant to the intended clinical applications. The major targeted cancers are lung, breast, colon and pancreas. This effort has resulted in the identification of novel protein products encoded in the genome, novel protein forms associated with disease development. Using training and testing approaches, panels of markers have been assembled that have utility for cancer early detection and assessment of treatment response. Success in discovery and initial validation is leading the way to prospective clinical trials to determine the utility of blood based cancer markers in complementing imaging modalities for cancer detection and for disease monitoring.

PL04-02 A Decade of Adventures of CNHUPO and HLPP

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Following its inception in 2002, CNHUPO organized the 1st international forum in liver proteomics and then sent delegates to attend HUPO 1st congress. In the congress, the HLPP was initiated as the first international cooperative project in organ proteomics. This marked the beginning of CNHUPO and HLPP. For the past decade, CNHUPO has been at the forefront of pushing forward proteomics in China and the world. We introduced the young proteomics field to Chinese people by organizing national/international meeting, training young investigators, establishing comprehensive collaborations in the nation and beyond. We are the strong advocates for funding support from the Chinese government. With the strong funding support and great collaborative efforts from scientists around the world, we have made proteomics widely applied in almost all fields of biological research in China. On the front of HLPP, we proposed the concept of looking at the liver proteome at physiological and pathological conditions by comprehensively measuring protein abundance, PTM, and PPI. The concept has become the cornerstone of the new HPP. Despite the limitation of technology development, we have developed a series of novel proteome strategies to draw the 1st version of a map of the human liver reference proteome at a depth of ~10k proteins. Reflect on the success of CNHUPO and HLPP, the following marks might deserve to be highlighted: 1) Proteomics is ready to take off in the age of grand discoveries. 2) Proteomics as a big science needs the support of big projects and big core facilities. 3) Supports from scientists and public are as important as the one from government. 4) Powerful organizations/consortiums play essential role in big "Omics" projects. 5) Close-collaboration between national and international organization/consortium can significantly accelerate progression of the proteomics field. 6) Technology is a driving force in proteomics. 7) Young investigators are the future of proteomics.

Keywords: CNHUPO, HLPP, Proteome

PL05-02 Evolution of Alternative Splicing and Transcriptional Regulation by Transposable Elements

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Transposable elements are present in numerous copies in mammalian genome and comprise up to 45% of the human genome. They can influence gene transcription and biological function through various mechanisms. Long terminal repeats (LTRs) of human endogenous retroviruses (HERVs) have been shown to influence the expression of neighboring genes. Solitary LTRs contain various transcriptional regulatory elements including promoters, enhancers, and polyadenylation signals. Functional LTR transcription start sites are located between the R and U5 region. Hypomethylation of the LTR element allows the neighboring functional gene to have tissue specific expression. Accumulated changes of the LTR elements in gene regulation are likely to be functional factors for the process of diversification, speciation and evolution consequences. A small minority of such sequences has acquired a role in regulating gene expression, and some of these may be related to differences between individuals, and to expression of disease. They seemed to be a source of alternative splicing, structural change of genomes, and could be related to genetic variation and epigenetic regulation linked to diseases.

PS01-01 Proteomic Approaches for Quantitative Protein Complex Analysis

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Analyzing the molecular architecture of native multi-protein complexes via biochemical methods has so far been difficult and error prone. Protein complex isolation by affinity purification in combination with mass-spectrometry (MS) is a commonly used technique and interaction data derived thereof are the basis for predictions of biological pathways or disease mechanisms concerning those proteins. Selectivity and specificity of pull-downs based on tag-fusion-proteins as well as immunoprecipitations (IP) suffer are compromised by non-specific binders to the capture agent or carrier beads. Due to this lack of specificity, a large proportion of reported protein interactions in the literature as well as in databases gathering interaction data are likely to be compromised by false positives.

Isotope labeling in combination with affinity-based isolation has increased accuracy and reproducibility; yet, larger organisms - including humans - are hardly accessible to metabolic labeling. With the aim to analyse protein interactions in at endogenous levels in primary tissue and combine the selectivity of affinity-based isolation with the accuracy and reproducibility of MS, we combined isotope coded protein labeling (ICPL) with IP and quantitative MS. ICPL-IP allows sensitive and accurate analysis of protein interactions from primary tissue including human specimens. Using a pull down strategy (SF-TAP) we have further refined a pre-existing method to destabilize protein complexes by treatment with very low concentrations of SDS. By combining SDS concentration gradient-induced decomposition of protein complexes coupled to quantitative MS and in silico elution profile distance analysis we can now determine protein complex composition as well as complex topology. By applying these new methods to a cellular transport module, the IFT/Lebercilin complex we demonstrate its ability to determine modular composition as well as sensitive detection of known and novel complex components.

PS01-02 Workflows and Solutions for Targeted Quantification to Increase Throughput and Sensitivity

Amol Prakash

Thermo Fisher Scientific, USA

A typical mass spectrometry based biological experiment requires large number of experiments, which translate to high throughput. We have developed some novel ideas on the hardware, sample prep and data analysis side which will unify all these thoughts into a multiplexing concept which will increase knowledge throughput without requiring many days of instrument time or analysis time. I will present some these via real world examples to show how we were able to translate these into practical improvements in our workflow to achieve both throughput and sensitivity.

PS01-03 Proteomic Analysis of Colon Cancer Phenotypes

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Microarray and next generation sequencing-based high-throughput genomic technologies have allowed comprehensive characterization of cancer phenotypes. These studies have resulted in improved understanding the biological behavior of cancer cells and important advances in personalized treatment of this disease. However, genes and transcripts execute most of their functions through the proteins they encode and insight into the global proteome remains relatively unexplored because of technical limitations in quantitatively assessing large numbers of proteins in complex mixtures. Because proteins are the ultimate effector molecules in cells and undergo changes that cannot be captured by genomics and transcriptomics, global proteomic analysis can serve as an orthogonal analysis strategy for biological discovery that complements genomic and transcriptomic analyses.

Recent technological advances allow thousands of proteins to be routinely identified from small quantities of tissues or cells through shotgun proteomics. Studies by the National Cancer Institute-supported Clinical Proteome Technology and Assessment of Cancer (CPTAC) network (proteomics.cancer.gov) have laid the foundation of a standardized and reproducible shotgun proteomic methodology. For this approach, proteins are digested in to peptides that separated using multi-dimensional liquid-chromatography and identified using mass-spectrometry. We employed this approach for the analysis of colorectal cancer cell lines and primary tumor tissues and derived proteomic signatures associated with different sub-types of the colorectal cancer phenotype using enrichment analysis (www.webgestalt.org). Expression profiles of proteins of interest were further quantified using targeted mass-spectrometry analyses using multiple-reaction monitoring (MRM). These analyses confirmed our initial observations by shotgun proteomics in the large majority of cases. Novel biological insights were confirmed using *in vitro* biological assays for specific biological properties of colorectal cancer cell lines.

Of particular importance is the combination of genomic and proteomic data to study the expression of predicted genomic features in the proteome. Examples are expression profiles of proteins located in amplified regions and the capability to identify variant protein sequences and the global proteomic effects of mutations. Our studies identified expression of important cancer-associated mutations such as K-ras codon 12 and B-raf codon 600 mutations at the protein level, the presence of somatically acquired peptide variants, in addition to known variants that could be mapped to single nucleotide polymorphisms (SNPs) in the genome. Proteomic profiles in cells with increased levels of variant peptides suggest a coordinated program of cellular adaptation resulting in degradation of abnormal proteins.

PS01-04 Quantitative CID Cleavable Crosslink Strategy on a Benchtop Instrument Using All-Ion-Fragmentation and SILAC

Richard Alexander Scheltema, Herbert Schiller, Matthias Mann

Max Planck Institute for Biochemistry, Germany

Mass spectrometry based crosslinking experiments have been one of the major successes in proteomics in recent years and have as a result become part of the standard proteomics toolkit. However, most current approaches rely on full database searches to determine the sequence identities of the crosslinked pair and are therefore limited in sample complexity, while lacking deterministic and quantitative properties. Alternative approaches use CID labile linkers, but these require expensive state-of-the-art mass spectrometry platforms and lack in sensitivity. Here we present a novel crosslinking approach designed to overcome these limitations with SILAC labeling in conjunction with CID cleavable crosslinkers on the Q Exactive benchtop mass spectrometry platform. As this platform lacks true ms3 capabilities, we utilize pseudo-ms3 as All-Ion-Fragmentation (AIF) to separate the crosslinked peptides prior to further analysis. The combination of full scans and AIF scans allows for deterministic assignment of the two crosslinked peptides both during acquisition as well as during the analysis of the recorded data. Analysis of the recorded data is performed with MaxQuant, which has been extended to support this type of data. The method is validated on the recently published TRIC/CCT chaperone complex for which we approach the sensitivity of non-cleavable crosslinking approaches, while opening up the possibility to analyze higher complexity samples for which MaxQuant Real-time is used to improve the acquisition.

Keywords: Crosslinking, LC/MS, Real-time control

PS02-01 Design, Operation and Application of an Automated High Throughput Monoclonal Antibody Facility to Support Global Proteomics Initiatives

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Monash Antibody Technolgies Facility, Monash University, Australia

Monoclonal antibodies (Mabs) are a key reagent to support the Chromosome-Centric Human Proteome Project which aims to define the full set of proteins encoded by each chromosome, including PTMs, ASTs and protease-processed protein variants. Fundamental to this initiative is the generation and validation of large numbers of high affinity renewable Mabs, and making them available to the research community at an affordable price. This will be particularly relevant for proteins which have not yet been formally identified by mass spectrometry or using currently available antibodies. Monash University has established a state of the art high throughput robotic platform (Monash Antibody Technologies Facility; MATF) to produce custom made, high quality, high-affinity mouse or rat Mabs against protein or peptide targets with the capacity to provide thousands of novel antibodies per year to a global clientele. Using Antigen-MicroArray (AMA) technology for primary screening, multiple antigens can be screened simultaneously for specific binding and, by differential staining, IgG secreting clones can be specifically selected. Chr 7 has been formally selected by a consortium of Australian and New Zealand researchers since it contains a number of disease-associated genes or loci, including the EGFR which has been a long term focus of a number of research groups in Australia. Preliminary bioinformatics screening has indicated that of the 946 protein encoding genes listed in neXtProt, 170 have not yet been experimentally confirmed. It is proposed that, using recombinant proteins or defined peptides (e.g. peESTs), customised antibodies would be raised to these proteins for use in affinity proteomics, western blotting, IHC and ELISA approaches. In this presentation we will describe the design and operation of the antibody facility, discuss operating and financial models, discuss antibody validation and a strategy for the use of such antibodies to identify "missing proteins".

Keywords: Monoclonal antibodies, Robotics, Missing Proteins

PS02-02 Exploration of the Protein Coding Genome

Caroline Kampf[1], Fredrik Ponten[1], Linn Fagerberg[2], Bjorn Hallstrom[2], Emma Lundberg[2], Kalle Jonasson[2], Mathias Uhlen[2]

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Since 2003 the Human Protein Atlas project has worked systematically towards completing a proteomic map of gene expression in a range of normal tissues, cancers and cell lines an antibody-based approach. The output of the project is a publically available Protein Atlas (www.proteinatlas.org), where all IHC data, images along with annotations and validation data are published. Recently, a large effort was made to also generate data on a transcript level using RNA-sequencing, and complete transcriptomic data is now available for 27 normal tissues and 44 cell lines included in the Human Protein Atlas. The quantitative transcriptomics approach across all major human tissues allows us to systematically explore the protein-coding genome, generating a first draft of a characterization of "house-keeping" as well as tissue-specific proteins. In addition, the large-scale profiling on both transcript and protein level allows for an additional level of antibody validation. The Human Protein Atlas project now aims to set up a new gene expression portal, built to serve as a repository of information on transcript expression levels combined with spatial cellular and subcellular information on the protein expression.

Keyword: Immunohistochemistry, Human Protein Atlas, proteomics, transcriptomics

PS02-03 Chemical Proteomics for Target Identification and for the Development of Therapeutic Antibodies: From the Bench to the Clinic

Dario Neri

Swiss Federal Institute of Technology (ETH Zürich), Switzerland

Antibodies can be used to deliver bioactive molecules (drugs, cytokines, photosensitizers, radionuclides, fluorophores etc.) to sites of disease (e.g, cancer or chronic inflammatory conditions), thus enabling molecular imaging applications or therapeutic interventions which spare normal tissues. The antibody-based targeting of markers of pathology which are readily accessible from the blood-stream (e.g., markers of angiogenesis or components of the modified extracellular matrix) is particularly attractive for biomedical applications.
In this lecture, I will present results of chemical proteomics methodologies developed in my laboratory, which have allowed the development of antibody-based therapeutic agents, which are currently being investigated in clinical trials. A main technology used in the lab for target identification purposes consists in the terminal perfusion of animal models of pathology (e.g., tumor-bearing mice) with a reactive derivative of biotin, which covalently modifies accessible proteins. Alternatively, the biotinylation reaction can be performed by *ex vivo* perfusion of surgically resected human organs with cancer. Biotinylated proteins can be purified on streptavidin resin in the presence of stronge detergents, digested and the resulting tryptic peptides separated by HPLC and analyzed by mass spectrometry in the presence of internal standards for quantification purposes.
I will show how novel targets have been discovered using chemical proteomics methodologies (in collaboration with Philogen, a Swiss-Italian biotech group) and how the corresponding monoclonal antibodies can be used for imaging and therapeutic applications.

PS02-04 Development of an Automated Immuno-MALDI Assay for the Clinical Measurement of Plasma Renin Activity

Robert Popp[1], David Malmstrom[1], Alexander G. Camenzind[1], Daniel T. Holmes[2], J. Grace van der Gugten[2], Christoph H. Borchers[1,3]

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The renin angiotensin aldosterone system (RAAS) is crucial for the regulation of blood pressure. Dysfunctions of the RAAS can lead to severe diseases related to hypertension. A well-established biomarker for the diagnosis of primary aldosteronism, a form of secondary hypertension, is plasma renin activity (PRA). It is commonly determined by radioimmunoassay (RIA), which has the disadvantages of using radioisotopes and the possibility of cross-reactivity.
To overcome these issues we have developed a mass spectrometric PRA assay based on immuno-MALDI (iMALDI) with the final goal of implementing it in the clinic. Plasma samples are split into two aliquots. The first aliquot is incubated at 37 ℃ for Angiotensin I (Ang I) generation, the second one is placed on ice as a blank. The aliquots are then incubated for 1 hour with stable isotope-labeled Ang I analogues and anti-Ang I antibodies bound to magnetic beads. The beads are washed and spotted directly onto a MALDI target
with the peptides being eluted from the beads by addition of HCCA matrix. Ang I quantitation of both aliquots allows for PRA determination.
64 clinical samples were prepared manually and analyzed using our iMALDI method. Results were compared with RIA (R^2 = 0.9412) and LC-MS/MS (R^2 = 0.9471) results determined at St. Paul's Hospital in Vancouver, Canada, exhibiting strong correlation to clinical measurement.
In order to achieve the requirements for clinical assays (robustness, high throughput and accuracy) we have optimized and automated the sample preparation on an Agilent Bravo liquid handling platform for analysis on a Bruker Microflex MALDI instrument.

Keywords: iMALDI, Plasma Renin Activity, Automation

PS03-01 Single Cell Membrane Protein-Protein Interactions on Human Cancer Cells May Change Antigen Availability to Immunohistochemical Detection

Mark S. Baker, David Cantor, Harish Cheruku, Rohit Saldanha, Leon McQuade, Abidali Mohamedali, Charlie Ahn, Iveta Slapetova, Samuykta Anand, Shoba Ranganathan, Ed Nice

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Proteomics delivers more comprehensive understand of biology. Recent proteomics studies on colorectal cancer (CRC) cell lines where integrins and protease receptor expression have been deliberately altered are presented in the context of attempts to understand changes associated with the invasive/metastatic phenotype.
Immunoprecipitation proteomics on epithelial cells in our lab has now identified uPAR- and $\alpha v\beta 6$ integrin interacting proteins (i.e., P-P metastasome) and showed how many of these proteins may be involved in the regulation of TGFβ1 activation and downstream effects. Subsequent detailed analyses of the sites of protein interaction between uPAR-$\alpha v\beta 6$ using overlapping peptide array binding blots, ELISA-type peptide competition assays, structural modelling and proximity ligation assays suggest interesting biologies may also be associated with these interactions.
In addition, we demonstrate a potential solution to a very old argument regarding whether the epithelial and/or the stromal cells found in human CRC tissues are $uPAR^+$. In detail, we can report data that suggests a reason why both sides of the "debate" may be accurate - namely that different anti-uPAR MAbs do indeed differentiate the apparent presence of uPAR on both of these cells types in CRC tissues. We show that uPAR is "visible" to different MAbs on both epithelial and stromal cells but not to the other MAb. We propose that uPAR-P interactions may "shield" epitopes recognised by certain epitope-specific anti-uPAR MAbs whilst allowing detection by others directed against distant epitopes. This data raises the conundrum that P-P interactions may shield detection of cell-surface antigens involved in P-P interactions a single cell type specific manner.

PS03-02 Single Cell Analysis of Proteins from Clinical Samples Relevant to Human Health

David R. Klug

Department of Chemistry, Imperial College London, UK

There are a range of motivations driving the development of single cell analysis in general, and single cell protein analysis in particular and I will discuss some of these. My group has up to now focussed on the development of microfluidic devices incorporating antibody capture and single molecule detection. I will outline how these work, their strengths and limitations and discuss some possible applications. This will be followed by some preliminary data on both cell lines and clinical material from prototype devices to highlight how one can put such devices into operation in the context of biological and biomedical research.

PS03-03 Analysis of Single Cell Signaling Through Time and Space by Mass Cytometry

Bernd Bodenmiller

IMLS, University of Zurich, Switzerland

Tissues and tumors are complex assemblies of multiple cell types that interact and communicate with each other to achieve disease states.
In cancer, many aspects of tumor development to metastasis depend on these cell interactions in unique microenvironments. Especially signaling networks, forming the core of cellular decision making, are shaped by cell interactions. To study and understand the decision making in the microenvironments, single cell analysis technologies are needed that allow to measure cell type, signaling network state and other cellular processes with spatial resolution.
Mass cytometry is a recent single cell mass spectrometry approach that enables to measure up to 100 molecules simultaneously using isotopically pure rare earth metals as reporters. Previously, only cells in suspension could be analyzed using mass cytometry, and thus essential information on cell location and cell-to-cell interactions was lost. We have now coupled immunocytochemical and immunohistochemical methods with high-resolution laser ablation to mass cytometry. The approach now enables the simultaneous imaging of up to 100 proteins and phosphorylation sites at a sub-cellular resolution. We mass cytometry to study the signaling networks activated during the epithelial-mesenchymal transition (EMT), a process driving the formation of metastasis, in model systems and within their native microenvironment in breast cancer tumors.
Imaging mass cytometry revealed an unexpected complexity of cell-to-cell interactions and cellular (EMT) states in the analyzed tumors. The approach also allowed to accurately classify patients based on the visualized single cell marker expression and cell interactions. Imaging mass cytometry will enable the analysis into how cellular assemblies generate phenotypes in health and disease and will support the transition of medicine towards individualized molecularly targeted therapies.

PS03-04 Monitoring Protein Synthesis in Living Cells with Fluorescent Labeled tRNA FRET Pairs

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We introduce Protein Synthesis Monitoring (PSM) - a technique to monitor protein synthesis in living cells. In PSM, we transfect cells with tRNAs labeled as FRET donors and acceptors. A FRET signal is generated only when a donor- and an acceptor-labeled tRNA come in close contact (< 7nM), as they do on the ribosome during elongation. The intensity of the FRET signal correlates with the number of ribosomes engaged in protein synthesis, providing a real-time, live-cell assay for measuring rates of protein synthesis. PSM can monitor general protein synthesis using bulk tRNAs, or the synthesis of a specific protein, using specific pairs of tRNA. PSM has sub-micron spatial and sub-second temporal resolutions. Cells continue to live and grow normally, and the synthesized proteins are unchanged since the labeling is on the tRNA itself and not on the amino acid. The cells uptake the tRNAs using liposomes or other common methods. The specificity of PSM arises from the large number of distinct tRNA pairs - 1176 in humans (corresponding to 48 isoacceptors). For about 83% of all proteins, an adjacent pair of tRNAs can be found that is enriched in the synthesis sequence of that protein compared to its frequency in the average, or background protein. The enrichment, or E-factor, can indicate the expected signal to background value for a given protein. With this approach, cells can be monitored for the exact timing of synthesis of a protein of interest, provided it is synthesized at sufficient rates (e.g. a sufficient number of ribosomes is engaged in synthesis of this protein). We have demonstrated specific PSM for monitoring synthesis of a viral protein (NS3) during viral infection using Isoleucine tRNA, and for monitoring synthesis of collagen during fibrosis in mouse fibroblasts using tRNA-Gly and tRNA-Pro. We will discuss these results as well as additional applications of PSM in stem cells, drug discovery, cell sorting and basic research.
Keywords: Protein Synthesis, ribosome, tRNA

PS04-01 (Keynote) Importance of Systems Glycobiology for Understanding Disease Onset, Biomarker and Therapeutics

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Glycans are implicated in the onset of infectious diseases, as well as life-style related diseases such as diabetes and chronic obstructive lung disease, autoimmune diseases, neuromuscular disease, and cancer. Glycosylated proteins are also used for cancer biomarkers. The use of modified recombinant glycoproteins can be useful for various therapeutics including antibody therapy against cancers. Our group has been focused on the role of glycosyltransferases in the biosynthesis of N-glycan branching and the identification of their target proteins in relation to diseases. We recently proposed the concept of the "Glycan cycle" as a functional unit of glycans for understanding glycan functions, which will permit the integration of glycan functions in relation to diseases. This conceptual "functional unit of the glycan cycle," such as just described for GlcNAc or fucose, is intended to help in developing our understanding of the integrative and dynamic analysis of glycan functions, an important approach to systems glycobiology. I will also discuss some of our recent studies dealing with the metabolism of nucleotide sugars using chemical biology techniques. In previous studies, we developed a method for the simultaneous analyses of nucleotide sugars, Moreover, in order to investigate the fate of UDP-GlcNAc, we developed a tracing method for following the synthesis of UDP-GlcNAc and its utilization, and GlcNAc utilization using $^{13}C_6$-glucose and $^{13}C_2$-glucosamine, respectively, followed by the analysis of mass isotopomers using liquid chromatography-mass spectrometry. Thus systems glycobiology is one of the promising approaches for understanding glycan functions.
Keywords: Systems Glycobiology, Glycans

PS04-02 Challenges of Disease Biomarker Discovery: A Perspective from Developing Countries

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Biomarkers are biomolecules that can aid in early disease diagnosis, discrimination between diseases with overlapping clinical manifestations as well as useful in monitoring disease progression. We are investigating proteomic alterations in various types of biological samples in different tropical infectious diseases and human brain cancers to gain mechanistic insight about the disease pathogenesis and identification of diagnostic and prognostic protein markers. Our comparative analysis of malaria, dengue and leptospirosis revealed alterations in expression levels of quite a few serum proteins in multiple diseases, which could be an upshot of inflammation mediated acute phase response signaling, while the uniquely differentially expressed candidates were also identified in each pathogenic infection indicating unique responses. With a panel of identified proteins we are able to distinguish different infectious diseases or different grades of brain tumors. There are several challenges associated with proteomics-based clinical research in developing countries. Considering over 80% of global burden of disease resides in developing countries, biomarker discovery research should be accelerated to improve diagnostic and therapeutics in countries with low-resource settings. The organized collection and storage of biospecimens thorough biobanking initiatives are required for "omics-based" translational studies. Apart from governmental funding resources, there is an urgent need of non-governmental funding resources in developing world to accelerate clinical research. Moreover, collective improvement of biomarker discovery and validation process requires sharing of scientific data among different research groups across the world. To this end, Data-Enabled Life Sciences Alliance is and important initiative to establish a common data bank for biological sciences to share scientific data among different research groups across the world.
Keyword: Biomarker, Cancer, Infectious diseases, Developing countries

PS04-03 Proteomics and Nephrolithiasis

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In addition to defining biomarkers for diagnostics and prognostics, biomarker discovery in nephrolithiasis (kidney stone disease) is also directed to characterizations of target molecules that play critical roles in kidney stone formation. Our group has performed proteomic investigations of kidney stone disease to screen for such target molecules that can modulate calcium oxalate (CaOx) crystals, which are the major crystalline compositions in kidney stones. Various functional analyses have been developed and optimized to address modulatory function (either inhibition or promotion) of these target molecules in CaOx crystal growth, aggregation, adhesion and invasion. In this session, some successful characterizations of such target molecules or kidney stone modulators will be highlighted. For example, the most recent findings were obtained from characterizations of changes in secretome of renal tubular epithelial cells after exposure to CaOx crystals. One of the altered secreted proteins identified from this study was enolase-1. To address functional significance of enolase-1 in the disease mechanisms, we have developed a novel assay to investigate CaOx crystal invasion through the renal interstitium [Chiangjong W, Thongboonkerd V. Talanta. 101:240-5, 2012]. The data revealed that enolase-1 could bind to CaOx crystals, resulting to crystal invasion through extracellular matrix (ECM) via plasminogen/plasmin system in a dose-dependent manner. These data may lead to further development of therapeutic targets for prevention of kidney stone formation in high-risk group and/or its recurrence.

PS04-04 High Throughput Cell-Based Studies and Protein Microarrays for Biomarker and Target Discovery

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One of the most compelling steps in the post-genomic era is learning the functional roles for all proteins. The DNASU repository accelerates discovery by providing nearly 15,000 full-length clones for human genes enabling high-throughput protein function studies. To exploit this resource, we developed a novel protein microarray method, called nucleic acid programmable protein array (NAPPA). In lieu of printing purified proteins, NAPPA translates proteins in situ from printed cDNAs by extracts containing human ribosomes and chaperones. This obviates the need to purify proteins, produces human proteins in their natural milieu, and ensures protein stability on the array as the proteins are made just-in-time for assay. NAPPA arrays have been used in the discovery of disease biomarkers, protein-protein interactions and enzyme substrates. Recent experiments have focused on the search for autoantibody responses in breast cancer patients. The prevalence of autoantibodies to specific proteins is typically in the 20% range; thus a panel of autoantibodies will be needed to achieve high sensitivity. Using 155 cases/130 controls in a three phase study, including a blinded validation, we have discovered a panel of 28 autoantigens in breast cancer with sensitivities ranging from 5-40% and specificities ranging from 80-100%. We are further advancing this methodology using photolithographically etched discrete silicon nanowells coupled with next generation piezoelectric printing to achieve very high densities. High density protein expression and display, as well as functional protein-protein interactions, was shown in 8000 nanowell arrays, the highest reported density of individual proteins in nanovessels on a single slide.

Keyword: autoantibodies, biomarker, cancer

PS05-01 (Keynote) Towards Novel Separation and Fragmentation Techniques for Top-Down Proteomics

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Top-down proteomics is based on separating proteins from complex mixtures, ionizing them and fragmenting their molecular ions inside a mass spectrometer. The bottlenecks of the approach appear to be the insufficiently potent methods of protein separation and fragmentation. Our laboratory works towards developing novel approaches to solving these bottlenecks.

Ideally, a separation method should be orthogonal to reversed-phase liquid chromatography (rpLC), an efficient separation technique often coupled directly to mass spectrometry. Electrophoretic focusing is such a method, but its use is restricted by the absence of a commercial device directly compatible with rpLC and suitable for proteomics. We have recently designed such a device based on a multiple-junction capillary fractionator [1]. The device separates a mixture of polypeptides by their isoelectric point into a desirable number of rpLC-orthogonal fractions (usually 4-20) that can be directly injected onto a chromatographic column. We have now thoroughly tested this device in 2D proteomics and achieved a significant increase in proteome coverage.

The ideal fragmentation method should efficiently cleave polypeptide bonds in large molecules that may be in low charge states, as in native mass spectrometry. Electron-capture based methods, such as ECD and ETD, are attractive candidates, but their efficiency for lower charge states needs to be improved. Increasing the precursor charge state in the gas phase before fragmentation (i.e. charge increase followed by charge reduction) would solve the problem. We are pursuing this goal using a novel combination of a plasma source and a high-resolution mass spectrometer (benchtop Orbitrap). Preliminary experiments are encouraging [2]. Recent results on such a combination will be presented.

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2. Chingin, K.; Makarov, A.; Denisov, E.; Zubarev, R. A. *Fragmentation of Positively Charged Biological Ions Activated with A Beam of High-Energy Cations, in revision*.

PS05-02 (Keynote) Top-down Analysis of Modified Proteins and Glycoproteins Using Various Dissociation Methods

Mark E. McComb, Roger Theberge, Sandrine Bourgoin-Voillard, Yan Jiang, Stephen A. Whelan, Xiaobin Xu, Xiang Yu, Christian F. Heckendorf, Jean L. Spencer, Cheng Lin, Catherine E. Costello

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For determinations that must take into account the presence of specific proteoforms, top-down sequencing provides the most information on amino acid sequence (including details on variants) and the type and location of post-translational modifications. It is thus ideal for proteomics and for clinical and functional studies that can reveal information about the inter-relationships of various potential modifications. We are optimizing the operating conditions for our 12-T SolariX hybrid Qh-FTICR MS (Bruker) utilizing several ExD modes, with or without IR activation, our Q-Exactive orbitrap MS with HCD (Thermo Fisher) and our UltrafleXtreme MALDI-TOF/TOF MS (Bruker) to perform top-down MS/MS studies of proteins and glycoproteins and their fragments produced by ISD. The techniques are also useful for characterization of chemically cross-linked proteins and peptides. To facilitate data interpretation, we have modified our BUPID software to accommodate the needs of top-down spectral interpretation (BUPID-Topdown). The experimental strategies will be illustrated with data from ongoing studies.

Acknowledgements: This research is supported by NIH/GM P41 GM104603, NIH/NHLBI HHSN268201000031C, S10 RR020946, S10 RR025082 and S10 OD010724.

Keyword: top down sequencing

PS05-03 Top-Down and Flexible Analysis of Protein Using MALDI In-Source Decay

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Mass spectrometry (MS) provided fascinating ionization tools such as ESI and MALDI methods. Recent topics relating to radical ion chemistry in MS are MALDI-ISD and ESI-ECD/ETD, resulting in c' - and z' -ions originated from the specific cleavage at N-Ca bond on the peptide backbone. MALDI-ISD is used for identifying intact proteins as a tool of top-down proteomics. MALDI-ISD uses the hydrogen radicals to form the protein radicals. Hydrogen radicals are produced from matrix activated with UV laser photons. The resulting hydrogen radicals bind to carbonyl oxygen on the backbone and result in protein radicals. Recent advance of MALDI-ISD gives information about susceptible amino acid residues to the N-C^{α} bond cleavage. The susceptible residues Xxx-Asp/Asn and Gly-Xxx which give relatively intense c' -ion peaks can be rationalized from a criterion that those residues are preferred in flexible backbone structures free from intramolecular hydrogen-bonded structures such as α-helix and β-sheet. This presence of more susceptible amino acid residues than the rest in protein is of interest from the standpoints of interaction(s) between peptide backbone and matrix molecules and of protein flexibility. Protein flexibility is relating to the interactions with drugs, nucleic acids, peptides and proteins. Such a so-called "intrinsically disordered protein [6]" is relating to wide variety of biological functions. Here I describe a relationship between ISD flexible residues and protein backbone flexibility which can be estimated by using hydrogen/deuterium exchange (HDX) by NMR spectroscopy and the B-factor by X-ray crystallography.

Keyword: MALDI, In-source decay, Flexible amino acid, Asp, Asn, Gly

PS05-04 Top-down Proteomic Analysis of Enzymes and Complexes in *Trichoderma* Fungal Secretomes

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Biomass degrading enzymes produced by fungi must be understood in relation to their structural and functional properties in order to become fully usable for biotechnological applications, including biofuel production. Recently, we showed that *Trichoderma harzianum* and *Trichoderma reesei* secretomes contain cellulases and hemicellulases assembled as multienzymatic complexes as revealed by blue native-PAGE and LC-MS/MS bottom-up techniques. Herein, we report on a novel use of top-down proteomics for the identification of intact proteins and native complexes in the *Trichoderma reesei* secretome. The sample was collected after nine days of growth on sugarcane waste, and separated using SDS-PAGE and BN-PAGE followed by electroelution. Fractions were processed using methanol/chloroform/water precipitation prior to nano-LC analysis. Top-down mass spectra were collected on an Orbitrap Elite mass spectrometer with fragmentation performed by ETD and HCD. Proteins were identified using ProSightPC 3.0. Data from SDS-PAGE coupled to denatured electroelution yielded top-down MS identification of 531 proteoforms within 95 different protein accession numbers so far. Current work is aimed at applying blue native PAGE coupled to native electroelution, followed by top down LC-MS/MS to identify the protein subunits of high molecular weight complexes present in the *T. reesei* secretome.

Keywords: Top-down proteomics, Trichoderma secretome, Complexes

PS06-01 Molecular Tissue Imaging Under Ambient Conditions Using a Novel Imaging Source Coupled to High Resolving Power Mass Spectrometry

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Mass Spectrometry Imaging (MSI) is used to simultaneously elucidate the spatial distributions of endogenous compounds (*e.g.*, metabolites, lipids, glycans, peptides, and proteins) as well as xenobiotics including drugs and their metabolites with unparalleled molecular specificity. While these analyses are typically performed under vacuum using MALDI-MSI, novel ambient ionization sources have been developed allowing for molecular imaging under ambient conditions. Ambient imaging permits the analysis of samples that are not amenable to vacuum with little to no sample preparation. Matrix-assisted laser desorption electrospray ionization (MALDESI) is the first hybrid ambient ionization source that combines features and benefits of MALDI and ESI. This technique involves using a pulsed laser to excite an endogenous or exogenous matrix that facilitates desorption of neutral analyte molecules from the sample. These neutral analytes partition into the charged solvent droplets of an electrospray plume and become ionized via an ESI-like mechanism. Different modes of the MALDESI technique have been demonstrated using various laser wavelengths including ultraviolet (UV-MALDESI) and infrared (IR-MALDESI). For each mode, a corresponding matrix is chosen that strongly absorbs in the wavelength region of the laser emission. In IR-MALDESI, with laser emission at 2.94 μm, liquid water or ice can be used as the laser absorbing matrix which nearly eliminates spectral interference from matrix related ions. The use of ice as a matrix for IR-MALDESI-MSI was systematically investigated using shadowgraphy imaging techniques correlated with corresponding mass spectra. IR-MALDESI-MSI has been applied to drug and metabolism studies as well as hypothesis driven and discovery based investigations to discern the dissimilarities between tissue sections from various biological states. In addition, a freely available and vendor neutral software has been developed to process MSI datasets.

PS06-02 Imaging Mass Spectrometry of Clinical Samples

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Development of molecular imaging technology has potently progressed biology and medicine. Main stream of such methodologies has been staining methods such as immunohistochemistry and genetic manipulation to incorporate fluorescent protein tags into the target proteins. Antibodies do not have one-to-one correspondence to the genetic codes. To this problem, imaging mass spectrometry is one of the solutions. We developed microscopy combined with matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS), a two-dimensional MS technique, in collaboration with Shimadzu. This is a molecular imaging technique to investigate directly the spatial distribution of biomolecules on tissue sections without any time-consuming extraction, purification, and separation procedures [1]. We have enabled visualization and direct on-tissue identification [2, 3]. Innovation in ionization methods and equipments supported these achievements [4]. Having started with microanatomical, physiological analysis of molecular distribution, we are currently extending the research target to the clinical fields including [5]. I would like to present the methodology, application, and recent advances in our MALDI-IMS study.

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PS06-03 A Study of Drug Distribution in Malignant Melanoma Tissue by MALDI Mass Spectrometry Imaging for Evaluation of Drug Efficacy

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Malignant melanoma (MM) is a disease with ever-increasing detection rates in the industrialized countries. However, the elevated incidence frequency has not been paralleled by the development of novel therapeutic agents with a significant impact on survival. Currently, limited therapeutic options exist for patients with metastatic melanomas, and all standard combinations used in metastasis therapy have low efficacy. Therefore, to develop the novel therapeutic agents is required for improving outcome of MM patients. In the process of drug development for therapy purposes, one of the key objectives is to optimize the efficacy and safety. Mass spectrometry imaging (MSI) is a powerful tool for pharmacokinetics/pharmacodynamics. MSI will provide a way forward in characterizing drugs and their spatial localization in tissue sections. The aim of this study was to examine the drug distribution within melanoma tissue sections using a pharmacokinetic model that allows quantitative analysis by MALDI MSI. MM tissues were prepared in sections of 10 μm in thickness prior to be exposed to drug by our in-house designed microdispenser platform. Samples coated with a matrix (α-cyano-4-hydroxycinnamic acid) were analyzed by a MALDI LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany). The drug fragment ions were visualized by the ImageQuest software (Thermo Scientific) and quantified by QUANTINETIX (Imabiotech, Loos, France). We examined the tissue localization of several drugs, which have previously reported in MM studies, and detected the parent and fragment ion mass signals with high sensitivity. These compounds were evaluated for further investigations to measure the drug efficacy in clinical settings.

Keywords: imaging, mass spectrometry, malignant melanoma

PS06-04 Peptide MALDI Imaging - How to Get Most Out of Your Sample?

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Introduction: Matrix-assisted laser desorption ionization (MALDI)-Imaging mass spectrometry (IMS) has become a powerful and successful tool for biomarker detection especially peptide IMS. The overall goal of this study was to test and compare various protocols of tissue digest in order to improve the quality of IMS data.

Material and Methods: In the presented work the MALDI-ImagePrep™ device from Bruker Daltonics was used for automated spraying of trypsin and matrix. Ten μm thin rat brain tissue sections served as samples. Subsequent measurements were carried out with an UltrafleXtreme instrument (Bruker Daltonics). The experimental set up consisted of different experiments varying the trypsin incubation time, the matrix, the protease and the raster width of the laser. Every obtained image was analyzed using the flexImaging™ and the SCiLSLab software (Steinbeis Innovation Center SCiLS, Bremen).

Results: A total of 73 slides were processed and measured. It has been noted that the type of matrix has a significant impact on the number and quality of resolved structures. Similar observations were made for the experiments with trypsin overnight digestion. However it also became clear that the vast amount of external factors can have a large impact on the overall performance and outcome of an on-tissue digestion protocol. The SCiLS Lab software facilitated the comparison of sample sets due to the analysis of more than one sample simultaneously.

Conclusion: A crucial aspect is without doubt the quality of the sample. Analyzing such a comprehensive dataset is time consuming and difficult due to the variability of the parameters and more importantly the lack of tools for objective post-processing. Help may be provided by the SCiLS Lab software allowing the analysis of many samples in parallel.

Keywords: MALDI imaging, method improvement, rat brain

PS07-01 Global Mapping of Mitochondrial Interaction Networks Connects Mitochondrial Function to Neurodegenerative Disease

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Proteomic approaches such as affinity purification in combination with mass spectrometry (AP-MS) and yeast two-hybrid have been proven successful in the isolation of native soluble or membrane protein-protein interactions and complexes in model organisms, including the yeast *Saccharomyces cerevisiae* and the eubacterium *Escherichia coli*. In this talk, I will discuss how the AP-MS purification strategy, in particular, can be a powerful tool for generating interactome maps for disease-causing mitochondrial proteins (MPs). MPs are involved in many cellular processes, as a result of their propensity to interact with each other and with other extra-compartmental proteins, and hence defects in their function have emerged as causative factors for diverse human disorders, particularly neurodegenerative (ND) diseases like Parkinson's, Alzheimer's and Huntington's. To date, biochemical and genetic investigations have uncovered only a small number of MPs involved in ND diseases. Given the diversity of processes affected by mitochondrial function, and because it is difficult to pinpoint the role of mitochondrial dysfunction in human diseases, many more remain unknown. We are addressing this deficit by focusing on over 600 putative disease-causing MPs, of which we have affinity purified roughly 60 lentiviral tagged proteins in the mammalian model human embryonic kidney (HEK293) cell line. Interactors of the purified proteins were then identified with a high performance Orbitrap Elite mass spectrometer. Our assay captured both previously known interacting proteins, as well as several new associations that have not been reported previously. The data we have generated so far provides new insight into the complex etiologies of ND disease, and opens avenues for identifying new therapeutic drug targets that could ameliorate many diseases all together.

PS07-02 Decoding Ligand Receptor Interactions

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Ligand-induced changes in cell surface receptors result in physiological responses, which constitute the biological activity of various ligands such as proteins, peptides, pharmaceutical drugs, toxins or whole pathogens. However, traditional approaches for the ligand-based identification of corresponding receptors are usually limited to non-transient, high affinity interactions and highly artificial experimental set-ups. Therefore, many signaling molecules remain orphan ligands without a known primary molecular target – invaluable information in understanding the respective mechanisms of signal transduction, drug action or disease. Previously, we have developed the cell surface capturing (CSC) technology for the unbiased identification and quantification of cell surface N-glycoproteomes by mass spectrometry (MS). This demonstrated the powerful applicability of chemical reagents in the tagging of cell surface glycoproteins at carbohydrate groups and the subsequent purification of the corresponding peptides for MS analysis. Based on these results we now synthesized trifunctional cross-linkers for the ligand-based tagging of glycoprotein receptors on living cells and the purification of receptor-derived peptides for MS analysis. Through quantitative comparison to a sample generated with an unspecific control probe, this ligand-based receptor capturing (LRC) approach allows for the highly specific and sensitive detection of ligand interactions with their corresponding receptors under near-physiological conditions. Experiments with ligands ranging from peptide hormones to clinical antibodies demonstrate the potential of this approach to specifically identify one or more target receptors for a given ligand with great statistical power. Advanced discovery-driven applications reveal potential receptors and receptor panels for ligands ranging from protein domains to intact viruses. Together, I will present a short summary of our recent biomedical research to understand the surfaceome as a cellular signaling gateway and a chemoproteomic technology for the unbiased detection of ligand-receptor interactions on living cells.

PS07-03 A Plasma Membrane Proteomic Analysis of Mouse and Human Cardiovascular Proteins

Parveen Sharma, Nicolas Bousette, Savo Lazic, Cynthia Abbasi, Nicole Dubois, Alex Ignatchenko, Vladimir Ignatchenko, Jie Liu, Allen Teng, Melissa Noronha, Robert Hamilton, Peter P. Liu, Peter H Backx, Gordon Keller, Igor Stagljar, Ian C. Scott, Thomas Kislinger, Anthony Gramolini

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We employed cationic silica-bead coating coupled with shotgun proteomics to enrich and identify cell-surface associated proteins from primary mouse neonatal and human fetal ventricular cardiomyocytes, endothelium, and smooth muscle cells. Human coronary artery smooth muscle and endothelial cells, and human cardiac muscle derived cardiomyocytes were cultured. Human fetal ventricular myocytes were acutely dissociated. Membrane proteins were cross-linked to cationic silica beads to isolate cytosolic proteins and a membrane fraction attached to the beads. Samples were analyzed by LC-MS MuDPIT strategies on a Thermo LTQ or LTQ Orbitrap. Shotgun proteomics identified >3,000 mouse and >2,500 human proteins. Organelle enrichments were confirmed by immunoblotting. Mapping of orthologous proteins between mouse and human resulted in 1717 proteins. In the cardiomyocytes, QSpec statistical analysis calculated differential spectral counts between proteins found in the membrane enriched and membrane depleted fraction and provided a dataset of 555 cardiomyocytes proteins. Bioinformatic integration with transmembrane helix predictions, Phenotype Ontology (PO), and available microarray data identified a rank ordered set of cardiac-enriched surface proteins; select examples of which the subcellular location were further confirmed using confocal microscopy, immunogold electron microscopy, and sucrose density gradients. For several of the highly ranked membrane proteins, lentiviral-based shRNA knock-down demonstrated significantly altered Ca^{2+} transient amplitude, release rates, and uptake rates. Initial knockouts in Danio rerio (zebrafish) support the cultured myocyte studies. In conclusion, we have provided the first comprehensive analysis of membrane cell surface-associated proteins in all three major cardiac cell types, and provided a pipeline to validate surface proteins in cardiomyocytes that might be involved in Ca^{2+} dynamics.

Keywords: cardiomyocyte, membrane proteomics, cell surface

PS07-04 Development of Novel Membrane Protein Marker for Lung Cancer Diagnosis and Therapy

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Lung cancer is the one of common cancers and the fetal disease causing high death rate. Lung cancer is histologically divided into four types: adenocarcinoma, large-cell carcinoma, small-cell carcinoma, and squamous-cell carcinoma. High death rate of lung cancer is because of late diagnosis and absence of effective treatment. Thus, it is important to develop an efficient method for diagnosis and treatment. Targeted therapy is a rising method for cancer therapy and has a potential clinical benefit. Especially, cancer specific membrane proteins are useful as biomarker. In this study, we found candidate proteins for biomarker of lung cancer and confirmed that they can target four types of human lung cancers.

We analyzed proteins expressed in normal human lung tissue and in four types of human lung cancer cell lines. We got the list of 1,340 proteins by MS/MS. Among them we chose four promising proteins which can be used as biomarker of lung cancer. For further validation, we prepared antibodies against candidate proteins and carried out various experiments to verify the specific expression of candidates in the membrane of lung cancer cell. Also, we performed antibody treatment in lung cancer cells and in the xenograft model to analyze whether antibody can regulate proliferation of lung cancer cells.

According to results of western blot and immunocytochemistry and immunohistochemistry, we concluded that candidate proteins are expressed in human lung cancer cell specifically. In addition, the result of flow cytometry showed candidate proteins are located in membrane of human lung cancer cell lines. Furthermore, we found that antibodies against candidate proteins can kill lung cancer cells and block the growth of lung cancer tumor in xenograft model. Besides, in vivo and ex vivo imaging represented antibodies can target lung cancer tumor. These results suggest four candidates can play a significant role in targeting lung cancer for diagnosis and treatment.

Keywords: lung cancer, membrane protein, biomarker

PS07-05 Proteomic Profiling of Ganglioside-Associated Microdomain in Malignant Melanomas

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Melanoma is difficult to cure because of its malignant properties. Ganglioside GD3 levels are highly elevated in melanomas. It has been shown that GD3 enhances cell proliferation and invasion using GD3 synthase-transfectant cells of a GD3-negative (GD3-) mutant line SK-MEL-28 N1. p130Cas, paxillin and FAK were identified as highly tyrosine-phosphorylated molecules involved in the increased cell proliferation and invasion with GD3 expression. However, remaining issue to be clarified is how GD3 interacts with known/unknown molecules in the vicinity of cell membrane. To clarify these mechanisms, we isolated the glycosphingolipid-enriched microdomain (GEM)/rafts with sucrose density-gradient ultra-centrifugation of Triton X-100 extracts from GD3+ and GD3- cells. We also labeled cell surface molecules present in the vicinity of a target molecule in living cells with EMARS reaction (Honke et al.). Isolated molecules as components in GEM/rafts and EMARS products were comprehensively analyzed with LC/MS (LTQ-Orbitrap). In the GEM/rafts, 73 membrane proteins were identified in GD3+ and GD3- cells. Among them, 50 membrane proteins were common. In the EMARS with GD3, 9 molecules such as neogenin, integrin α3, β1 and MCAM were identified as GD3-interacting molecules. They were also identified in the GEM/raft fraction. To check the results of LC/MS, we performed immuno-blotting and confocal microscopy analysis. Neogenin and MCAM were found in GEM/raft fraction of GD3+ cells and labeled with EMARS reaction in immuno-blotting. They were co-localized with GD3 in confocal microscopy analysis. Differences in the molecular profiles identified in GEM/rafts and as EMARS products suggest the heterogeneity in GEM/rafts.

Keywords: Lipid raft, Glycosphingolipid, EMARS

PS08-01 Integrated Site Specific Mapping of N-linked Glycopeptides for Cancer Biomarker Discovery in Human Plasma by Mass Spectrometry

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The glycoprotein analysis (GPA) system was developed to identify the N-linked glycopeptide including glycan compositions and amino acid sequences and quantify each glycopeptides. Three scoring algorithms was used for automated high-throughput glycopeptide identification; M-score for glycopeptide selection, S-score for glycopeptide match, and Y-score for glycopeptide identification. The GPA platform were basically designed to utilize MS and MS/MS data from most type of MS spectrometer such as QTOF-MS, Orbitrap-MS, and FT-MS.

Typical standard glycoproteins were digested with trypsin and analyzed by LC/MS to obtain high resolution MS and MS/MS spectra with HCD or CID fragmentation. In order to select N-glycopeptides in the GPA system, the glycan oxonium ions and accurate MS spectra were used. Label-free quantification of the identified N-glycopeptides between normal and cancer was performed.

This GPA platform has been successfully evaluated for the site-specific mapping of different N-glycoproteins for cancer biomarker discovery to identify and quantify different glycoproteins in human plasma including medium and low abundant proteins, such as TIMP-1 with HILIC enrichment and LC/MS/MS analysis. The increased levels of fucose, sialic acid, N-acetyl glucosamine, and highly branched N-linked glycans were found to be associated with cancer more than normal case.

PS08-02 Nerve Growth Factor and Its Precursor as Cancer Biomarkers and Targets: Using Proteomics from Discovery to Validation

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Nerve growth factor (NGF) and its precursor (proNGF) are well know for their role in the development of the nervous system where they participate in neuron survival and differentiation. We have discovered that NGF can stimulate breast cancer cell growth and using proteomics as well as other molecular biology approaches we have shown that it is overexpressed in breast tumours. Moreover, ProNGF is also secreted by breast cancer cells and has the effect of enhancing tumour cell invasion. In animal models, targeting NGF and proNGF or their receptors TrkA, p75^{NTR} or sortilin resulted in an inhibition of tumour growth and metastasis and therefore NGF and proNGF are potential clinical biomarkers and targets in breast cancer. Bench to bedside translation of biomarkers involves validation studies with large cohorts of cancer samples as well as the development of tools for clinical applications. An integrative approach involving immunohistochemistry, ELISA, and multi reaction monitoring (MRM) mass spectrometry has been developed to analyse tumours and blood samples and to explore correlation between the levels of proNGF/NGF and their receptors with clinicopathological parameters including tumour subtype, estrogen and progesterone receptors, HER2, node invasion and patient survival. These studies have highlighted a number of features, such as a higher level of proNGF in the blood of breast cancer patients as compared to normal controls, suggesting proNGF as a possible biomarker for diagnosis, and a relationship with node invasion indicating that it may also be a prognosis biomarker for the risk of developing metastasis. Interestingly, overexpression of NGF/ProNGF was also found in lung, thyroid, and prostate tumours, expanding their clinical value to other cancers.

Keywords: Cancer, Breast, Lung, Thyroid, Prostate, Biomarkers and Therapeutic Targets

PS08-03 Convergence of Genomics with Proteomics to Better Understand Cancer Biology - The NCI Clinical Proteomic Tumor Analysis Consortium

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The National Cancer Institute (NCI), part of the National Institutes of Health (NIH), has established a consortium of research centers that are focusing on identifying proteins that derive from cancer genomes. The Clinical Proteomic Tumor Analysis Consortium (CPTAC) adds to NCI's ongoing initiatives in molecular biology technology-based research programs, such as The Cancer Genome Atlas (TCGA), that comprehensively characterize tumors and make its findings available to the public. Initiatives such as TCGA have characterized and sequenced the genomic alterations from several types of cancer, providing a catalog of alterations in a cancer genome and setting the stage for the development of more molecular interventions that attack cancer cells based on their specific genetic makeup. CPTAC is leveraging its state-of-the-art, proteomic technologies to comprehensively connect genomic alterations to cancer biology with proteomics. A unique feature of CPTAC is the utilization of genomically characterized biospecimens (such as those from TCGA) in conjunction with an independent prospective biospecimen cohort to confirm unique biological findings. Data, assays, and protocols produced from this program will be made available to the public. This seminar will discuss the questions being addressed by the program and what it hopes to accomplish in furthering our understanding of cancer biology.

Keyword: genomics, proteomics, proteogenomics, cancer

PS08-04 Label-free Quantitative Personalized Tissue Membrane Proteomics and Targeted Membrane Glycoprotein Profiling for Gastric Cancer Biomarker

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The glycoproteins and/or membrane proteins hold promises for discovery of cancer biomarker because most FDA-approved cancer biomarkers are secreted glycoproteins. Unfortunately, some of the current cancer biomarkers, such as CEA, CA 19-9, and CA 72-4 have low sensitivity and specificity for gastric cancer (GC) detection. Recent studies suggest that analysis of tumor tissues can directly lead to the investigation of the origin of cancer and generate potential markers with higher specificity and sensitivity. In this study, we applied an informatics-assisted label-free quantification method for personalized membrane proteomics analysis of paired of cancerous and adjacent normal tissue from patients with gastric cancer. In this study, individual membrane proteins were first purified from paired tumor and adjacent normal tissues of 24 GC patients with different stages. The purified membrane proteins were mixed with internal standard protein, subjected to gel-assisted digestion. Finally, extracted peptides were analyzed in triplicate by LC-MS/MS. The analysis quantified 1752 proteins across different patients; 1058 were classified as membrane proteins or membrane-associated proteins including 525 plasma membrane proteins annotated by Gene Ontology, Ingenuity Pathway Analysis Knowledge Base, and TMHMM prediction. There are 497 significantly up-regulated expressed proteins in the four different stages of GC patients including the well-documented GC biomarkers: EGFR, ENO1 and PPIA. These previous biomarker candidates show up-regulation in <40% patients, suggesting their low sensitivity in for clinical utility. Therefore, we select 8 biomarker candidates which satisfy criteria including structural characterization as glycosylated membrane proteins and ability to secret or shed in serum; five of these proteins had been reported to associate with GC. Those candidates will be validated extensively in well-defined retrospective and prospective clinical samples.

Keywords: membrane protemoics, gastric cancer, biomarker

PS09-01 Towards a Comprehensive Characterization of a Human Cancer Cell Phosphoproteome

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We recently demonstrated that by using a refined strong cation exchange (SCX) chromatographic system one can isolate multiply phosphorylated, N-acetylated and singly phosphorylated peptides. Analysis of these near pure populations, once again, emphasized their inherent complexityand the deficiencies of the final LCMS step. In order to make these pools more palatable by LCMS we developed additional fractionation strategies based on hydrophilic interaction liquid chromatography (HILIC) or weak anion exchange (WAX). Taking a single SCX fraction and then subjecting it to WAX we were able to dramatically increase the number of phosphosites identified.

The SCX system does not allow purification of phosphopeptides containing multiple basic residues (basic phosphopeptides). Such peptides are often products of phosphosites that are adjacent to basic residues and are often generated by basophilic kinases. Unfortunately these peptides are often poorly enriched by chelation strategies since there is frequently a salt bridge present between the phosphate and amino/guanido groups hindering coordination and enrichment. Here, we show a careful choice of chelation material (Ti-IMAC) and solvent conditions can dramatically improve enrichment. In our hands we have found that ETD of such peptides is far superior to classical HCD or CID sequencing.

Our removal of biases in phosphopeptide enrichment allowed a protocol to be formed that allows over 90% enrichment on a lysate. I will demonstrate that enrichment followed by a 3 hour single LCMS analysis one can obtain approx. 5000 sites or 15,000 phosphosites in a simplified 2DLC strategy.

I will finish by discussing a new sequencing method we have developed (we refer to as EThcD) that provides excellent tandem mass spectra of phosphopeptides and often allows unambiguous site localization. In this presentation I hope to show that there are many (complementary) tools available to interrogate the phosphoproteome.

PS09-02 Sequential Phosphoproteomic Enrichment through Complementary Metal-Directed Immobilized Metal Ion Affinity Chromatography

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There have been recent advances in instrumentation and analytical strategies aimed at the identification and quantitation of protein phosphorylation. Despite these advances, methodologies to enrich heterogeneous types of phosphopeptides are critical for comprehensive mapping of the under-explored phosphoproteome. Taking advantage of the distinct binding affinities of Ga^{3+} and Fe^{3+} for phosphopeptides, we designed a tip-based, metal-directed immobilized metal ion affinity chromatography (MD-IMAC) procedure for the sequential enrichment of phosphopeptides. In Raji B cells, up to 92% of the 6,283 phosphopeptides were uniquely enriched in either the 1st Ga^{3+}-IMAC (41%) or 2nd Fe^{3+}-IMAC (51%). The complementary properties of Ga^{3+} and Fe^{3+} were further demonstrated through the exclusive enrichment of almost all of the 1,214 multiply phosphorylated peptides (99.4%) in the Ga^{3+}-IMAC, whereas only 10% of the 5,069 monophosphorylated phosphopeptides were commonly enriched in both fractions. The application of our sequential Ga^{3+}-Fe^{3+}-IMAC approach to human lung cancer tissue allowed the identification of 2,560 unique phosphopeptides. In addition to the above mentioned monophosphorylated peptides and multiply phosphorylated peptides, this fractionation ability was also demonstrated on the basic and acidic phosphopeptides This MD-IMAC strategy provided complementary mapping of different kinase substrates and their phosphorylation sites in multiple cellular pathways related to cancer invasion and the metastasis of lung cancer. Given the fractionation ability, reproducibility, sensitivity and ease of tip preparation demonstrated for this Ga^{3+}-Fe^{3+}-IMAC technique, we propose that this strategy allows more comprehensive characterization of the phosphoproteome both *in vitro* and *in vivo*.

PS09-03 Enhanced Phosphopeptide Identification in Escherichia Coli by Stepwise Hydroxy Acid-Modified Metal Oxide Chromatography with Elevated Sample Loading Capacity

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Protein phosphorylation in bacteria is as important as in other living organisms to regulate physiological processes. However, the much lower phosphoprotein stoichiometry severely hampered the progress in phosphoproteomic field in microorganisms. Here, the stepwise enrichment with large-scale hydroxy acid-modified metal oxide chromatography (HAMMOC) was integrated into phosphoproteomic analysis in gram-negative model organism, *Escherichia coli*. The large-scale HAMMOC (200-μL C8 StageTip) showed the comparable enrichment efficiency with the conventional one (10-μL C8 StageTip). From 100 μg protein lysates, 31 and 38 phosphopeptides accompanied with 137 and 122 non-phosphopeptides were identified in conventional and large-scale HAMMOC, respectively. Also, these two methods had similar enrichment specificity with an average of 48.8 % and 44.6% in conventional and large-scale HAMMOCs. As increasing the lysates from 500 to 1000μg with 100μg interval, 998 unique phosphopeptides accompanied with 4,186 non-phosphopeptides were identified in total by large-scale HAMMOC. 268 serine, 104 threonine, and 38 tyrosine phosphorylation sites were found from 318 phosphoproteins yielding a Ser/Thr/Tyr phosphorylation ratio of 65.4/25.4/9.2%. Since the large number of non-phosphopeptides would suppress the phosphopeptide ionization and thus hindering the identification of phosphopeptides, further development on the stepwise strategy was adopted to reduce the non-phosphopeptides. Successfully, the enrichment specificity was significantly improved to higher than 95%. This approach exhibited its advantages in higher enrichment efficiency in aspect of less sample amount and LC-MS/Ms analysis time and simpler manipulation with higher number of identified phosphopeptides and phosphoproteins. Therefore, this method displayed its feasibility in bacterial phosphoproteomic analysis.

Keywords: bacterial phosphoproteomics, phosphopeptide enrichment

PS09-04 Unravelling Cell Signaling Events with Sub-Minute Temporal Resolution

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Large-scale phosphoproteomics studies enable the profiling of more than 15000 phosphosites from mg size of cell extracts. Investigation of dynamic changes in protein phosphorylation is a promising approach to dissect signaling events and correlate interactions between kinases, phosphatases and their substrates. Many protein phosphorylation/dephosphorylation events take place rapidly in response to environmental perturbation and thus require special sample handling techniques. Here, we present a novel sample collection protocol tailored for phosphoproteomic analysis of fast signaling events. We measured changes in phosphorylation within the first minute following osmotic shock in *Saccharomyces cerevisiae* with a temporal resolution of 5 sec. Our approach provided an unprecedented temporal resolution enabling the collection of high quality phosphorylation profiles for ~5,500 phosphosites on ~1,600 proteins. Evolutionary analysis showed that our dynamic phosphosites are more conserved than static ones, and are more functionally significant. We also found high enrichment of these sites on regulatory proteins such as kinases and phosphatases. Particularly we detected dynamic changes in phosphorylation on more than 25 % of the 128 kinases from *S. Cerevisiae* proteome. Temporal resolution achieved in our experiments enabled the dissection of dynamic events within a MAPK pathway responsible for high osmolarity including dual phosphorylation of MAPK Hog1. We also for a first time identified dynamic changes in phosphorylation on the majority of key players such as Sla1 (S785, S996), Abp1 (S357), Akl1 (S12, S985), and Syp1 (S347, S405) involved in clathrin mediated endocytosis. Subsequent site mutagenesis experiments confirmed our findings and allowed us to get deeper understanding of interplay between osmotic shock and endocytosis events.

Keywords: phosphoproteomics, dynamics, osmotic shock

PS10-01 Dissecting the Sub Cellular Proteome

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Many proteins exist in highly controlled micro-environments, which range in scale from organelles, sub-organelle compartments, and clusters of membrane bound proteins to multi-protein complexes. The components of such micro-environments vary in a role-dependent manner increasing functional diversity. Our knowledge of the constituent proteins in all of these niches is important to the analysis of protein function and our understanding of cellular systems. Moreover, the ability to look at changes in niche components is vital to the elucidation of the mechanisms associated with disease.

The characterization of a protein's location has traditionally involved relatively low throughput microscopy or co-isolation of proteins in complex coupled with antibody based detection methods. Such approaches tend to be targeted and require high quality reagents to ensure robust data. More recently, the emergence of high throughput quantitative mass spectrometry has allowed analysis of both location and interaction partners on a proteome-wide scale.

The comprehensive determination of sub-cellular niches is beset with numerous technical challenges including the necessity to express proteins at non-physiologically relevant abundance levels, or to express tagged proteins which may behave differently than their native counterparts. Moreover, it is often impossible to purify sub-cellular niches and complexes without a significant level of contamination from unrelated proteins leading to false discoveries. Furthermore, many interactions of interest are weak or transient, and highly dynamic and thus difficult to capture.

In this presentation, I will describe emerging quantitative proteomics tools that specifically target membrane proteins giving information about their sub-cellular locations and binding partners. These methods allow simultaneous and accurate protein localization of membrane proteins across multiple locations and do not rely on the complete purification of any niche of interest. I will describe tools that we have developed which employ novel machine learning methods for data analysis. I will also introduce a novel method for defining the local environments of proteins within membranes.

PS10-02 How to Submit MIAPE Compliant Data to ProteomeXchange Repository in the Context of the Human Proteome Project

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The Chromosome-Centric HPP is one component of the HPP and focuses on constructing the proteomic catalog in a chromosome-by-chromosome fashion (www.c-hpp.org). This implies to generate and create publicly accessible data and informational resources supporting better proteomics experiments for a wider range of the life science community. It is expected the amount of submitted data will continually grow as HPP begins to produce data from many teams. Metadata about sample processing and experimental procedures becomes crucial for HPP project where information about how each proteoform has been experimentally detected from a certain sample/tissue should be part of the aimed human proteome map. The ProteomeXchange consortium was launched with the aim of providing scientific community a unique entry point for sharing proteomics data, developing the appropriate tools based on HUPO-PSI standards. Metadata about sample and experimental equipment and protocols that is currently required seems to be crucial for the HPP submissions.
Here we present the ProteoRed MIAPE Extractor, which prepares data for a ProteomeXchange submission and additionally provides the way for assuring MIAPE compliance of the submitted data.
The tool is able to firstly extract MIAPE data from PSI standard data files. Secondly, it provides a way for completing the required metadata to reach the MIAPE compliance. Thirdly, it compiles and integrates data coming from several experiments, allowing its filtering, keeping just the reliable data. Finally the MIAPE Extractor prepares all data for a ProteomeXchange submission by compiling all required files in an output folder and creating a PRIDE XML file from each processed experiment data, also including human readable MIAPE compliant reports. Then, an automatic submission using the ProteomeXchange submission tool can be done. This tool is being used by the Spanish Human Proteome Project (SpHPP) consortium and its open for its use from any other consortium.

PS10-03 SOMAmer Capture Coupled to Mass Spectrometry for Plasma Protein Quantification

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Proteins can be quantified with high specificity and sensitivity by mass spectrometric measurement of immunoaffinity-enriched proteins or proteotypic peptides. Such assays are either termed mass spectrometric immunoassays (MSIA) or stable isotope standards and capture by anti-peptide antibodies (SISCAPA) depending on whether the quantification is performed at protein or peptide level.
However, capture molecules are still the limiting resource despite extensive activities in the field of affinity reagent generation. Slow off-rate modified aptamers (SOMAmers) are synthetic protein binders, which are isolated from artificial binder libraries. Availability of such binders is virtually unlimited and their production is more cost efficient compared to e.g. antibody binders. We demonstrate a workflow where plasma proteins and their isotopically labeled counterpart are precipitated using SOMAmers. The enriched proteins are digested on-bead and quantified by selected ion monitoring mass spectrometry with peptide redundancy for each protein. Advantages and disadvantages compared to peptide-centric approaches such as SISCAPA will be discussed by means of comparative data generated with SOMAmers.

Keywords: Mass spectrometric Immunoassay, Slow off-rate modified aptamers, Plasma proteins

PS10-04 Protein Quantitative Trait Locus (pQTL) Analysis in Mouse by Targeted Proteomics

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The hallmark of targeted proteomics is the quantification of a set of predefined peptides in a complex sample by the acquisition of specific precursor ion to fragment ion transitions over time. As the main implementation of this concept, selected reaction monitoring (SRM) has become a technology that ideally complements some limitations of shotgun strategies by its unique potential of SRM for reliable and reproducible quantification of proteins of low abundance in complex mixtures. To correlate protein abundance with genetic variations, a protein quantitative trait locus (pQTL) analysis relies on consistent and precise quantification of a set of peptides throughout a large number of samples. Here we applied SRM to a pQTL analysis across 58 liver samples of 29 diverse mice strains (BXD type derived from C57BL/6J and DBA/2J strains) to study the genetic control of 155 metabolic proteins. In total, we found 130 QTLs under chow diet or high fat diet, among which DHTKD1 and 1433B map significantly to the same QTL under both conditions. DHTKD1 is one component of the 2-oxoglutarate dehydrogenase complex, and its mutations cause 2-aminoadipic and 2-oxoadipic aciduria in human. We found a strong correlations between DHTKD1 protein levels in the liver, α-aminoadipic acid levels in plasma, and α-ketoadipic acid in the urine. These modulated metabolite levels, which are also observed in humans with malfunctioning DHTKD1, appear to be dependent on sequence variants in the Dhtkd1 gene between the two parent strains. Furthermore, most proteins were not cis-regulated (i.e. by their own gene) and were often differentially regulated between the two dietary conditions. Our results suggest targeted proteomics-based QTL analysis as a powerful strategy to study correlation among genetic variance, protein abundance and phenotypes in complex systems and to reveal regulatory networks of metabolic diseases.

Keywords: protein quantitative trait locus (pQTL), targeted proteomics, murine genetic reference populations

PS11-01 (Keynote) The Ubiquitin-Proteasome System

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The ubiquitin-proteasome system (UPS) controls diversed cellular processes - such as progression through the cell cycle, signal transduction, cell death, immune responses, metabolism, protein quality control, and developmental programs - by degrading short-lived regulatory or structurally aberrant proteins. I have been studying the UPS for the past 30 years, focusing on the clarification of the ubiquitin chain puzzling and the structure and functions of the proteasome. The former ubiquitin functions as a key molecule in an elegant post-translational protein modifying system. The latter proteasome is a 2.5-MDa sophisticated multisubunit complex that contains a catalytic core particle (CP) and two terminal regulatory particles (RPs), which associate with the termini of the central CP at opposite orientations. The CP consists of four heptameric rings (two outer α-rings and two inner β-rings), which are made up of seven structurally related α and β subunits. The CP contains catalytic threonine residues (β1, β2, and β5 with caspase-like, trypsin-like, and chymotrypsin-like activities, respectively) on the surface of a chamber formed by two abutting β-rings. The RP recognizes polyubiquitylated substrate proteins and then unfolds and translocates these proteins into the interior of the CP for degradation. One longstanding question is how the complex structure of the proteasome is organized with a high fidelity. Recently, we proposed a novel assembly mechanism that is assisted by multiple proteasome-dedicated chaperones. Moreover, we found the diversity of proteasomes named the immunoproteasome and the thymoproteasome whose catalytic subunits are replaced by homologous counterparts. These two isoforms perform specialized functions that help discriminate self from non-self in cell-mediated immunity. Collectively, emerging evidence suggests that the UPS plays an important role in various intractable diseases that have been increasing in today's aging society.

PS11-02 (Keynote) Molecular Dissection of Autophagy - Intracellular Recycling System -

Yoshinori Ohsumi

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Every cellular process is mediated by a balance between synthesis and degradation of proteins. Recently it has become clear that degradation is a highly regulated process, playing critical roles in cell physiology. Proteasome degrades every target protein after strict recognition by ubiquitination reactions, while the lysosome/vacuole system, autophagy, facilitates bulk and non-selective degradation. Under starvation recycling of own proteins becomes crucial for survival. Research also indicates that selective elimination of harmful proteins, organelles, and intracellular bacteria via autophagy is important for maintenance of cellular homeostasis.

More than 20 years ago we first observed autophagy induced by nutrient starvation in the yeast, *S. cerevisiae*, under a light microscope. Taking this advantage of the yeast, we succeeded in isolation of many autophagy-defective mutants. We know now that 18 *ATG* genes are essential for starvation-induced autophagy. These Atg proteins function concertedly in the sequestration of cytoplasmic constituents during the formation of a specialized membrane, known as the autophagosome. The Atg proteins consist of six functional units, namely the Atg1 protein kinase and its regulators, the PI3 kinase complex, the Atg2-Atg18 complex, the membrane protein Atg9, and two unique ubiquitin-like conjugation systems. Since these *ATG* genes are well conserved from yeast to mammals and plants, a vast range of studies in autophagy have recently been undertaken in various organisms.

We are attempting to elucidate the function of these Atg proteins, helping us to understand the unique membrane dynamics during autophagosome formation using the yeast system. To unveil the remaining mysteries of autophagy comprehensive analyses are required, including by microscopy, biochemistry, molecular biology, cell biology, and structural biology. Our present knowledge on the molecular mechanism and physiological roles of autophagy will be presented.

PS11-03 How Unspecific is Protein Degradation by Stress-Induced Autophagy?

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Stress-induced autophagy is regarded as a model for unspecific, autophagosomal bulk degradation of proteins by lysosomes. However, the autophagic flux is generally measured by western blot analysis of single proteins, microscopic techniques addressing autophagosome formation/degradation, or "long-lived protein degradation" assays using radioisotope tracers. These techniques monitor single proteins, whole organelles, or bulk protein degradation, but are unable to characterize the degradation of multiple individual proteins. Using mass spectrometry-based proteomics, we have studied protein abundance differences, turnover, and degradation in cells undergoing autophagy. In addition, we monitored the proteomic composition of autophagosomes by organellar proteomics approaches. Depending on the stress-inducing stimulus as well as the timeframe of stimulation, proteins from different subcellular compartments exhibit distinct dynamics. Also the composition of the autophagosome reflects the cellular needs under the specific stress conditions. Thus, global proteomics analyses highlight stimulus-specific aspects of stressed-induced autophagy. Currently, we aim at deciphering underlying autophagosomal targeting mechanisms responsible for specific protein degradation by autophagy.

Keywords: autophagy, degradation, SILAC

PS11-04 The True Story of How MHC Peptides are Produced

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Cellular protein degradation can be followed by analysis of their degradation products, the HLA peptides. This immunopeptidomes provide an unsurpassed outlook on the degradome of the cells and enables following the dynamics of degradation of the cells' proteins. We performed large-scale immunopeptidome analyses, using dynamic-SILAC labeling by immunoaffinity purification of the HLA molecules and Orbitrap MS. Using specific inhibitors we were able to define the main proteolytic pathways that lead to the formation of these immunopeptidomes. The more interesting effects were observed by inhibiting the proteasomes with inhibitors, such as epoxomicin and bortezomib (Velcade), which affected in a complex manner the rate of synthesis of the cellular proteins and of their degradation, and formation of MHC peptides. While (as expected) the proteasome inhibitors reduced the rates of degradation of many cellular proteins, they increased the degradation (and synthesis) rates of others. Correlating between the rates of production of the source proteins and their derived HLA peptides suggests that the contribution of the proteasomal proteolysis to the production of the HLA immunopeptidome should be reevaluated and that it may contribute more significantly to the production of HLA peptides derived from newly synthesized proteins, possibly including defective ribosome products (DRiPs) and short lived proteins (SLiPs), while a non-proteasomal pathway (such as the autophagosomal pathway) may contribute more to the production of HLA peptides derived from stable, long-lived proteins.

Keywords: HLA peptidome, dynamic-SILAC, proteasome

PS12-01 Quantitative Assessment of the Interactome, Phosphoproteome and Regulome in Bacterial Infection

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Interactome maps are typically generated using affinity purification-mass spectrometry, yeast two-hybrid screening or protein-fragment complementation assay. We have developed a novel method to monitor the interactome that combines SILAC and size exclusion chromatography to derive protein complex composition based on chromatographic co-elution; the use of triplex SILAC also allows measurement of the interactome's response to stimuli. This approach is as sensitive and as specific as more conventional techniques but avoids tagging artifacts and requires two orders of magnitude less work and instrument time. We apply it here to measure the response various interactomes to diseases states, including infection by the facultative intracellular bacterium *Salmonella enterica*, serovar Typhimurium. Presumably through its type 3-secreted effectors, *Salmonella* infection leads to wholesale rearrangement of the interactome. Many of the changes occur in proteins and complexes that are known targets of *Salmonella* effectors but several novel complexes also appear to be targeted. We will also present how the method can be adapted to monitor membrane protein complexes, such as those from the mitochondria and how they change during apoptosis.

Keyword: Interactome

PS12-02 Surrogate Markers for Abeta as Biomarkers for Alzheimer and T-ALL

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The 42 amino acid amyloid β-peptide (Aβ42) is the major constituent of senile plaques, which is the pathological hallmark of Alzheimer disease (AD) and is generated by proteolytic processing of the β-amyloid precursor protein (βAPP). Remarkably, in the CSF of AD patients Aβ42 levels are low, whereas this peptide accumulates within the brain. It has been believed that this discrepancy may be due to the fact that Aβ42 is largely deposited in insoluble plaques within the brain and that clearance into the CSF is therefore reduced. Although the reverse relationship of Aβ42 levels can be used as a biomarker to some extent after disease onset, better surrogate markers specifically for presymptomatic diagnosis are desperately required.

The canonical Notch signaling is transmitted intracellularly via sequential endoproteolysis of Notch receptors in a ligand-dependent manner. The receptors undergo extracellular shedding by metalloproteases, followed by intramembrane proteolysis by presenilin (PS)/g-secretase. As a result, the intracellular signaling molecule Notch intracellular domain (NICD) is liberated. Because the degradation of the Notch-1 transmembrane domain is thought to require an additional cleavage near the middle of the transmembrane domain, extracellular small peptides (Notch-1 Aβ-like peptide [Nβ]) may be produced. We discovered APL1β28 as a novel and highly sensitive biomarker. This peptide is generated by the same proteolytic mechanism as Aβ42, except that it is derived from a divergent substrate, namely the βAPP-like protein, APLP1. Non-amyloidogenic APL1β28 can be detected in the CSF and its levels correlate with Aβ42 production. Remarkably, the ratio of APL1β28 to total APL1β are significantly increased in familial and sporadic AD patients. We propose using the levels of APL1β28 as a surrogate marker for Aβ42 production in the central nervous system. This has clinical importance for the diagnosis and early detection of sporadic AD.

Similarly, we found that the Nβ species are aberrantly secreted in T-lymphoblastic leukemia (T-ALL) cells. The overexpression of pathogenic Notch-1 mutants or the artificial upregulation of S2 cleavage increased the levels of Nβ secretion. Therefore, we considered that high levels of Nβ may be secreted from human T-ALL cells, with abnormally enhanced Notch signaling due to the Notch-1 mutations. These results indicate that Nβ is a candidate surrogate marker for Notch signal transduction, and potential biomarker for T-ALL.

PS12-03 In Deep Characterization of the Host Pathogen Interaction during Internalization of *Staphylococcus aureus* by A549 Cells

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S. aureus, a Gram-positive coccus, can cause a broad range of diseases in humans ranging from milder skin infections to severe diseases such as the toxic shock syndrome or systemic infections like sepsis or endocarditis. Though widely considered an extracellular pathogen, it became recently obvious that *S. aureus* is able to invade and persist in non-professional phagocytic cells. We therefore developed a protocol which allows the quantification of proteins from bacteria and host cells with cell sorting and LC-MS/MS based proteomics. The host model chosen in the study presented here employs the A549 cell line isolated from a human lung cancer. First, we observed an increase of the intracellular number of bacteria per infected cell until 6.5 h post infection. Second, 842 *S. aureus* proteins were identified and quantified using our proteomics workflow. As part of this data set, bacterial proteins related to the uptake of iron or to the peptidoglycan biosynthesis showed a higher level during infection, while virulence factors and ribosomal proteins were reduced in abundance. Third, proteomic analysis of the host side resulted roughly in the identification of 2,000 and quantitation of 1,000 proteins. Ten proteins belonging to the pigment granule and 27 proteins of the membrane-enclosed lumen were up-regulated. Contrarily, proteins of the non-membrane-bound organelle and of protein biosynthesis were down-regulated when compared to non-infected control cells. Finally, we quantified twelve different cytokines in the cell culture supernatants and observed increased levels of IL-8, IL-6 and IFN-γ in a time frame of 48 h post infection.

Keywords: Host-Pathogen Interaction, isotopic labeling, Voronoi Treemap Visualization

PS12-04 Proteomic Study of Biomarkers for Amyotrophic Lateral Sclerosis in Human Muscle Biopsies

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Amyotrophic lateral sclerosis (ALS) is a serious neurodegenerative disorder characterized by progressive loss of motor neurons causing muscle atrophy. Many research groups looked for ALS protein biomarkers applying proteomic and genomic approaches, using different tissues and body fluids, animal models and real patients. In present study we used muscle biopsies from patients diagnosed with ALS in order to find the altered proteins specific for this disease. We compared three groups of patients: ALS diagnosed, control objects, and patients with other diseases causing denervation (one of them is post-polio syndrome). Our gel-free proteomic approach involved detergent-based protein extraction from the muscle tissue, in-solution digestion of proteins, and isotope dimethyl labelling of tryptic peptides, followed by protein identification and relative quantification by nano-liquid chromatography - high resolution mass spectrometry (nanoLC-MS/MS, Fourier-transform ion cyclotron resonance mass spectrometer, FT-ICR, has been used). We found an array of protein candidates which were: (i) downregulated exclusively in ALS patients' biopsies, e.g. FHL1, Four and a half LIM domains protein 1, (ii) upregulated exclusively in ALS patients' biopsies, e.g. PADI2, Protein-arginine deiminase type-2, (iii) altered in biopsies of patients with other diseases, but not changed in ALS patients' biopsies. These proteins, individually or in combination, could be used as disease biomarkers revealing molecular mechanisms of ALS development. Importantly, after additional verification, these potential biomarkers can be used for diagnosis of ALS from muscle biopsy without a risk to confuse ALS with other denervation (presently ALS cannot be diagnosed though muscle biopsy).

Keywords: ALS, muscle, proteomics

PS12-05 Mass Spectrometry Methods for Surrogate Biomarker Discovery in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is one of the most common and severe form of childhood muscular dystrophies affecting about 1 in 3,500 boys. The need to define surrogate biomarkers to monitor DMD progression and response to treatments is becoming crucial as promising treatment strategies for DMD are entering phase II and III clinical trials. The most commonly used surrogate endpoint for DMD to date is the 6 minute walk test. However, this test seems to be not sensitive enough for clinical trials with a short a period of time and also impractical for DMD patients who lost ambulation. In this study we have initiated proteome and metabolome profiling on serum samples from both DMD patients and dystrophin deficient mouse model (mdx). Using nano-hydrogel particle proteome profiling on serum samples from DMD donors ($n = 10$) and age matched healthy controls ($n = 10$) we identified 11 candidate biomarkers that were highly elevated (p value < 0.05) in serum of DMD patients relative to controls. Whole serum proteome profiling of mdx ($n = 3$) and wild type mouse ($n= 3$) using SILAC mouse spike-in strategy led to the identification of an additional 19 candidate biomarkers that were associated with dystrophin deficiency. Of these 8 were validated in human serum samples. Furthermore metabolomics analysis of DMD serum ($n = 5$) versus healthy control serum ($n = 5$) revealed dramatic alterations in the levels of specific amino acids and lipids. Biomarkers associated with disease progression were evaluated and validated in the mouse model.

Keywords: Duchenne muscular dystrophy, serum, surrogate biomarkers

PS13-01 Quantitative Mass Spectrometry (SRM/MRM) to Amyloid Peptides, Tau Protein, and Apolipoprotein E in Human Cerebrospinal Fluid for Alzheimer Disease Diagnosis

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BACKGROUND: Recent improvements in mass spectrometry (MS) allow this technology to quantify with clinical grade analytical sensitivity and specificity, peptides and proteins in biological fluids. We believe that in some cases MS will represent a valuable alternative to immunodetection methods. We followed this path for biomarkers in Alzheimer disease (AD) which represents major cause of dementia. AD is associated with specific apolipoprotein E (ApoE) isoforms, and with alteration of cerebrospinal fluid (CSF) biomarkers. As a matter of fact, the decrease of amyloid peptides (Aß) and the increase of Tau proteins in CSF are currently use for AD diagnosis. Many isoforms of these molecules exist and MS represent an interesting tool to quantify their diversity, and therefore, to improve AD diagnosis and follow-up.

METHODS: For this purpose, quantitative targeted mass spectrometry (SRM/MRM) was developed using a triple quadripole. SPE, trypsic digestion and sample clean-up were realised using an automated liquid handling robot. Quantotypic peptides (Aß1-40, Aß1-42, tau, ApoE..) were synthetized in light and heavy (^{13}C/^{15}N) versions and used in calibration curve to evaluate LOD and LOQ. Experiments were run on series of human biological samples.

RESULTS: Optimal MRM methods for the different analytes were developed. Detection of specific Apo E peptides resulted in a rapid method for e2/e3/e4 phenotyping. Different isoforms of Aß and Tau proteins were detected with sensibility compatible with pathophysiological variations. Correlation with immunodetection methods and validation of the clinical relevance of the results are on-going.

CONCLUSIONS: The MS detection of several isoforms of Aß, Tau protein, and Apo E in CSF represents an important achievement that opens new avenue for quantitative Clinical Chemistry Proteomics (qCCP). The perspective is to exploit these results to improve phenotyping, diagnosis and follow-up of dementia.

Keywords: Quantitative Clinical Mass Spectrometry, Neurodegenerative diseases, Alzheimer

PS13-02 High Resolution MRM Quantification of 300 Tear Proteins Using MS/MS^ALL with SWATH™ Acquisition and Its Application to Biomarker Discovery

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Many studies have demonstrated that the tear fluid is an accessible and useful source in studying ocular surface disorders and biomarker discovery. The aim of this study was to establish a robust, reproducible and rapid quantitative method for tear protein biomarker study from a large number of clinical samples. Human tear samples were collected from 1000 patients with no eye complaints (411 male, 589 female, average age 55.5 years, SD 14.5 years) using the Schirmer tear test strips and pooled into a single global control sample. A 2-hour nanoLC-MS/MS run was used to separate the tryptic peptides and MS data was recorded using MS/MS^ALL with SWATH acquisition on a TripleTOF® 5600 system. Data from three information dependent acquisition (IDA) experiments were combined and used as ion library for subsequent processing of SWATH acquisition data. This method was applied to compare the levels of tear proteins in both post-trabeculectomy glaucomatous eyes (n=11) and normal control eyes (n=8). For quantitation, 1487 peptides representing 474 proteins from IDA were used and the coefficient of variation (CV) of 808 peptides representing 298 proteins was below 20% for five replicates. Quantitative analysis revealed that 27 tear proteins were upregulated (ratio > 2.0) and 20 tear proteins were downregulated (ratio < 2.0) in post-trabeculectomy group as compared to control group. Tear proteomics results suggest that prolonged ocular surface inflammation after trabeculectomy may lead to increased wound healing and reduced surgical success. This study demonstrated that high resolution MS/MS based quantification of hundreds of tear proteins using MS/MS^ALL with SWATH acquisition is a powerful workflow for biomarker discovery.

Keywords: SWATH, Tear Proteomics, High Resolution MRM

PS13-03 Development of an LC-MRM Based Assay of Prostate Specific Antigen (PSA) in Blood Samples

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Introduction Quantitative determination of Prostate Specific Antigen (PSA) in the human serum has been used for the early diagnostics and prognostics of prostate cancer. Most of the existing methods utilize enzyme-linked immunosorbent assay (ELISA) to measure the PSA in the serum. The ELISA method, while providing good sensitivity and high throughput for the PSA diagnostics, suffers from low specificity. There is a great deal of interest to find a more reliable diagnostic and prognostic method. In this paper we reported a method to quantify PSA in serum using a simple sample preparation followed by the LC-MS/MS analysis operated in multiple reaction monitoring (MRM) mode. The MRM method provides high sensitivity, high accuracy and good specificity. **Methods** PSA standard was trypsinized and analyzed for peptide identification on an ion trap mass spectrometer initially. The obtained MS/MS data was searched using Mascot software. Two signature peptides from PSA standard were selected for MRM analysis. The MRM method and ion source parameters were further optimized for sensitivity and interference, and linear responses. For serum samples, high abundant proteins such as albumin were removed by solid phase extraction (SPE) to reduce the matrix interference. After trypsin digestion, the PSA peptides were analyzed with the MRM method with external calibration. **Preliminary results** An initial MS/MS study on an ion trap mass spectrometer was carried out on the tryptic digested PSA standard. Based on the high relative abundance and spectra quality and other reported exclusion criteria, two peptides were chosen with their MRM transitions for further optimization. For PSA standard, the LOQ (S/N>10) of the developed MRM assay are at pg/mL level. For PSA in serum after the removal of high abundance protein, the LOQ is at high pg/mL level, sufficient for clinical diagnosis purpose.

Keywords: Clinical diagnostics, MRM triple quadrupole, Biomarker

PS13-04 Improved PhosphoTau SRM Assay Sensitivity Enables Multi-site Tau Phosphorylation Quantitation in a Preclinical Model of AD Treated with Novel Small Molecule Inhibitors of Casein Kinase 1 Delta

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Neurofibrillary tangles, comprised of paired helical filaments (PHFs) of hyper-phosphorylated tau, are a pathological characteristic of Alzheimer's disease (AD). We previously characterised PHF tau isolated from post-mortem AD brain tissue by mass spectrometry. Subsequently, we developed the Phospho-Tau SRM assays to measure tau phosphorylation levels in preclinical AD models. The Phospho-Tau SRM 6plex assay enables the quantitation of total tau plus five phosphorylation sites; distinct human and mouse pThr181 measurements, pSer199, pThr231, pSer262 and pSer396 (human 2N4R numbering). The Phospho-Tau SRM 7plex assay quantifies six phosphorylation sites, pSer46, pThr50, pSer113, pSer396, pSer404, pSer433, as well as distinct measurements covering the R406W mutation, present in the TMHT tau transgenic mouse model. Prior to SRM analysis, phosphopeptides were resolved by microflow (100 μL/min) reversed phase chromatography (XBridge C18 3.5 μM, 1.0 x 100mm, Waters). The linear working range of the microflow-PhosphoTau SRM 6plex assay was 5-1000fmol on column (o/c), with CVs ranging from 5-20%. Per analysis up to 10 μg total protein per sample was consumed. Herein we describe the miniaturisation of the PhosphoTau SRM assays to nanoflow (200nl/min; Easy C18 3 μM ID 75 μM x 100mm, ThermoFisher). Preliminary data demonstrates a 50 fold improvement in linear working range, down to 100 atmol o/c, and detection of endogenous Tau phosphorylation levels in preclinical AD models from as little as 1 μg of material. The utility of the nanflow-PhosphoTau SRM assays to quantify multiple site specific phosphorylation events will be demonstrated in a tau transgenic mouse model treated with novel small molecule inhibitors of Casein Kinase 1 delta.

Keywords: Selective Reaction Monitoring, Targeted Quantitative Phosphoproteomics, Alzheimer's, disease

PS14-01 The Structural Proteomics of Glycoproteins

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It is estimated that approximately half of all proteins encoded by the human genome have attached sugars. Many of these molecules are important cell surface receptors and the target of disease modifying antibodies. Obtaining structural information from glycoproteins presents presents two major technical challenges for structural biology (1) protein production requires eukaryotic expression which is time-consuming and relatively expensive and (2) products are heterogeneously glycosylated which generally militates against crystallization. We have developed methods to address these issues and have assembled a pipeline for producing and crystallizing glycoproteins. The pipeline includes parallelized ligation independent cloning, semi-automated small scale transient expression screening in HEK 293 cells; scale-up of protein production in HEK cells; automated protein purification; quality assessment by mass spectroscopy; and nanodrop crystallization. The application of these processes to solving the structure of a number of glycosylated proteins will be presented.

Review

Aricescu AR, Owens RJ. Expression of recombinant glycoproteins in mammalian cells: towards an integrative approach to structural biology Curr Opin Struct Biol. 2013 Apr 25. pii: S0959-440X (13) 00055-9. doi:10.1016/j.sbi.2013.04.003. [Epub ahead of print]

PS14-02 Structural Insights into the EGFR Pathway Substrate Dephosphorylation by Protein Tyrosine Phosphatase N3

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Reversible tyrosine phosphorylation is an essential post-translational modification in the regulation of numerous cellular activities. This fundamental mechanism is controlled by protein tyrosine kinases, protein tyrosine phosphatases (PTPs) and thousands of their substrates in cells. PTPs represent a large family of evolutionarily conserved enzymes, which are responsible for the phosphate removal via a phosphoryl-cysteine intermediate. In this project we have focused on PTPN3, a member of the membrane-associated tyrosine phosphatase sub-family contains an N-terminal FERM domain, a PDZ domain and a C-terminal PTP domain. Our recent findings have indicated that PTPN3 is a specific phosphatase for an epidermal growth factor receptor (EGFR) pathway substrate (EPS). Dephosphorylation of EPS by PTPN3 is important for the regulation of EGFR internalization in non-small cell lung cancer. To determine the molecular basis of PTPN3 substrate recognition, we have solved the crystal structure of PTPN3 catalytic domain in complex with EPS phosphopeptide at 1.6 Å resolution. Binding of EPS phosphopeptide to the PTPN3 active site reveals a novel conformation, which is different to other PTP-phosphopeptide structures. Our phosphatase activity confirmed a high level of substrate specificity between PTPN3 and EPS. Employing the biochemical approach and structural analyses, we have identified several key PTPN3 residues involved in the recognition of EPS. Its effect *in vivo* is currently being investigated. In a parallel study, we have also determined the crystal structure of PTPN3 in complex with the MAPK12 phosphopeptide at 2.5 Å resolution. The interaction between PTPN3 and the MAPK12 phosphopeptide reveals a similar binding pattern to other PTP-phosphopeptide structures, which further emphasize the novelty of EPS recognition by PTPN3.

Keywords: Dephosphorylation, Phosphatase, EGFR

PS14-03 Cooperative Formation of a Multiple Transcription Factor Assembly on an Enhancer DNA and Its Regulation by Phosphorylation of a Transcription Factor via a Cell Signaling

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Eukaryotic transcription factors (TFs) activate target genes by forming specific TFs-DNA complexes on target gene enhancers. These events are characterized by a cooperative TFs binding on the target DNA and also considered to be regulated by chemical modifications of TFs within the complex.

To understand the mechanism for regulation of higher order TFs-DNA complexes, we focused on a complex formed on the *TCRα* gene enhancer, which is comprised of multiple hematopoietic TFs including Ets1, Runx1/CBFβ and LEF1. Among these TFs, Ets1 is known to be phosphorylated via a Ca^{2+}-dependent cell signal, and the phosphorylated Ets1 appreciably inhibits its own DNA binding activity. We recently performed crystallographic and biochemical analyses of the Ets1-Runx1-CBFβ-DNA complex formed on the *TCRα* gene enhancer and found that Runx1 allosterically modulates the conformation of Ets1 through the DNA molecule, thereby enhancing the DNA binding activity of Ets1 and counteracting the autoinhibitory effect of phosphorylation of Ets1 for the DNA binding.

Now, to understand the mechanism in detail, we have engaged in analyses of the inhibitory mode by which phosphorylation of Ets1 affects its DNA binding. In parallel, to reveal a complete picture of the TFs-DNA complexation on the *TCRα* gene enhancer, we proceed with crystallographic analyses of the LEF1-Runx1/CBFβ-DNA and the Ets1-$(Runx1/CBF\beta)_2$-DNA complexes.

We will discuss a possible mode of cooperative DNA binding of these TFs and their interplay with phosphorylation.

PS15-01 Chemical Proteomics for Drug Discovery: Drug Targets, Drug Selectivity, Drug Mechanism of Action and Drug Resistance

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Preclinical stages in the drug discovery process require a multitude of biochemical and genetic assays in order to characterize the effects of drug candidates on cellular systems and model organisms. Early attempts to apply unbiased proteomic techniques to the identification of protein targets and off-targets as well as to elucidate the mode of action of candidate drug molecules suffered from a striking discrepancy between scientific expectations and what the technology was able to deliver at the time. Dramatic technological improvements in mass spectrometry-based proteomic and chemoproteomic strategies have radically changed this situation in the past few years. On the example of kinase inhibitors, this presentation highlights chemical proteomic approaches suitable for differnt aspects of drug discovery. These are illustrated by examples from my laboratory including the identification of drug targets, the determination of the selectivity of drugs, the elucidation of the mechanism of action of a drug and the detection of resistance mechanisms.

Reference:

Schirle, Bantscheff and Kuster (2012) Mass Spectrometry-Based Proteomics in Preclinical Drug Discovery. *Chemistry & Biology*, **19** 72-84.

PS15-02 Systems-Level Analysis of Inflammatory Proteolytic Events In Vivo by N-Terminomics Analyses

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During inflammation, local tissue responses are augmented by complement and acute phase proteins that exude into the tissue because of increased blood vessel permeability mediated by bradykinin, which is proteolytically released from kininogen. We quantified changes in the proteome and the nature of protein N-termini (the N-terminome) and the altered abundance of murine proteases and inhibitors during skin inflammation. Through analysis of the N-terminome by iTRAQ-terminal amine isotopic labeling of substrates (TAILS), we identified cotranslational and posttranslational αN-acetylation motifs, quantitative increases in protein abundance, and qualitative changes in the proteolytic signature during inflammation. Of the proteins identified in normal skin, 50% were cleaved, which increased to 60% during inflammation caused by phorbol esters, including chemokines and complement in which we identified previously uncharacterized cleavage sites. In mice deficient in matrix metalloproteinase 2 (MMP2), exudation of serum proteins was diminished compared to that in wild-type mice, and their proteolytic networks differed. Quantitative analysis of the neo-N terminal peptides revealed a novel MMP2 cleavage site in complement 1 (C1) inhibitor that was detected *in vivo*. Cleavage and inactivation of the C1 inhibitor by MMP2 increased complement activation and bradykinin generation by plasma kallikrein in wild-type mice, leading to increased vessel permeability during inflammation. In the absence of MMP2, the intact C1 inhibitor levels rose and exerted negative regulatory effects on generation of bradykinin by reducing plasma kallikrein activity and kininogen cleavage. The knock out mice also had reduced complement activation through pathways controlled by C1 inhibitor. Our degradomics analysis dissecting proteolysis in skin inflammation demonstrated perturbance of the proteolytic signaling network and its functional consequences arising from lack of a single protease. The roles of proteases in vivo can therefore be unravelled and either the protease or the pathways they regulate can then be targeted for drug development to restore normal homeostasis.

PS15-03 Cell-Surface Proteins as Drug Targets

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Cell-surface receptors represent attractive targets for drug intervention and numerous cancer therapies are directed towards these molecules using either monoclonal antibodies or low molecular weight inhibitors thereby modifying the functionality of these receptors. Analytical proteomics provide the tools to identify quantify and structurally characterize these cell-surface proteins, of which the majority is glycosylated. A detailed characterization is essential as post-translational modifications, mutations, deletions, and the formation of protein complexes are frequently associated with the biological activity of the receptors.

Targeted high-resolution mass spectrometry methods have been developed to precisely analyze glycosylated cell surface proteins. These have been applied to monitor changes in glycosylation patterns of the primary cell surface receptor targets in health and disease, as well as their downstream "partners" involved in signaling pathways.

The methodology based on the specific isolation of glycoproteins from isolated cells or tissues and the LC-MS characterization will be demonstrated on the EGF-receptor, an important drug target in various forms of cancer. Furthermore, down-stream signaling pathway profiling of wild-type EGF receptor and clinically relevant mutated EGF receptor forms using reversed protein arrays have been employed to trace sensitivity and acquired resistance of tumors towards EGF receptor selective tyrosine kinase inhibitors.

PS16-01 Integrative Multi-Platform Analysis of Cancer Kinome Networks

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Biological systems are composed of highly dynamic and interconnected molecular networks that drive biological decision processes. The goal of network biology is to describe, quantify and predict the information flow and functional behaviour of living systems in a formal language and with an accuracy that parallels our characterisation of other physical systems such as Jumbo-jets. Decades of targeted molecular and biological studies have led to numerous pathway models of developmental and disease related processes. However, so far no global models have been derived from pathways, capable of predicting cellular trajectories in time, space or disease. The development of high-throughput methodologies has further enhanced our ability to obtain quantitative genomic, proteomic and phenotypic readouts for many genes/proteins simultaneously. Here, I will discuss how it is now possible to derive network models through computational integration of systematic, large-scale, high-dimensional quantitative data sets. I will review our latest advances in methods for exploring phosphorylation networks. In particular I will discuss how the combination of quantitative mass-spectrometry, systems-genetics and computational algorithms (NetworKIN [1] and NetPhorest [4]) made it possible for us to derive systems-level models of JNK and EphR signalling networks [2,3]. I shall discuss work we have done in comparative phospho-proteomics and network evolution [5-7]. Finally, I will discuss our most recent work in analysing genomic sequencing data from NGS studies and how we have developed new powerful algorithms to predict the impact of disease mutations on cellular signaling networks [8,9].

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PS16-02 The Genetic Landscape of a Cell

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We are constructing a genome-scale genetic interaction map, examining all ~18 million gene-gene pairs for synthetic genetic interactions, which has generated quantitative genetic interaction profiles for ~75% of all genes in the budding yeast, *Saccharomyces cerevisiae*. A network based on these profiles reveals a functional map of the cell in which genes of similar biological processes cluster together in coherent subsets and highly correlated profiles delineate specific pathways to define gene function. Most recently, we've been focussing on the essential gene network, mapping genetic interactions for conditional temperature sensitive alleles of essential genes. The resultant global network identifies functional cross-connections between all bioprocesses, mapping a cellular wiring diagram of pleiotropy. Genetic interaction degree correlated with a number of different gene attributes, which may be informative about genetic network hubs in other organisms. Large-scale genetic interaction mapping in human cancer cells carrying defined mutations revealed networks resembling the yeast network and identifies potential drug targets for synthetic lethal cancer therapies.

PS16-03 Ca²⁺ Induced Phosphorylation Signalling in Nerve Terminals

Phillip J. Robinson

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Synaptic plasticity is how neurons adapt over time, as occurs when the brain is learning and storing memories. The main adaptation is the amount of synaptic transmission between presynaptic nerve terminals and postsynaptic dendrites. Presynaptic nerve terminals are small organelles of about 1 micron in diameter containing all the machinery and subcellular organelles required for synaptic transmission. They respond to differing depolarising stimulus by increasing exocytosis of synaptic vesicles (SVs), and produce new SVs by two recycling paths: clathrin-mediated endocytosis or bulk endocytosis following by budding of new SVs. Initially phosphorylation of dynamin was found to mediate bulk endocytosis. We have now shown that depolarising stimuli of different intensities stimulates unique patterns of global phosphorylation changes in the nerve terminal. The proteins are involved in multiple SV functions including different endocytic modes and SV biogenesis. We have uncovered evidence for the global integration of these signal transduction pathways by protein phosphatase regulatory proteins. We aim to increase knowledge of presynaptic plasticity at the molecular level. Better understanding the molecular mechanisms of synaptic transmission may lead to new therapeutic approaches for treatment of disorders of synaptic transmission.

PS17-01 Conformational Changes of Multi-Protein Complexes Determined by Mass Spectrometry

Juri Rappsilber

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Life is a marvellous and complex network of dynamic processes. Structural biology currently provides wonderful insights on highly purified proteins (often trimmed to remove flexible domains) yielding snapshots that reveal much about how protein domains interact and how ligands are bound. However, I cannot help but imagine how much more we could learn if we were to study intact proteins in their native environments as parts of dynamic processes possibly within huge macromolecular assemblies. I think with quantitative cross-linking/mass spectrometry (CLMS) we start holding a tool in our hands that will make important contributions to this. We have shown recently that cross-linking analysis is compatible with quantitation by stable isotope labelling (Fischer et al. 2013). Quantitative CLMS shows its full power as part of an integrated structural biology approach that includes tools that outline the shape of protein complexes (cryoEM and SAXS) and tools that reveal high-resolution structures of subunits (X-ray crystallography, NMR). I will present here how quantitative CLMS as part of integrated structural biology can provide structural and biological insights into key proteins.

Fischer L, Chen ZA, Rappsilber J. Quantitative cross-linking/mass spectrometry using isotope-labelled cross-linkers. J Proteomics. 2013 Aug 2;88:120-8.

PS17-02 Protein-Phosphoprotein Complexes in DNA Damage and Cancer Signaling

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The forkhead-associated (FHA) domain is the only known phosphoprotein-binding domain that specifically recognizes phosphothreonine (pThr) residues, distinguishing them from phosphoserine (pSer) residues. In contrast to its very strict specificity toward pThr, the FHA domain recognizes very diverse patterns in the residues surrounding the pThr residue. Also, the FHA domain exists in various proteins with diverse functions and is particularly prevalent among proteins involved in the DNA damage response and cancer signaling. This lecture will highlight the diversity of biological functions of two FHA domain-containing proteins in DNA damage response and cancer signaling. Phosphorylation sites and binding proteins are identified by mass spectrometry, and then the structures of protein-phosphoprotein or protein-phsphopeptide complexes are solved by NMR or X-ray crystallography.

Keyword: FHA

PS17-03 From the Top Down; Mass Spectrometry and Its Role in Studying Intact Proteins and Protein Complex Structure, Dynamics, and Assembly

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Around for more than a century the analytical technique of mass spectrometry is blooming more than ever, and applied in nearly all aspects of the natural and life sciences. In the last two decades mass spectrometry has become routine for the high-throughput analysis of peptides. However, also intact proteins and even complete protein complexes can nowadays be analyzed, enabling MS to enter the field of structural biology. Here, I will describe the emerging role of mass spectrometry with its different technical facets in structural biology, focusing especially on the analysis of viruses, dynamic protein assemblies and therapeutic antibodies. I will describe how mass spectrometry has evolved into a tool that can provide unique structural and functional information about viral protein and protein complex structure, conformation, assembly and topology, extending to the direct analysis of intact virus capsids of several million Da in mass. Mass spectrometry is now used to address important questions in virology ranging from virus structural topology to how viruses assemble. The unique features of native MS will further be highlighted in a study of the macromolecular complex controlling circadian timing. I will also describe recent developments in mass spectrometry technology that have allowed us to analyze intact native proteins and protein complexes using Q-ToF and Orbitrap mass analyzers with very high sensitivity and mass resolving power, and how that has enabled us to profile therapeutic antibody quality and structure in their native state without requiring any sample preparation. Prospectively, I will describe what I think will be the huge contribution of this breakthrough for the future of proteomics.

Keywords: protein assemblies, top-down proteomics, native mass spectrometry

PS17-04 In Vivo Crosslinking Combined with Label-Free Quantitative Proteomics to Determine the Subcellular Distribution and Stoichiometry of Proteasome Complexes

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The proteasome is a large protein complex involved in the degradation of intracellular proteins. It thus plays a crucial role in the regulation of many cellular processes and in antigenic peptides presentation. A 20S core particle, that contains the catalytic activity, can be associated to one or two regulatory particles (RPs) of identical or different protein composition. Several RPs exist but their precise subcellular distribution remains to be determined. Here we present an efficient integrated workflow combining *in vivo* crosslinking with cell fractionation and an affinity purification-mass spectrometry strategy using the 20S core particule as a bait and label-free quantitative proteomics to determine the endogenous subcellular distribution of human proteasome complexes[1].

Labile proteins interactions of RPs with the 20S complex have been successfully stabilized by *in vivo* crosslinking using formaldehyde before cellular factionation while maintaining proteasome activity. Label-free quantitative data were then acquired using a high sequencing speed, high resolution Orbitrap mass spectrometer and analyzed using the home-developped MFPaQ software. In two leukemic cell lines we showed that there was a high proportion of 20S complex not associated with RPs and that the 19S RP was the main associated activator in all cellular compartments (cytosol, microsomes, nucleus). This result was confirmed in total cell extracts of 7 various additional cell lines. However, the determination of the precise distribution of free and RPs associated 20S complexes revealed differences that could be correlated with variations in proteasome activity, including in fractions where proteasome complexes are present in low amounts (nucleus, microsomes). Moreover, this optimized workflow allowed to follow the dynamics and to highlight the specificity of RPs association with the 20S complex.

1. Fabre et al, Mol Cell Proteomics 2013, 12, 687

Keywords: Protein complex, Human Proteasome, Crosslink

PS18-01 Utilizing Carbohydrate Fragmentation Database (UniCarb-DB) for Glycoresearch Discovery

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Glycomic analysis of *O*-linked oligosaccharides using mass spectrometry are generating large amount of data that is difficult to overview and extract the important information that could be used to answer biological questions. The exercise can simplistically be described as being able to identify the core types (usually core 1-4), branching (I and i) and/or elongation (type 1 and type 2) pattern, and terminal epitopes (eg sialylation, blood groups). Branching/elongation analysis also includes the exercise in identifying non-terminating such as sulfation, and internal sialic acid.

In order to address these question we have accumulated structural information together with mass spectrometric data (MS^2 spectra) and metadata in the resource UniCarb-DB (www.unicarb-DB.com). This resource is now the platform for future development of softwares for interpretation of glycomic LC-MS and LC-MS^2 data. The utilization of peak matching tools for MS^2 spectra allows identification of oligosaccharide candidate structures based on matches found with structurally assigned spectra in the database The peak matching tool can also be used to identify the type of terminal structures present. This is exemplified by the approach to identify 3 or 6 linked sulfate based on *m/z* regional matching of MS^2 spectra.

Including appropriate metadata to UniCarb-DB would allow advanced bioinformatic mining of the database. For instance, including also intensity information and type of instruments in the database, datasets using similar analytical methods can be compared for such things as tissue typing and biomarker discoveries, where it is obvious from comparison pulmonary infection, that its is not only a switch between sulfate and sialic acid but also an alteration of the core type.

The challenge for the future is to utilize current glycomic resources, such as UniCarb-DB to develop the workflows for glycomic analysis to reach the sophistication to be utilized for biodiscovery.

PS18-02 Challenges & Solutions for Glycoproteomics

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Glycoconjugates are well known to be key players of intercellular communication [1]. Comprehensive knowledge on these important molecules and their particular glycan structures present on the cell's surface is a fundamental requisite for deciphering glycan mediated intercellular communication signals. Glycoproteomics provides this information by identifying, characterising and cataloguing glycoproteins present on cell surfaces or biological fluids [2, 3, 4]. In this context detailed glycoproteomic maps of major immunoglobulins such as sIgA [5] and IgM [Kolarich *et al*, manuscript in preparation] have just been acquired recently.

Despite tremendous recent advances in qualitative glycoproteomics applications particular quantitative questions regarding the MS signal intensity relationships between various forms of un-/de-/glycosylated peptides remained unanswered. To determine the quantitative information label free techniques provide on glyco-microheterogeneity an in-house produced synthetic library of peptides and N-glycopeptides was investigated using various ionisation sources/MS detector combinations [4]. These N-linked glycopeptides were found to exhibit up to 10x lower signal intensities compared to their unglycosylated counterparts when equimolar mixtures were analysed. This finding emphasises the importance of glycopeptide specific sample preparation steps prior analysis.

Combining glycoproteomics findings with well-established solid phase (glyco) peptide synthesis provides unique opportunities for numerous glycobiology aspects such as method development, quantitative glycoproteomics and functional glycobiology.

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PS18-03 Connecting Proteomics with Glycomics

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As the proteomics field starts to encompass glycomics and glycoproteomics, specific new methodologies and informatics databases and tools need to be developed to interpret the data and ultimately relate it to the function of these glycans and their glycoconjugates.

For glycoproteomics analysis of a complex biological mixture of proteins in sputum we present a novel glycomics and glycoproteomics strategy for the site-specific analysis of glycoproteins. N-glycosylation heterogeneity was determined by combining global N-glycome characterization followed by the analysis of deglycosylated and glycosylated peptides with orthogonal fractionation, complementary tandem mass spectrometry (MS/MS) and advanced data-processing tools.

For glycoprotein data analysis, in 2011 we introduced UniCarbKB as an international initiative that aims to collect, distribute and extend resources and practices from glycobiologists to the whole biological research community (1). The mission is to provide a comprehensive, high quality catalogue of published and experimental information on the carbohydrates attached to proteins, and to integrate this data with the other '-omics' knowledgebases. I will present on behalf of many participants, a summary of the start we have made on establishing the infrastructure and content of the publically available UniCarbKB. The establishment of a glycomics bioinformatics hub on the ExPASy server and the linking of UnicarbKB to the proteomics knowledgebase UniProt will be described. Other data initiatives, such as the establishment of UniCarb-DB an annotated data repository of MS/MS spectra, currently in its infancy, will also be reported on. In the future it is hoped that the UniCarbKB knowledgebase, centered on a reference database of curated glycan structures, will become the key resource of quality information for glycoproteomic research.

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PS18-04 N-Glycoproteome Analysis from Identification of Glycosylation Sites to Intact Glycopeptides

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The comprehensive analysis of N-glycoproteome requires the high-throughput determination of glycans structures and their precise attachment sites on protein sequences. By using the combined technology and method, we have identified about 5,000 glycosylation sites to generate the largest dataset of protein glycosylation for human liver. We also developed the solid-phase based technology by integrating all of the digestion, enrichment, deglycosylation together with LC-MS analysis for glycoproteome analysis, and by which both the identification sensitivity and through-put was improved greatly. Almost no glycopoteomic method has been developed for identification of both glycan structures and peptide backbone, finally we present a novel high throughput strategy that combines MS spectra of deglycosylated peptides and MS spectra of intact glycopeptides to identify glycopeptide sequences, glycan structures, and specific attachment sites. Application of this strategy to a complex proteome led to the identification of the largest dataset of glycosylation from mammalian samples.

Keywords: Glycoproteomics , N-glycosylation site, Glycan structure

PS19-01 (Keynote) Exploring Communication in the Tumor Microenvironment

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Myeloid-derived suppressor cells (MDSC) inhibit both innate and adaptive immunity to cancer cells. Inflammation enhances accumulation and activity of MDSC in the tumor microenvironment. We postulate that exosomes, nanoscale membrane-bound extracellular vesicles that are shed by MDSC, participate in chemotaxis and immunosuppression in the tumor microenvironment. In search of mechanistic insights to test this hypothesis, qualitative and quantitative proteomic strategies have been used to identify the protein cargo of these exosomes and to analyze differential effects of inflammation on these proteins. The activities of proteins found to be of high interest have been confirmed biologically and tested for activity.

Exosomes were shed by MDSC induced in BALB/c mice by the 4T1 mammary carcinoma and developed in either a conventional tumor microenvironment or a microenvoronment with increased inflammation due to high levels of the pro-inflammatory cytokine IL-1β. More than 1000 proteins have been identified from MDSC exosomes thus far. The majority of these proteins are assigned as originating from the cytosol, nucleus, cytoskeleton and plasma membrane in parental MDSC. Functional ontology assigned the majority as protein binding, nucleotide binding, ion binding and hydrolytic. In replicate iTRAQ experiments, the concentrations of S100-A9, S100-A8 and neutrophilic granule protein were found to change in concert in the exosomes, though not in the parental MDSC. Functional studies showed that S100 A8/A9 proteins in exosomes mediate MDSC chemotaxis and alter the function of macrophages. Abundances of histones and several enzymes involved in the production of NADPH+ were also found to be altered by inflammation.

Keyword: exosomes, cancer

PS19-02 Oncogenic H-Ras Reprograms Madin-Darby Canine Kidney (MDCK) Cell-Derived Exosomes During Epithelial-Mesenchymal Transition

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Epithelial-mesenchymal transition (EMT) is a highly conserved morphogenic process defined by the loss of epithelial characteristics and the acquisition of a mesenchymal phenotype. EMT is associated with increased aggressiveness, invasiveness, and metastatic potential in carcinoma cells. To assess the contribution of extracellular vesicles during EMT, we conducted a proteomic analysis of exosomes released from Madin-Darby canine kidney (MDCK) cells, and MDCK cells transformed with oncogenic H-Ras (21D1 cells). Exosomes are 40-100 nm membranous vesicles originating from the inward budding of late endosomes and multivesicular bodies (MVBs) and are released from cells upon fusion of MVBs with the plasma membrane. Exosomes from MDCK cells (MDCK-Exos) and 21D1 cells (21D1-Exos) were purified from cell culture media using density gradient centrifugation (OptiPrep™), and protein content identified by GeLC-MS/MS proteomic profiling. Both MDCK- and 21D1 Exos populations were morphologically similar by cryo-electron microscopy and contained stereotypical exosomes marker proteins such as TSG101, Alix and CD63. In this study we show that the expression levels of typical EMT hallmark proteins seen in whole cells correlate with those observed in MDCK- and 21D1-Exos - i.e., reduction of characteristic inhibitor of angiogenesis, thrombospondin-1 and epithelial markers E-cadherin, and EpCAM, with a concomitant up-regulation of mesenchymal makers such as vimentin. Further, we reveal that 21D1-Exos are enriched with several proteases (e.g., MMP-1, -14, -19, ADAM- 10, DAMTS1), and integrins (e.g., ITGB1, ITGA3, ITGA6) that have been recently implicated in regulating the tumour microenvironment to promote metastatic progression. A salient finding of this study was the unique presence of key transcriptional regulators (e.g., the master transcriptional regulator YXB1) and core splicing complex components (e.g., SF3B1, SF3B3 and SFRS1) in mesenchymal 21D1-Exos. Taken together, our findings reveal that exosomes from Ras-transformed MDCK cells are reprogrammed with factors which may be capable of inducing EMT in recipient cells.

PS19-03 EVpedia: An Integrated Proteome Database for Systemic Analyses of Prokaryotic and Eukaryotic Exosomes

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The secretion of extracellular vesicles, also known as exosomes and microvesicles, is a common cellular activity observable not only in simple unicellular organisms (e.g. archaea, Gram-positive, and Gram-negative bacteria) but also in complex multicellular ones. Extracellular vesicles are spherical bilayered proteolipids with a mean diameter of 20-1,000 nm, which are known to contain various bioactive molecules including proteins, lipids, and nucleic acids [1]. Here, we present EVpedia ((http://evpedia.info), which is an integrated database of high-throughput datasets (proteins, mRNAs, miRNAs, and lipids) from prokaryotic and eukaryotic extracellular vesicles [2]. EVpedia provides an array of tools, such as the search and browse of vesicular components, Gene Ontology enrichment analysis, network analysis of vesicular proteins and mRNAs, and a comparison of vesicular datasets by ortholog identification. Moreover, publications on extracellular vesicle studies are listed in the database. This free web-based database of EVpedia might serve as a fundamental repository to stimulate the advancement of extracellular vesicle studies and to elucidate the novel functions of these complex extracellular organelles.

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PS19-04 Extracellular Vesicle (EV) Array: Microarray Capturing of Exosomes and Other Extracellular Vesicles for Multiplexed Phenotyping

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Based on the technology of protein microarray, we hereby present a highly sensitive Extracellular Vesicle (EV) Array capable of detecting and phenotyping exosomes or other microvesicles from unpurified starting material in a high-throughput manner. The EV Array utilizes the possibilities to detect and profile microvesicles for 21individual surface exposed antigens simultaneously using only small amounts of starting material.

Exosomes are extracellular vesicles (40-100 nm) secreted by various cell types. The quantity and molecular composition of exosomes shed from different cell types differs considerably. Until now, the "gold standard" for quantification, characterization and phenotyping of exosomes is either by WB or FACS. These types of analyses requires considerable amounts of exosomal material (20-30 μg of protein derived from appr. 10^8 cells) and are only capabale of producing phenotypical data on one antigen per experiment. The EV Array exploits the potentials to detect and profile exosomes for 21 antigens simultaneously using unpurified exosomes from cell culture medium from 10^4 cells.

The EV Array is based on the antibody capture of microvesicles and subsequent detection of the captured subtypes of microvesicles by labeled anti-tetraspanin antibodies (CD9, CD63 and CD81 for exosomes). Antibodies used to capture these targeted exosome biomarkers are specific to membrane proteins for: exosomes in general (CD9, CD63, and CD81), and exosomes from cancer cells (EpCam, CD276) and 11 other membrane markers. Compared to NanoparticleTracking Analysis (NTA), EV Array revealed a higher specificity and sensitivity for exosomes, regardless of the pre-analytical circumstances as two-steps centrifugation or freezing/storage of the samples. Among others, the method was used to generate an extensive phenotyping of plasma-derived exosomes from 80 healthy donors.

Keywords: Protein Microarray, Exosome, Extracellular Vesicle (EV) Array

PS20-01 Phosphoproteomics and Cancer

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Lung cancer is the leading cause of cancer-related death in the USA and worldwide. Due to the late stage of diagnosis, poor efficacy of chemotherapies, and development of drug resistance, the 5-year survival rate is 16%. Genomic sequencing has revealed mutations in human lung cancers, e.g. EGFR, KRAS, ALK, and RET, that control oncogenic processes including evasion of apoptosis, cellular proliferation and cellular invasion. Unfortunately, targeted therapies for these kinases ultimately fail due to acquired resistance. To identify additional targets for treatment inherently transparent to genomic sequencing, new technologies must be developed, implemented and validated at the level of proteins and post-translational modifications.

Quantitative proteomics and phosphoproteomics are poised to improve our understanding of oncogenic signaling. We implemented a quantitative phosphoproteomic strategy to identify mitotic substrates of two families of mitotic kinases, Polo-like kinases and Aurora kinases, which are amplified or overexpressed in lung cancer. We increased the number of known phosphorylation loci attributed to Polo-like kinase 1, Aurora A, and Aurora B by 10-fold.

We then implemented a robust and accurate quantitative phosphoproteomics strategy to interrogate the global phosphorylation profile of primary human lung tumors. We quantified ~9000 phosphorylation sites across signaling pathways including KRAS, PI3K, and ERBB2, and identified the enrichment of PLK1-specific substrates in one of these human tumors. To ensure that the proteome and phosphoproteome of patient tumors was stable during processing by pathologists, we quantified protein and phosphopeptide abundances after tumor resection. Having established a robust analytical workflow for kinase network profiling by quantitative phosphoproteomics, it should now be possible to identify patient-specific phosphoproteomic profiles to aid informed decision making for personal treatment strategies.

PS20-02 How Targeted Therapies Exploit Signaling (Src) and Metabolic (Glucose) Over-Dependencies in Cancer Homeostasis

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Unbiased inquiries into signaling and metabolism using mass spectrometry-based phospho-proteomics have repeatedly pointed us to networks involving negative and positive feedback, cross-talk, synergy, and unexpected results. Examples include negative feedback of Src signaling in kinase inhibitor-resistant Bcr-Abl-driven leukemias (Rubbi et al.); feed-forward, synergistic amplification of signaling upon metabolic stress leading to catastrophic death (Graham et al.); synergistic co-treatments that can prevent kinase inhibitor resistance; and the identification of druggable tyrosine signaling in prostate cancer, a tumor type in which tyrosine signaling mutations are rare (Drake et al.). In sum, these examples provide illustrations of how the signaling and metabolic states of cancer cells, while homeostatic, can be relatively unstable to disruptive perturbations.

PS20-03 Development of Universal Protocols for Clinical Tissue Phosphoproteomics Optimized for Formalin-Fixed Paraffin-Embedded Specimens

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Archives of clinical tissues have been stocked typically in formalin-fixed and paraffin-embedded (FFPE) state in almost all clinical institutes for several decades, and therefore the accumulated specimens can be useful for retrospective research. This fact motivates us to achieve proteome and phosphoproteome analysis of FFPE clinical samples. However, FFPE specimens have inadequate properties to be analyzed, e.g. low recovery of proteins and artificial modifications by formaldehyde-induced cross-linking, leading to the low efficiency in protein identification as well as the significant decrease in quantitative accuracy. Recently, we introduced a novel protocol for protein extraction and digestion to maximize the proteome coverage, which we call phase transfer surfactant (PTS)-aided method. PTS method made it possible to extract 100 μg of protein from a few FFPE slides and identify more than 1500 phosphopeptides in combination with hydroxy acid-modified metal oxide chromatography (HAMMOC) using titania. In this study, we further improved our PTS method by utilizing novel deparaffinization protocols, to achieve more effective protein extraction and reduce the processing time. The optimized protocol was applied to FFPE slides of various human cancers and healthy control samples, and the obtained phosphoproteomes were compared. Furthermore, phosphoproteomes of freshly frozen tissues from cancer patients and healthy controls were also analyzed, and the difference between FFPE specimens was evaluated. In our system, almost all clinical samples including FFPE and freshly frozen specimens can be retrospectively analyzed without any analytical and biological discrimination.

Keywords: phosphoproteomics, FFPE, clinical tissue

PS20-04 Identification of Dysregulated Kinase-Mediated Pathways in Hepatocellular Carcinoma by a Quantitative Phosphoproteome Approach

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Phosphorylation is one of most common post-translational modifications in mammalian cells. It regulates protein activity, cellular localization, conformation, and binding affinity and further mediates diverse key physiological functions. Numerous literates have established the close association between aberrant phosphorylation and many diseases, including cancer.
Hepatocellular carcinoma (HCC) is a common and poor prognostic cancer worldwide. Surgical resection is the most effective modality, but there are many limitations, and unfortunately, the recurrence rate is about 50% even after resection. In previous studies, various kinases and phosphorylation-mediated signaling pathways such as HGF/c-Met signaling pathway, ERK/MAPK pathway and Wnt/β-catenin signaling pathway have been found to be significantly altered in HCC patients. Conceivably, phosphorylation plays an important role in these molecular mechanisms that can affect tumorigenesis. Therefore, exploring the tissue phosphoproteome profiles will facilitate the identification of critical factors involved in HCC.
In this study, we established a technology platform for quantitative phosphoproteome analysis via combining stable isotope dimethylation labeling and online SCX-TiO_2/RP-LTQ-Orbitrap, and then compared tissue proteome and phosphoproteome between tumor tissues and paired adjacent non-tumor counterparts in three HCC patients. The results yielded 3100-4700 quantifiable phosphopeptides corresponding to over 2600 proteins with high confidence. In order to check the accuracy of our platform, we proceeded to confirm the quantitative results by Western blotting using phospho-specific antibodies.
Furthermore, key upstream protein kinase(s) responsible for the phosphorylation of those phosphosites dysregulated in tumor tissues were predicted, enabling us to unravel aberrant active kinases as biomarkers, as well as discover potential therapeutic targets for HCC patients.

Keywords: phosphoproteome, hepatocellular carcinoma, dimethylation labeling

PS21-01 Integration and Data-Mining of Human Transcriptome and Proteome Databases in H-InvDB

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Based on comprehensive analyses of human transcriptome, we developed the H-Invitational Database (H-InvDB; http://hinv.jp/), an integrated database of all human genes and transcripts. The latest release of H-InvDB defines 36,789 protein-coding genes, 692 transcribed pseudogenes, and 8,366 potential non-protein-coding genes. The protein-coding genes include 13,320 genes for hypothetical proteins that require further experimental validation at the protein level. H-InvDB also defines a total of 61,403 alternative splicing (AS) isoforms of human protein coding genes. Detailed annotation of human AS isoforms has been compiled in a specialized database, H-DBAS (http://hinv.jp/h-dbas/). These information resources are useful for future experimental identification of novel human proteins, especially in C-HPP. We thus developed the H-Inv Extended Protein Database (H-EPD; http://hinv.jp/hinv/h-epd/) that provides comprehensive, non-redundant human protein sequences, including both curated and predicted human proteins in H-InvDB and other protein databases. Another newly released database that we constructed is a protein complex database (PCDq; http://h-invitational.jp/hinv/pcdq/) that provides information for both curated and predicted human protein complexes based on protein-protein interaction data and literatures. The predicted protein complexes can provide functional clues about hypothetical human proteins. We also provide useful tools for data-mining from these databases, including H-InvDB Enrichment Analysis Tool (HEAT) and ID Converter System (http://biodb.jp/).

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PS21-02 What is a Proteomic Publication?

Robert Chalkley

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The amount of information submitted to accompany a proteomic publication has changed dramatically in the last ten years, largely due to the implementation of journal publication guidelines. Authors are now expected to provide access to annotated spectra of at least the results that have the highest potential for mis-interpretation, and it is increasingly expected that the raw data files themselves are also submitted to a public location. In this presentation I will discuss options for how data associated with a publication is made available, tools for viewing the different results formats, including a spectral viewer that supports many results file formats, and highlight examples of how this data is mined. As part of this I will touch on the graying boundary between what should be in a journal article and what is supplementary information and whether data that is not peer-reviewed should be submitted to proteomic repositories.

Keywords: Journal Guidelines, Spectral Viewers, Repositories

PS21-03 Estimation of Protein Species Number for Mammalian, Bacteria, Insecta and Yeast

Elena Ponomarenko, Stanislav Naryzhny, Ekaterina Poverennaya, Mikhail Pyatnitskii, Andrey Lisitsa, Alexander Archakov

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The sequencing of the human genome was completed over 10 years ago during the Human Genome Project. As a logical continuation of this project, Human Proteome Project was launched in 2010. At the same time, the target size of the human proteome is still obscure: from 10 000 (Adkins et al., 2002) to 1 billion (Kelleher, 2012) of different protein species assumed. The diversity of protein species arises from the appearance of single amino-acid polymorphisms (nsSNP, SAP), alternative splicing variants (AS) and post-translational modifications (PTM). During last 3 years the numbers of entries in UniprotKB concerning SAP, AS and PTM stable for *Drosophila melanogaster* and *Saccharomyces cerevisiae* S288c, while for human there is a slight increasing SAP-related entries, and number of PTM-related entries are increased for *Rattus norvegicus* and *Escherichia coli.* Multiplying the average number of variations per gene, we could estimate the number of protein species coded by one gene; applying this calculation to all genes, we could expect the number of protein species. For human, rat and E-coli the minimal number of protein species estimated as 1,9 mln., 390 000 and 45 000 respectively. We obtain approximately 307 000 protein species for *Drosophila melanogaster* and 290 000 for *Saccharomyces cerevisiae* S288c (UniprotKB, v.03_2013). As the number of possible protein variants will definitely increase due to growth of the database, here we use the term «minimal number of protein species» for mammalian and *E. coli.* The number of protein species is necessary for understanding the target size of the proteome of each organism. Moreover, such kind of data is necessary for determination the each protein species abundance. Dependence of the number of detected proteins and the analytical sensitivity could be based on a comparison of the theoretical calculations with experimental results of number of protein species detected using 2DE with various dyes types.

Keywords: Data analysis, Number of protein species, Human Proteome Project

PS21-04 Isobar: Making Sense out of Protein and Modified Peptide iTRAQ/TMT Quantitative Data

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CeMM - Research Center for Molecular Medicine of the Austrain Academy of Sciences, Austria

While current mass spectrometry is able to measure thousands of non-modified and posttranslationally modified peptides, the identification of significantly regulated molecules remains challenging. We have introduced a new software package - isobar - to address this problem both at the protein and the modified peptide levels for iTRAQ and TMT data.
The isobar platform relies on carefully tested statistical models (Breitwieser et al., J Proteome Res, 2011) that separate technical variability, originating from the instrumentation, and biological variability. Significantly regulated proteins can be naturally selected by requiring a clear measure and biological significance. Isobar can also take advantage of replicates present in a single iTRAQ or TMT experiment.
We have extended isobar to analyze the regulation of PTMs (Breitwieser & Colinge, J Proteomics, 2013) by assessing the statistical methods in this special condition and by introducing necessary new features. In particular, isobar provides a generic mechanism of validating the localization of PTMs by means of the Mascot Delta Score approach or specialized external tools such as PhosphoRS. The navigation of peptide regulation results is facilitated by a sophisticated hyperlinked spreadsheet user report which integrates references to known PTMs from neXtProt and PhosphoSitePlus.
Isobar can be run without programming skills and it is released as a Bioconductor R package thus allowing more advanced users and bioinformaticians to fully exploit its rich repertoire of functions. It can parse the most common file formats (Mascot, Phenyx, MSGF+, MGF, Rockerbox, MzIdentML). Isobar also supports label free quantitation by computing emPAI and dNSAF protein abundance indexes.
Web site: http://www.ms-isobar.org
This work is supported by the Austrian Science Fund (FWF) grant P 24321-B21.
Keywords: quantitative proteomics, bioinformatics, PTM

PS21-05 FindPairs - The Protein Quantification Module of the PeakQuant Software Suite

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Accurate protein quantification is a major task in Proteomics. A wide range of stable isotope labeling techniques allow simultaneous quantification of thousands of proteins by using mass spectrometry. Here, the FindPairs module of the PeakQuant software suite is presented. It automatically determines peptide and protein abundance ratios based on the automated detection of isotopic peak patterns in stable isotope-coded mass spectrometric data. Hence it also works with SILAC and iTRAQ, the practicability of FindPairs is shown on the quantitative analysis of proteome data acquired in $^{14}N/^{15}N$ metabolic labeling experiments. This works on the one hand "database-driven", when sequences are known. On the other hand, a special feature of FindPairs is the application of an average mass shift factor to identify isotope patterns of $^{14}N/^{15}N$ peptide pairs, even if no sequence information is known. This is interesting especially for Quantitative Proteomics in unknown species, i.e. "Meta-Proteomics". Furthermore we give an overview of the features of FindPairs and compare these with already existing quantification packages. The software is publicly available at http://www.medizinisches-proteom-center.de/software and free for academic use.
Keyword: Bioinformatics, Quantitative Proteomics, metabolic labeling, 14N/15N labeling

PS22-01 Immunoaffinity Separations and Intelligent PRISM Fractionation for Achieving Ultrasensitive Targeted Protein Quantification

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Selected reaction monitoring (SRM) has been regarded as a promising high throughput targeted protein quantification technology; however, one of major limitations for current SRM technology is the lack of sufficient sensitivity for detecting low-abundance proteins present at sub-ng/mL in human plasma/serum. To address these challenges, we have developed and integrated different levels of immunoaffinity depletion techniques to enhance the overall sensitivity. More recently, we developed an antibody-free strategy that involves high-pressure, high-resolution separations coupled with intelligent selection and multiplexing (PRISM) for highly sensitive SRM-based targeted protein quantification. The strategy capitalizes on high resolution high pH reversed-phase LC separations for analyte enrichment, intelligent selection of target fractions via on-line SRM monitoring of internal standards, and fraction multiplexing prior to LC-SRM quantification. We demonstrated accurate and reproducible quantification of proteins at concentrations in the 50-100 pg/mL range in plasma/serum by coupling with IgY14 immunoaffinity depletion. PRISM-SRM provided up to ~1000-fold improvement in the LOQ when compared to conventional SRM measurements and a moderate throughput of quantification (~50 samples/week) with the use of fraction multiplexing. Applications to clinical serum samples illustrated the detection of several endogenous ng/mL-level proteins, including prostate-specific antigen (PSA), and an excellent correlation between the results obtained from the PRISM-SRM assay and those from clinical immunoassay for PSA. This approach was also applied for the verification of a number of prostate cancer candidate biomarkers in human urine, including anterior-gradient 2 (AGR2), where the data displayed a preliminary discrimination between prostate cancer and non-cancer subjects. Our results demonstrate that PRISM-SRM is an effective method for quantification of low-abundance endogenous proteins in highly complex biofluid samples. We anticipate broad applications for targeted quantification of low-abundance proteins in systems biology and candidate biomarker verification studies.

PS22-02 A High-Throughput and Reproducible Workflow for Targeted Mass Spectrometry Applied to Large Scale Analysis of Clinical Samples

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Multiple Reaction Monitoring (MRM or SRM) is a targeted mass spectrometry (MS)-based method for quantifying peptides that are unique for specific proteins in biological/clinical samples. The challenge with MRM is speed and accuracy of processing proteins into peptide and subsequent LC/MS/MS analyze of 100-1000s of samples. Numerous steps in sample preparation (protein denaturation, reduction, alkylation, digestion, peptide desalting) introduce analytical errors beyond acceptable limits for bioanalytical methods. Here, we describe the implementation of automated robotic sample processing and a dual column LC/MS/MS system to improve the reproducibility and throughput of MRM. A single or dual reverse-phase chromatography systems (MPX™-2 System, Shimadzu) was linked to a triple quadruple MS instrument (QTRAP® 5500 or 6500, AB Sciex). Pooled human sera was processed for MRM in a 96-well format using a Biomek NX^P automated liquid handling system (Beckman Coulter). The accuracy of a traditional manual MRM workflow was measured by spiking a constant amount of β-gal into 171 plasma samples. Each sample was reduced, alkylated, digested with trypsin, and spiked with heavy-isotope peptide internal standards, and desalted on a 96-well HLB™ solid phase extraction plate (Waters): 60 samples were processed per day = 70 hours for 171 samples. Samples were run in triplicate on a QTRAP® 6500 system with %CV for β-gal of 31%. While, robotic processing using a Biomek NX^P liquid handler reduced the total sample preparation time (excluding desalting) to < 18 hours including overnight trypsin digestion (which can be reduced to 2 hours) with a %CV of 5%. Moving from manual or 96 well plate desalting (%CV >20%) to LC online desalting using an online trap column or direct divert method where the salt fraction is diverted to waste prior to the QTRAP system had %CV of 9 and 4%, respectively. Furthermore, throughput for LC/MS/MS MRM assays doubled using a two parallel C-18 reversed phase columns LC system. Here peptides from one column elute into the MS while the other column is washed, regenerated and loaded. The %CV between LC columns was <5%. In conclusion, an automated high-throughput workflow with sample processing robotics and multiplexed LC/MS/MS for accurate and reproducible large-scale analysis of biological/clinical samples.

PS22-03 Mark Twain: How to Fathom the Depth of Your Pet Proteome

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The present lecture will highlight recent progresses in the technique of combinatorial peptide ligand libraries (CPPL), a methodology that has much to offer for the detection of low- to very-low abundance proteins in any proteome. In particular, advances in exploration of the urinary, plasma, CSF and tissue proteomes are discussed and evaluated. It will be shown that when treating biological fluids, such as plasma, with CPLLs, the detection sensitivity, which in the control only reaches 10ng/mL, can be enhanced to as high as 10pg/mL, with an increment of sensitivity of three orders of magnitude. Exploring such extreme low concentration intervals will allow access to those most sought after biomarkers that so far have been much elusive. Even in tissue proteome extracts, up to the present not analyzed via CPLLs, massively overloading the CPLL beads will allow exploration of very low-abundance proteins in presence of a set of highly abundant tissue proteins. The possibility of using CPLLs as a two-dimensional pre-fractionation of any proteome is also evaluated: on the charge axis, CPLL capture can be implemented at no less than three different pH values (4.0, 7.2 and 9.3), thus permitting a capture of proteinaceous analytes bearing a net positive or net negative charge, respectively. When capture is performed in absence of salts or at high levels of salts, one can favour capture of hydrophilic vs. hydrophobic proteins, respectively. This would thus be a genuine 2D protocol, working on orthogonal separation principles (charge vs. hydrophobicity). As the horizon of CPLLs is expanding, we expect major breakthroughs in, e.g., biomarker discovery, a field that as suffered a decade of failures.

Boschetti E, Righetti PG. Low-Abundance Proteome Discovery. State of the Art and Protocols, Elsevier, Amsterdam, 2013, pp. 1-341.

PS22-04 Automated Native Sample Preparation for Proteome Analysis

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Multidimensional chromatography has been adapted for fractionation of body fluids for proteomic analysis and biomarker search. Our method combines serial native size exclusion (1D), followed by parallelized anion exchange (2D) and lectin affinity (3D) chromatography. Parallelization and automation is realized with separation, spectrophotometric readout, temporary storage, hit picking, medium exchange, digest, desalting, and finally storage within an autosampler of LC-MS. The central unit is an experimental setting enabeling multichannel pipetting and robotic handling of microplates, reservoirs, and column arrays. Some tools have been developed to improve medium exchange, desalting, re-concentration, and readout. The protein input is scalable from 2 to more than 100 mg and from 2 to 1000 kDa molecular weights. Globally, protein recoveries from the corresponding load are 93.7 ± 2.7, 97.1 ± 11.2, and 88.8 ± 3.6% and best precisions are 3.3, 3.8, and 5.1% CV with 1D-, 2D-, and 3D-fractionation, respectively. For process control and all-round analytics a software package was developed. Besides automation, this method has several advantages: high proteome coverage, flexible dynamic range with respect to molecular weight and sample amount, and optional enzymatic and immunological analytics additional to mass spectrometry. All intrinsic components and information are preserved after fractionation including complex formation, fragmentation, and biological activities beneficial for biomarker search as well as for efficient evaluation. Preliminary applications show versatility with profiling plasma proteomes of humans, cattle, goat and mouse, and human cerebrospinal fluid. Application to biomarker search yields several candidate markers that could already be validated in severe inflammation and Alport syndrome.

Keywords: automated native multidimensional fractionation, parallel sample preparation

PS23-01 (Keynote) New Technology in Integrated Omics: Challenges: Concepts, Methods and Tools

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The Human Proteome Project is on its tracks and many if not most human proteins will be found in biological samples in the coming years. However, although the Human proteome project aims at the detection and the description of all human proteins encoded in the genome, it will remain a tremendous challenge to convert this new information into an increased knowledge on human biology and human physiopathology. I would like to challenge the fact that recent findings in molecular biology will be sufficient to explain biology as a whole. On the contrary, I' ll argue that systemic definition and global characterization of living systems will be usefully combined with molecular studies for a better understanding of biological functions in isolation or taken as a whole for a complete organism. For example, we need more integrative sciences between mathematics modelling, bio-mechanics, molecular biology, medicine and cognitive sciences to better address the question of heredity versus acquired traits (and possibly disorders) in cognitive brain functions. New representation of living systems will emerge in coming years, integrating new knowledge and new concepts coming from various fields. This fruitful combination will be the driving force for developing new technologies for a better monitoring of human health and, whenever necessary, better tools fur disease treatment.

Keyword: Integrated biology - interdisciplinarity

PS23-02 Insights from a Multi-Omic Analysis of a Single Human Cell Type

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Advances in the field of genomics, epigenetics, transcriptomics and proteomics have provided researchers the technical capability to carry out unbiased genomewide studies. These strategies are often used in isolation while investigating biological problems. It is now apparent that a unified approach that allows us to study genome, epigenome, transcriptome and proteome would provide better insights than any single Omics study. However, the challenging aspect of such a unified approach is the lack of appropriate computational tools to deal with such data. In an effort to determine the feasibility of embarking on a multi-Omics approach, we carried out whole genome sequencing, genomewide methylation profiling, mRNA sequencing, miRNA sequencing, proteomic and phosphoproteomic profiling of naïve CD4+ T cells from a single individual. We have generated one of the most extensive proteomic catalogs of any primary cell type in humans by obtaining data regarding protein expression, acetylation and phosphorylation of naïve CD4+ T cells. This study establishes expression of >8,000 proteins in naïve CD4+ T cells along with their various alternative forms including splice variants and post-translationally modified versions. We have obtained novel, and surprising, insights into transcription and translation of genes/proteins by correlating the methylation patterns with mRNA, miRNAs and proteins. For example, although recent studies have reported abundant, widespread RNA editing in cultured human cell lines, our analysis reveals RNA editing as a relatively infrequent event and highlights some of the caveats of next generation sequence data analysis. Overall, we believe that such multi-Omics profiling studies can inform us about basic molecular mechanisms in addition to paving the way for personalized medicine.

PS23-03 Chr 18 Quantitative Proteome and Transcriptome

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The current stage of C-HPP is focused on constructing the chromosome-centric proteomic catalogues. Representative proteins should be mapped in the tissues and quantitatively annotated. SRM is recommended for C-HPP as the method for targeted protein detection and quantification in complex samples due to its high sensitivity at the level of few protein copies per cell. Using SRM, the fragment ion maps for 277 proteins were assayed by SRM in depleted plasma, liver and HepG2 cells. Summarized SRM results for all types of biomaterial comprised in total 250 detected proteins which covered 90% of protein-coding genes of Chr 18. Analysis of tissue distribution demonstrated that a significant portion of detected proteins (51%) was observed both in human plasma and liver tissue. There were also proteins featured for plasma only (33%) or exclusively observed in the liver (16%). For 27 proteins, we observed the absence of the SRM signal or poorly reproduced signal.

Concentration of 50 proteins encoded by Chr 18 was measured by SRM using external calibration with purified nonlabeled peptides. The results on the quantitative label-free analysis of proteins in biological samples were validated using the stable isotopelabeled peptides. Generation of the correlation curve for 30 light- and isotope-labeled peptides measured in plasma, liver and HepG2 cells illustrates applicability of such an approach. The high correlation coeffcient (R^2= 0.96) between protein concentrations estimated by external calibration with light peptides and by isotope-labeled standards was observed. Therefore, we expect the external calibration with light peptides provides the suitable estimation for protein abundance.

PS23-04 Firmiana: An Integrated Platform for Mass Spectrometry-Based Proteomics Studies Based on Galaxy Framework

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Data analysis is essential and critical for proteomics studies. However, increasingly large amouns of high through-put data produced from mass spectrometry experiments pose significant challenges for computational analysis. Although lots of bioinformatics tools have been available, they are difficult to use, especially for experimental biologists with no programming experiences. Meanwhile some computational analyses are complex and integrative. Thus, user-friendly data analysis platforms integrating a broad range of tools are quite necessary.

Here we present a web-based platform called Firmiana for mass spectrometry-based proteomics study, built upon open source Galaxy framework which was originally designed for genomics research but allows easy integration of tools used in proteomics study as well as rapid configuration of proteomics data analysis workflows. This platform has integrated a collection of current and newly developed representative tools involved in most steps of mass spectrometry proteomics data analysis, from preliminary experiment management, file format conversion, spectrum identification with database searching, statistical validation of peptide and protein identification results, peptide and protein quantification, to downstream analysis such as significance test and Gene Ontology analysis. In addition, more new functions and tools can be included in this platform.

The user-friendliness of Firmiana platform will greatly facilitate mass spectrometry-based proteomic studies. The intuitive graphical user interface of tools liberates researchers from complex software operations, and the flexible construction of workflows simplifies complex data analyses. Besides, benefit from the scalability of Galaxy framework, tools can be configured to run on clusters or cloud to utilize more powerful computational resources.

Keywords: Proteomics Data Management & Analysis System, Galaxy, Mass Spectrometry Experiments

PS24-01 A Robust Method for the Relative Quantitative Analysis of Dual Labeled N-Glycans

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With the development of glycoproteomics, relative quantification of glycosylation has received considerable research attention, which is important for discovering glycosylation changes and finding potential biomarker. High throughput glycan quantitation was developed to investigate quantitative glycan changes in structure type, composition, linkage and conformation of glycans from different biological samples. In this research, we developed a novel relative quantitation method for glycans. The novel sodium borohydride assisted enzymatic ^{18}O labeling added the reducing end of all released glycans with 3 Da. After this label, the mass gap increased to 3 Da, and the partial overlap of isotope envelopes was largely reduced. Moreover, the glycan became more stable, and the labeled ^{18}O would never exchange with ^{16}O again in normal water. We further tested the stability and overlap of this label, which showed good results. We optimized the labeling, and made the method more suitable for quantitative glycomics by mass spectrometry. Moreover, we utilized this method to quantitatively determine the glycomic changes in sera from healthy individuals and patients diagnosed with hepatocellular carcinoma (HCC), and got the good results

Keywords: Dual Labeled N-glycans, Relative Quantitative, Mass spectrometry

PS24-02 An Integrated Approach of Proteomics, Glycoproteomics and Glycomics for the Structural and Functional Study of Glycoproteins

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Glycosylation is one of the most common forms of protein modifications. Each glycoprotein can be glycosylated at different glycosites and each glycosite may be modified by different glycans. This structural heterogeneity provides additional functions for each glycoprotein in physiological and pathological processes. However, the structural heterogeneity also complicates the studies of structure-function relationships of glycoproteins. To rapidly identify and quantify the glycosylation on each glycosite from complex biological mixtures and to understand the functions of the glycosylations, an integrated approach for global proteomics, glycoproteomics, and glycomics was used. We performed quantitative analyses of proteins, glycosylation site occupancy and glycan structures at specific site by the isolation of glycopeptides and glycans using chemical-enzymatic approaches and analysis of glycopeptides and glycans by liquid-chromatography followed by quantitative analysis by mass spectrometry. We showed by specific examples that upon the identification of specific changes in glycosylation, glycosites containing aberrant glycans were readily identifiable and quantifiable using the combination of quantitative global proteomics, glycoproteomics and glycomics. The application of integrated approaches may facilitate our understanding of how perturbed glycosylation impacts upon disease progression and could be used to discover glycosylated proteins that could serve as biomarkers or therapeutic targets to improve clinical outcomes of patients.

PS24-03 Use of Zinc Finger Nuclease Gene Targeting for Functional Glycomics

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Glycosylation is the most abundant and diverse posttranslational modification of proteins. While several types of glycosylation can be predicted by the protein sequence context, and substantial knowledge of these glycoproteomes is available, our knowledge of the GalNAc-type O-glycosylation is highly limited. This type of glycosylation is unique in being regulated by 20 polypeptide GalNAc-transferases attaching the initiating GalNAc monosaccharides to Ser and Thr (and likely some Tyr) residues. We have developed a genetic engineering approach using human cell lines to simplify O-glycosylation (SimpleCells) that enables proteome-wide discovery of O-glycan sites using "bottom-up" mass spectrometric analysis. We implemented this on 12 human cell lines from different organs, and present a first map of the human O-glycoproteome with almost 3,000 glycosites in over 600 O-glycoproteins as well as an improved NetOGlyc4.0 model for prediction of O-glycosylation. The finding of unique subsets of O-glycoproteins in each cell line provides evidence that the O-glycoproteome is differentially regulated and dynamic. The greatly expanded view of the O-glycoproteome should facilitate the exploration of how site-specific O-glycosylation regulates protein function.

PS24-04 Breast Cancer Tumour Transformation from Primary Tumour to Secondary Site

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Breast cancer is a very heterogeneous disease and some patients are cured simply by surgical removal of the primary tumour while other patients suffer from recurrence and spreading of the disease. A number of treatment predictive factors have been identified such as tumour size, estrogen (ER) and progesterone (PgR) receptor status and human epidermal growth factor receptor 2 (HER2) status. Lymph node involvement is also assessed during surgery to determine if the tumour has started spreading and thus determine if lymph node stripping is required. The predictive factors assessing the nature of the tumour are all based on the status of the primary tumour. However, it could be anticipated that the cancer cells undergo a molecular transformation allowing the spreading to a secondary site. If the lymph nodes are positive for cancer cells or if distant metastases are identified, this disease would likely be more successfully treated by assessing predictive markers characterizing the cells having undergone spreading. We are analysing a unique tumour material comparing a set of 18 primary breast cancer tumours with matched axillaries positive for breast cancer cells and a set of 20 primary tumours with matched distant metastases spread to different sites in the body to further understand the molecular changes during the spreading and identify novel predictive markers. We are analysing these tumours for glycoproteins. Protein glycosylation is predominant in both membrane proteins and secreted proteins. Importantly, changes in glycosylation of these proteins have been shown to correlate with cancer states. Glycopeptide capture was used in this study to selectively isolate and quantifies N-linked glycopeptides from mixtures of glycoproteins. The captured glycopeptides were subjected to mass spectrometry analysis. Glycopeptide capture gave the most satisfactory results with 1145 proteins identified in total, all samples combined.

Keywords: Glycosylation, Breast cancer, Biomarker discovery

PS25-01 Functional Validation of Biomarker Discovery from Mouse Models to Translational Research

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Animal model is very useful to discovery the disease-candidate biomarkers instead of human patients. However still there has been a couple of issue to define what can be the best model for human disease for discovering biomarkers. We found differentially expressed proteins and transcripts during adipocyte differentiation using proteomics and DNA micrarray.

To validate these proteins, we developed several lines of knock-out mice. Among them, we found obesity-related phenotypes from AHNAK knockout mouse. To investigate the functional role of AHNAK in lipogenesis, HFD (High fat diet) and LFD (Low fat diet) were fed for 12 weeks to AHNAK knock-out mice and its age matched wild type mice. Even though AHNAK knock-out mice revealed a reduced body weight at birth compared with wild type mice, ratios of major organ weight to body mass was almost same of wild type mice. Body weight of HFD-fed AHNAK mice showed significantly reduced with the rate of weight gain compared to HFD-fed wild type mice despite an identical food intake when normalized to body mass. HFD-fed AHNAK mice display a reduced epididymal fat mass. From discovering differentially expressed proteins in disease models to validate its function involved in the onset of disease, phenotypical characterization of knockout mice is one of essential issues leading to disease.

PS25-02 Biomarkers of Diabetes in Plasma of NOD Mice

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Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia and it has become a global epidemic in the past years. The heterogeneity in the determining factors for the development of diabetes and its complications represent a big challenge in the advance of new strategies for prevention and treatment. In the present study we propose the investigation of potential biomarkers of diabetes, as well as biomarkers of early stages of diabetic nephropathy in plasma of non-obese diabetic mice using proteomic analysis. Plasma samples from adult diabetic (n=6) and non diabetic NOD (n=4), as well as Swiss mice (n=5), were collected and processed. Individual protein samples were analyzed by NanoUPLC tandem nanoESI-LC MS^E. A total of 141 proteins were identified in all three groups but 10 proteins were exclusively expressed in the diabetic group. Among those ten, candidates had already been associated to type 1 diabetes prevention, endothelial function impairment, retinopathy, diabetes related oxidative stress and one had been described as early biomarker for the disease. Seven proteins were only present in the Swiss strain and 8 were exclusive of the NOD mouse lineage, independent of the diabetic state. NOD mice are not born diabetic and the incidence on the colony is not 100% so our next step will be to follow NOD from early age to adulthood collecting samples at different time points to better understand the change in the proteomic profile that occurs in the diabetic state.

Keywords: Diabetes, NOD mice, biomarkers

PS25-03 Proteomic Analysis of Left Ventricular Tissues in Dilated Cardiomyopathy Mouse Models

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Dilated cardiomyopathy (DCM) is an intractable disease, and neither its radical treatment other than cardiac transplantation nor its differential diagnostic procedure has been established. To develop these methods for DCM, it is important to understand its pathogenic mechanism. Because phenotypes of causative gene mutations in the DCM are rather common regardless of the genes, it is essential to analyze proteomic changes associated with its progression. In this study, we performed proteomic analysis of left ventricles (LVs) of 4C30 mouse, a DCM model overexpressing Gal-β-1,3-GalNAc-α-2,3-sialyltransferase 2. 4C30 mouse shows a severe symptom of DCM around 24 weeks. Frozen LV tissues were pulverized, denatured, and digested with trypsin in the presence of sodium deoxycholic acid. After desalting, the digests were analyzed by nanoLC-MS/MS (Triple TOF5600) and differential protein expression was quantitatively evaluated with a 2DICAL software. Among 894 identified proteins, 186 and 145 proteins showed significant increases or decreases in 4C30 mice, respectively, compared with age-matched wild-type mice. Proteins associated with fibrosis, remodeling of extracellular matrix and cytoskeleton, and endoplasmic reticulum stress were increased, while energy metabolic enzymes involved in the glycolysis, TCA cycle, and β-oxidization were remarkably decreased. Since β-oxidization system in particular is a main energy source for the heart, this energy depletion might underlie conditions leading to DCM. In addition, regulatory proteins of intracellular calcium levels were also decreased, suggesting malfunction of the cardiac contraction and relaxation. These proteins in the energy metabolism and calcium regulation may be useful for diagnosis and treatments of DCM. We are currently analyzing another mouse model to find common features for understanding pathogenesis of the DCM.

Keywords: dilated cardiomyopathy, 4C30 mouse

PS25-04 Plasma Proteomic Pattern Analysis for Murine Experimental Autoimmune Encephalomyelitis (EAE) Model

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Background: Multiple Sclerosis (MS) is the most common demyelinating disease of the CNS where current treatments have limited effectiveness. Experimental autoimmune encephalomyelitis (EAE), has developed with pathology including demyelination and axonal damage and clinical events such as relapsing and remitting episodes of paralysis, all of which are features common to MS. Here we adopt a plasma proteomic pattern analysis using murine EAE model. **Methods**: For active induction of EAE, female SJL/J mice were immunized with myelin basic protein (MBP) derived peptides at 10 wk of age. Mice were observed for clinical signs of EAE and scored on a scale according to the severity of the clinical signs. Murine plasma peptides/proteins were purified with C8 magnetic beads using a robotics (ClinProtrobot system) and obtained spectra by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Autoflex II). The spectra were analyzed and multivariate statistics and receiver operation characteristics were calculated using ClinProTools 2.2™. **Results and Discussions**: EAE have been developed with pathology including demyelination and axonal damage and clinical events such as relapsing and remitting episodes of paralysis, all of which are features common to MS. Plasma proteomic pattern was clearly altered with progression of EAE symptoms by machine learning method. By building a support vector machine classifier, an effect on plasma proteomic pattern of EAE was clearly observed with good cross validation accuracy from 13 post immunization day (pid). Of note, some peaks enabled an annotation of clinical stages such as remission and relapsing of the EAE. **Conclusion:** Plasma proteomic pattern analysis is a promising and a reliable biomarker strategy in applying murine EAE model.

Keywords: multiple sclerosis, Experimental autoimmune encephalomyelitis, proteomic pattern analysis

PS25-05 Integrated Proteomics for the Study of Metastatic Human Tongue Cancer Development in a Heterogeneous Microenvironment

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Tongue cancer has been shown to be the most metastatic of oral cancers in patient cases. To understand the behavior of metastatic cells and the mechanism of metastatic cancer cell development in a heterogeneous tumor microenvironment, we studied human tongue cancer cell lines with highly metastatic (HM) and non-metastatic (NM) properties, established from the same patient. We created an orthotopic xenograft mouse model via co-injection of dsRed NM and GFP HM clones, and observed that HM cells grew aggressively in the tumor center and formed metastases, while NM cells translocated to the tumor margin without metastasis. The cell lines were then compared using differential proteomic (iTRAQ) and transcriptomic (DNA chip) analyses, followed by network analysis (KeyMolnet) of the combined mRNA and protein data. With the statistically highest score, we detected the HIF (hypoxia inducible factor) signal pathway as being specifically upregulated in HM cells. Detailed analysis of identified signal network showed that 30 (75%) of 41 proteins displaying higher expression in HM were directly or indirectly related to HIF signal transduction. Immunoblotting and immunocyto-chemistry further revealed up-regulation of the expression and activation of HIF-1α in HM. For biological validation, we prepared HIF-1α knockdown HM (HM-HIF^{KD}) cells and observed the cellular proliferation and motility in 2-/3-dimensional co-culture systems of NM and HM or HM-HIF^{KD} clones. The results showed that growth of NM cells was suppressed with the co-culture of HM cells, while the NM cell growth normalized with HM-HIF^{KD} cells. In time-lapse video analysis, HM cells showed aggressive growth with crowding-induced exclusion of NH cells. HM-HIF^{KD} cells, however, had no effect on NM cell growth. These results demonstrate that HIF-1α related signals are important in the development of highly metastatic tumors in a heterogeneous microenvironment.

Keywords: cancer, metastasis, heterogeneity

PS26-01 Label-Free Proteomics for Biomarker Discovery and Validation in Proximal Fluids: Applications in Colon Cancer and Alzheimer's Disease

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Label-free mass spectrometry-based proteomics applied to biomarker-rich sub-cellular compartments in (pre)-clinical samples and human proximal biofluids in vivo is a powerful, versatile approach for discovery of tissue-derived biomarkers with close association to the disease and potential for non-invasive applications.

Our label-free workflow is based on GeLC-MS/MS and spectral counting-based quantitation combined with dedicated statistics (Pham et al., Bioinformatics 2010, 2012). With correct study design and sample handling, this workflow is reproducible (Piersma et al., JPR 2010 and associated news highlight) and allows for quantitative protein profiling in simple and more complex workflows (reviewed in Pham et al., Expert Rev Mol Diagn. 2012). We introduced the 'whole gel' procedure to speed up the in-gel digestion procedure when handling multiple samples and many gel bands (Piersma et al., Proteome Sci 2013). More importantly, we have successfully applied this workflow in multiple biomarker discovery projects, most notably in colon, lung and breast cancer and in neurodegenerative diseases.

In my talk, I will present the steps that we consider important for reliable biomarker discovery and show results from our on-going efforts in colon cancer and Alzheimer's Disease (AD). In both examples patient proximal fluids were the starting point for discovery of early detection markers: stool for colon cancer and cerebrospinal fluid for AD. Proteome profiling of stool revealed a set of proteins significantly enriched in colon cancer compared to control stool samples, of which candidate biomarkers could be verified by MRM. CSF proteomics identified promising candidates for identifying patients with mild cognitive impairment at risk of developing AD, that were validated in independent samples using proteomics as well as western blotting.

PS26-02 Clinical Proteomics MRM

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Protein quantitation is essential for diagnostic and prognostic monitoring of disease biomarkers, and for verifying and validating the candidates in human biofluids. A targeted multiplexed approach involving MRM/MS in conjunction with isotopically labeled peptide standards has demonstrated great utility in delivering precise and accurate protein concentrations. Although the methods are currently employed in pre-clinical screening studies, they were developed with an eye toward clinical translation to aid diagnostic accuracy through multiplexed marker analysis. To be presented is an overview of our methodological advancements and disease-specific applications for the pre-clinical evaluation of putative disease protein biomarkers in human biologics, including blood plasma. These enable large (>150 proteins) or condensed (*e.g.*, 40 proteins in a CVD-focused study) protein panels to be interrogated against patient samples in a rapid and inexpensive manner, while maintaining high robustness (average CVs: 7% for signal and 0.05% for retention time) and sensitivity (concentration ranges: 6 and 8 orders-of-magnitude for 1D and 2D LC-MRM/MS) for precise and relative quantitation of high-to-low abundance protein biomarkers.

PS26-03 Proteomics Strategies for Analysis of Liver Cancer

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Cholangiocellular carcinoma (CCC) is a major subtype of liver cancer that arises in the bile ducts. It is known to have poor patient outcomes, with a 5-year survival rate of about 5 %. The incidence of, and mortality from, CCC are increasing worldwide. At present, extensive surgical resection or transplantation remain the only potentially curative treatments, although most patients are considered inoperable at the time of diagnosis. Sensitive and specific biomarkers for the early detection of CCC are therefore needed.

In order to identify such biomarkers, malignant and healthy tissue from 8 CCC patients was analysed using two techniques from quantitative proteomics. 2D-DIGE (two-dimensional differential in-gel electrophoresis) was performed with minimal labelling of disrupted tissue. Differential spots were detected using DeCyder Software (GE Healthcare) and proteins of interest were identified by MALDI-TOF mass spectrometry on an Ultraflex II (Bruker). Parallel to this, a mass spectrometry-based label-free approach was adopted. For this, an RP-HPLC-MS/MS method was used to analyse the samples, with an Ultimate 3000 RSLCnano system (Dionex) online coupled to an LTQ Orbitrap Elite (Thermo Scientific). Progenesis software (Nonlinear) was used to detect differentially expressed proteins. Additionally, the proteome of bile from five CCC patients was analysed to check for the presence of proteins found to be regulated in the tissue. The bile was treated with Cleanascite Lipid Removal Reagent (Biotech) and a 2-D Clean-Up Kit (GE Healthcare) to eliminate salts and charged detergents. The proteome was subsequently analysed using RP-HPLC-MS/MS, as described above.

As expected, both strategies show complementary results, which indicates that a combination of both techniques may be very promising in the identification of biomarker candidates for CCC. Some of the proteins found using both these techniques were also detected in the bile of CCC patients. These overlapping results, from the tissue and the bile, make them especially promising candidates for minimally invasive biomarkers. In future projects these potential biomarkers will be validated using western-blots, immunohistochemical techniques and ELISA.

PS26-04 From a Brain Damage Proteomics Model to a Blood Time Indicator of Stroke Onset: The Glutathione S-Transferase-Pi Proof of Concept

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Vascular cerebral accident, or stroke, is a leading cause of death and disability in industrialized countries. An early diagnostic blood marker of stroke would allow immediate therapeutic interventions and hence reduce the extent of brain damage and risk of death. However, the discovery of novel diagnostic markers directly in the blood is limited and presents a real challenge for the development of new diagnostic tools. This can be overcome by the use of appropriate models and experimental designs. Here, we will report the use of post-mortem cerebrospinal fluid and cerebral extracelullar microdialysis fluid as models of stroke brain damage events for the discovery of potential early markers of stroke. The validation of 5 proteins found differentially expressed in model samples by proteomics strategies were compared to 25 known brain markers by immunoassays on a prospective cohort of stroke patients. Among the 30 molecules tested, GST-pi concentration was the most significantly elevated marker in the blood of stroke patients ($p<0.001$). More importantly, GST-pi displayed the best area under the curve (AUC, 0.79) and the best diagnostic odds ratios (10.0) for discriminating early (<3 h of stroke onset) vs. late stroke patients (>3 h after onset). The ability of GST-p to predict the time window after stroke onset and consequently the potential eligibility for thrombolytic therapies may open new avenues for the management of stroke patients but also more specifically for the wake-up stroke patients, representing up to 30% of stroke events and traditionally excluded from treatment because of unknown time of symptom onset.

PS26-05 Diagnosis of Male Reproductive System Disorders with Protein Biomarkers Quantified in Seminal Plasma

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Non-invasive methods for differential diagnosis of male reproductive system disorders present urgent unmet needs in the urology clinics. In this presentation, we will introduce our biomarker discovery platform, discuss in detail development of male infertility biomarkers and suggest approaches for identification of biomarkers of prostate cancer and prostatitis. In our search for biomarkers, we focus on the proteome of seminal plasma (SP), a proximal fluid suitable for identification of novel biomarkers and for development of non-invasive diagnostic tests. Using mass spectrometry, we identified more than 3,100 proteins in SP of healthy men and men with infertility, prostate cancer and prostatitis.

To develop markers for differential diagnosis of male infertility, we selected 79 biomarker candidates based on differential proteomics of SP from fertile men and men with obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Using quantitative SRM assays, we verified 30 proteins and then validated 18 proteins in 148 SP samples. We identified two proteins, epididymis-specific ECM1 and testis-specific TEX101, which differentiated between OA, NOA and normal spermatogenesis with near absolute specificities and sensitivities. The performance of ECM1 was confirmed by ELISA in 188 samples, and a 2.3µg/mL cut-off distinguished OA from normal spermatogenesis with 98% specificity, and OA from NOA with 74% specificity, at 100% sensitivity. Immunohistochemistry and immunoaffinity-SRM assays revealed differential expression of TEX101 in distinct histopathological subtypes of NOA. As a result, we proposed a simple 2-biomarker algorithm for differential diagnosis of OA and NOA, and, in addition, for the differentiation of NOA subtypes. Clinical assays for ECM1 and TEX101 will replace diagnostic testicular biopsies and improve the prediction of testicular sperm retrieval, thus increasing the reliability of assisted reproduction techniques.

PS27-02 Development of iPS Cell Technology for Clinical Application

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Pluripotent stem cells have been thought to be useful sources for regenerative medicine. Although embryonic stem (ES) cells have abilities to differentiate into several kinds of somatic cells and grow infinitely in vitro, there are several problems with using ES cells for clinical application such as ethical issues and rejection after transplantation. To overcome these problems, pluripotent stem cells were generated from somatic cells by introduction of defined factors. These have been termed induced pluripotent stem cells (iPSCs). iPSCs have raised hopes for a new era of regenerative medicine because they can avoid the ethical problems and innate immune rejection associated with ES cells.
iPSCs have been generated from mouse and human fibroblasts by retroviral transduction of four transcription factors, Sox2, Oct3/4, Klf4, and c-Myc. Both mouse and human iPSCs are indistinguishable from ES cells. We found that the chimeras and progenies derived from mouse iPSC showed an increased incidence of tumor formations, primarily due to the reactivation of the c-Myc retrovirus. To overcome this issue, we have developed the methods for generation of safer iPSCs by episomal vectors containing L-Myc instead of c-Myc.
Human iPS cells (hiPSCs) are typically generated and maintained on feeder cells. Mouse feeder cells (SNL or MEF) are conventionally used for hiPSC culture. These cells are prepared with FBS-containing medium. For clinical use, feeder-free (Ff) and xeno-free (Xf) culture conditions seem to be better than the conventional ones. We succeeded to develop the Ff-culture conditions by using recombinant proteins and Xf-medium for hiPSC establishment and long-term culture.
We believe that this system is useful not only for producing clinical-grade hiPSCs in Cell Processing Centers for future application, but also for research, such as basic stem cell research.

PS27-03 Directed Differentiation of Stem Cells by Changing Protein Dynamics

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A holy grail of proteomics is to identify the cellular locations of all proteins. Several studies have sought to identify protein locations systematically, but an even larger task is to map and validate which of these proteins dynamically translocate as well as interact in development and disease (the translocalizome, the interactome).
We developed the novel algorithm for predicting condition-specific subcellular locations of the gene coding proteins at genome-wide level using only limited and condition-unspecified known locations. With systems biological mRNAs analysis of human stem cells using this method, the key target genes and their coding proteins which involved in maintaining pluripotency and differentiation process were predicted. Molecular biological experiments for target genes and proteins as well as novel protein function validation methods such as FCCS (Fluorescent Cross Correlation Spectroscopy), PLA (Proximity Ligation Assay), molecular imaging, gene transcription will be demonstrated for visualizing the validation process of key mechanisms. Finally, artificial translocation and interaction of novel protein complexes induced directed differentiation of human stem cells will be introduced.

PS27-04 Deep Proteome Profiling Identifies Distinct Pluripotent Cellular Populations During Somatic Cell Reprogramming into Induced Pluripotent Stem Cells

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Somatic cell reprogramming into induced pluripotent stem cells (iPSCs) is a process in which a complex network of molecular events generates a wide spectrum of either transient and stable cell states. Understanding the molecular mechanisms underlying the reprogramming phases is essential to increase its efficiency and will have important implications for improving our knowledge of cellular plasticity. Here we applied an in-depth high-resolution quantitative MS-based analysis to probe dynamic proteome changes during reprogramming. Protein levels were quantified at 13 different states over the reprogramming time-window by using isobaric labeling (i.e. TMT), SCX pre-fractionation and high-resolution LC-MS/MS (i.e. Orbitrap Velos). Using this approach, we successfully identified 504,060 PSMs at a false discovery rate lower than 1% which corresponded to 7,265 unique protein groups. Most importantly, a total of 6,915 proteins were quantified and for 65% of them we obtained temporal profiles uncovering the whole set of samples included in the analysis. Our data reveal a proteome resetting step taking place already 48 hours after transgene expression, which involved specific biological processes linked to the c-Myc transcriptional network. A second wave of major proteome reorganization occurred in a later stage of reprogramming, where we could distinguish and characterize two distinct pluripotent cellular populations. One of these represented a stable transgene-dependent cell type, which showed an altered pattern of adhesion proteins and a partial activation of pluripotency markers. The other population represented embryonic stem cell (ESCs)-like cellular precursors and showed a higher level of proteins involved in cell adhesion, epigenetic mechanisms and pluripotency.

Keywords: Cellular Reprogramming, Induced Pluripotent Stem Cells, Quantitative Proteomics

PS28-01 Whole Proteome Resources: Access and Sharing of Data through the Peptide and SRMAtlas Proteomics Suites

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A major goal of the human proteome project is to advance the characterization of all coding gene products to complete the human proteome and to enable the application and dissemination of proteomic technologies that will support the comprehensive identification and quantitation of all the proteins, their isoforms, and post-translational modifications contained within the human proteome. With recent advances in comprehensive resources in technology, informatics and reagents, the ability to measure proteins in all its forms is now realized. These developments of new technology for multiplexed quantitative protein measurements, with a throughput consistent with the needs of iterative measurements of perturbed systems, has enabled the building and sharing of tools that are generally applicable to all proteomic-based studies. The advancements in proteomic informatics provides highly curated databases that enable standardization across workflows and form a solid basis for the widespread deployment of multiplexed assays and reporting of their results in a community based manner. This sharing and re-use of data and results provides confidence in the field of proteomics. I will discuss tools that are capable of generating complete, reproducible and quantitatively accurate datasets of entire proteomes.

PS28-02 Building Your Knowledge Base of Empirically Measured Peptides with Skyline and Panorama

Brendan MacLean

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Targeted proteomics is becoming an increasingly popular method of measuring peptide quantities in experiments. It was recently chosen as method of the year by Nature Methods. Targeted proteomics relies heavily on the principle that by accumulating prior knowledge of peptides of interest, a mass spectrometrist can make highly precise quantitative measurements of the peptides, with high confidence. That prior knowledge frequently takes the form of empirical measurements of physiochemical properties of the peptide molecules which are hard to derive from the amino acid sequence alone, such as: peptide retention time on column, relative peptide expression for a protein, linear range in a given sample matrix, relative product ion expression under fragmentation, optimal collision energy for each fragment and collisional cross section. Existing tools have made storing and reusing this critical information cumbersome and error-prone. To improve this situation, developers of the targeted MS desktop application, Skyline, and collaborators at LabKey Software have implemented the Panorama repository software for laboratories already accumulating targeted proteomics experimental results in the form of Skyline documents. The Panorama software helps labs organize results, collaborate securely, and build collections empirically derived targeted assay properties to inform future experiments. Skyline now supports both publishing a document directly to a server running Panorama, and also downloading chromatogram libraries of targeted assay properties from a Panorama server. Developed as a module in the LabKey Server biomedical data management platform, Panorama is freely available and open source. Free hosted Panorama projects are also available on http://panoramaweb.org.

PS28-03 Computational Proteomics Enables Accurate Label Free Quantification of Proteins and Posttranslational Modifications

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Protein quantification without isotopic labels has been a long-standing interest in the proteomics field and is becoming a feasible alternative to label-based quantitative proteomics workflows. However, accurate and robust proteome-wide label-free quantification of pre-fractionated samples remains a challenge. Here we report on a new intensity determination and normalization procedure called MaxLFQ that is fully compatible with any peptide or protein separation prior to LC-MS analysis. Protein abundance profiles are assembled using the maximum possible information from MS-signals given that the presence of quantifiable peptides varies from sample to sample. On a benchmark dataset with two proteomes mixed at known ratios, we accurately detect the mixing ratio over the entire protein expression range, with higher precision for abundant proteins. On a second benchmark dataset, we accurately quantify fold changes over several orders of magnitudes, a task that is challenging with label-based methods.

An even more challenging task the quantification of posttranslational modification sites without labeling. We introduce a computational strategy for extracting site occupancies from label-free datasets with multiple samples. For sites that are changing between biological conditions high quality occupancy estimates are obtained. A quality parameter is introduced which indicates the reliability of the site occupancy quantification

MaxLFQ is a generic label-free quantification technology that is readily applicable to tackle many biological questions and it is compatible with standard statistical analysis workflows, and it has been validated in many and diverse biological projects. Our algorithms can handle very large experiments of 500+ samples in manageable computing time. It is implemented in the freely-available MaxQuant computational proteomics platform and works completely seamlessly at the click of a button (www.maxquant.org).

PS29-01 Site-Specific Glycan-Peptide Analysis for Determination of *N*-glycoproteome Heterogeneity in Bacteria and Mammalian Systems

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The major limitation in glycopeptide analysis is the need to separate glycans from modification sites, thus losing the context of protein glycosylation. We developed a glycan-peptide analysis schema using zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) and fragmentation by collision induced dissociation (CID) and higher energy collision dissociation (HCD) MS/MS to identify >100 intact *N*-glycopeptides from the pathogen *Campylobacter jejuni*. Analysis was simplified by the conserved nature of the *C. jejuni* heptasaccharide, although the analytical depth achieved also identified targets of a phosphoethanolamine-modified *N*-glycan. We next combined glycomics and glycoproteomics for site-specific analysis of *N*-glycosylation heterogeneity in a complex mammalian protein mixture. Initially, *N*-glycomics was performed using porous graphitized carbon LC-MS/MS and the data used to create an *N*-glycan database. Tryptic glycopeptides were then enriched using ZIC-HILIC and fractionated by reversed-phase LC. Each fraction was separated into two aliquots. The first set of aliquots was treated with PNGase F, and former *N*-glycopeptides analyzed by nano-RPLC-MS/MS. This enabled the creation of a glycopeptide-centric concatenated database for each fraction. The second set of aliquots, containing intact glycopeptides, was analyzed by nano-RPLC-MS/MS, employing CID and HCD. Assignment of glycan compositions to peptide sequences was achieved by searching the intact *N*-glycopeptide HCD MS/MS spectra against the glycopeptide-centric databases employing the *N*-glycan database. Semi-automatic annotation of HCD spectra enabled false discovery rate calculations based on the frequency of peptide backbone fragment ions that matched with high mass accuracy. Finally, CID spectra were used to assign glycan structures identified in the glycomic analysis to peptide sequences. This approach identified 863 unique intact *N*-glycopeptides from 161 rat brain proteins.

PS29-02 Tackling Sulfoglycomics and Realizing the Prospects of Sulfoglycoproteomics

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The proper functioning of a diverse range of terminal glyco-epitopes as cell surface recognition codes is not only dependent on the underlying glycan chain and protein carriers, which dictate their accessibility and valency, but also modulated by specific non-sugar substituents. Sulfation at strategic position is one such critical determinant. Time and again, glycan array screening revealed that many endogenous glycan-binding proteins including members of the selectins and Siglecs, bind the sulfated counterparts of their cognate ligands at significantly higher affinity. The prime examples being sialyl 6-sulfo-LeX displayed on high endothelial venules serving as homing ligand for leukocytes expressing L-selectins, and α2-6-sialyl 6-sulfo-LacNAc for CD22/Siglec-2 on B cells. In the presence of multiple sialylation on the termini of most complex N- and O-glycans, the extra negative charge imparted by sub-stoichiometry sulfation often renders these sulfated glycans cryptic in single dimensional glycomic mapping. In analogy to phosphoproteomics, which requires extra experimental steps to enable global mapping of otherwise under-represented phosphopeptides, we have taken the lead to establish the enabling techniques for MS-based sulfoglycomics and shown that sulfation is more widespread than currently appreciated. Many previously unknown sulfo-glycotopes were identified *de novo* and yet, paradoxically, several others predicted based on antibody staining or glycan array data remain undetectable. Unlike the latter approaches, only MS-based sulfoglycomic identification will inform the underlying glyco-structural context. A demanding next step is to further localize the expression of these functionally important sulfated glycans on key cell surface and adhesion molecules by glycoproteomics. However, several technical hurdles remain in realizing this aim and our current advances in sample preparation, MS data acquisition and analysis will be presented.

PS29-03 A New Method for Glycopeptide Assignment by Duplex-LC/MS Analyses

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Assignment of glycopeptide on both peptide sequence and glycan moiety remains technically challenging. Although some approaches including MS/MS analysis employing electron-transfer/capture dissociation (ETD/ECD) have been reported to enable the assignment, these methods are basically applicable to isolated or mixture of a small number of glycoprotein(s) and are still hard to apply for a complex mixture of glycopeptides. Thus far, we have developed a method to identify a large number of peptide sequences carrying *N*-glycan(s) by coupling the affinity capture of glycopeptide subset, enzymatic deglycosylation-mediated stable isotope-labeling on their glycosylated Asn residues, and LC/MS identification of the labeled peptides (IGOT method). Then, we designed a new method to find a series of glycopeptide signals as clusters of glycoforms from LC/MS data, and to predict the peptide sequence of each glycoform cluster by matching the observed accurate mass of glycopeptides and mass of the peptide portions identified by IGOT method. This method allows easy assignment of glycopeptide glycoforms derived from a single model glycoprotein.

Keywords: Glycoproteome, Glycoform, Glycan heterogeneity

PS30-01 Target Deconvolution of Bioactive Small Molecules via Multi-Omics Based Approach

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Many efforts have focused on target deconvolution of bioactive small molecules from phenotypic screens to explore mechanisms of action (MOA) of small molecules and target proteins towards cellular and medical applications. We have developed Multi-Omics based Target Identification and Validation (MOTIV) for harnessing target deconvolution of bioactive small molecules. MOTIV includes a direct affinity-based target protein identification using phage display biopanning or Drug Affinity Responsive Target Stability (DARTS), an indirect genomics-based profiling using yeast ORFeome, and combinations of computational network analysis and validations of these target candidates leading to identification of the biologically relevant target proteins and cellular mechanism of small molecules of interest. We have applied this approach to explore MOA of bioactive small molecules from phenotypic screens of angiogenesis and autophagy. Accordingly, new target proteins and pathways have been identified and validated to provide new insights into the biology of angiogenesis and autophagy leading to biotechnological and medical applications. In this presentation, recent results of our group using MOTIV for target deconvolution of bioactive small molecules targeting angiogenesis and autophagy will be introduced.

PS30-02 Reverse Chemical Proteomics as a Tool in Drug Discovery

Peter Karuso

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Functional proteomics has an important role to play in drug design and development. Not only for mapping proteins in health and disease but also in uncovering the mode of action of drugs (chemical proteomics).[1] Our research focuses on *reverse chemical proteomics (RCP)*, which promises to be a fast, efficient and enabling technology for understanding drug action, identifying the most avid binding partners for small molecules and the discovery of new protein drug targets for medicinal chemistry.
In *RCP* the proteome is tagged with its encoding gene and the bait tagged with and affinity reagent such as biotin. Using T7 bacteriophage as the genetic tag of the proteome has many advantages but the most important is that the proteome can be amplified as many times as needed. The most avid binding partners are purified through multiple rounds of affinity purification (biopanning).
Here, we will outline the concepts of *RCP* and our results for the discovery of protein binding partners for the natural products FK506, artemisinin, kahalalide F and daptomycin. FK506 has well characterized interactions and acts a positive control while artemisinin is an antimalarial with recently described anticancer activity. Daptomycin is a recently introduced antibiotic with notable side effects that suggest off-target associations with human proteins. The natural products were biotinylated at sites known not to affect the compound' s biological activity and the resulting probes were screened against cDNA libraries. This work aims to support our understanding of the underlying biochemical pathways of these compounds and to pave the way for better structure-based drug design and development through chemical protemics.

[1] P. Karuso, in *Comprehensive Natural Products II: Chemistry and Biology, Vol. 9* (Eds.: L. N. Mander, H. W. Lui), Elsevier, Oxford, **2010**, pp. 513-567.

PS30-03 Target Identification of Novel Anti-inflammatory Compound Using Chemical Proteomics Approach with Bait Compound

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Interleukine-12 (IL-12) and IL-23 are pro-inflammatory cytokines which induce serious pathological conditions in diverse of inflammatory diseases such as inflammatory bowel disease (IBD), psoriasis and rheumatoid arthritis. The therapeutic potential of IL-12/23 inhibition has been validated in clinical by Ustekinumab which is an approved monoclonal antibody neutralizing IL-12/23. Through the cell-based phenotypic screening and extensive medicinal chemistry campaign, we generated APY0201 as a potent and unique inhibitor for IL-12/23 production from activated macrophages, possessing significant selectivity over other cytokines including TNF-α. As a result of chemical proteomics approach using a bait compound equipped with FLAG peptide, PIKfyve kinase was identified as a biological target of our promising IL-12/23 production inhibitor. APY0201 is a potent, highly selective and ATP-competitive PIKfyve kinase inhibitor, which ameliorated inflammation in experimental model of colitis. We will disclose the design of bait compound equipped with FLAG peptide, strategy of target identification, SAR, and unique character of this novel drug target, to treat autoimmune and inflammatory diseases.

Keywords: Target identification, PIKfyve, IL-12/23

PS31-01 An Overview of Label-Free Quantitative Shotgun Proteomics in Rice and Grapes

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In recent years we have performed a number of label-free quantitative shotgun proteomics studies in rice and grape plants and cells that have revealed new information on how plants respond at the molecular level to abiotic stress in different environments. In a detailed study of temperature stress in rice cells, we observed tightly controlled switching of sugar metabolism pathways occurring in response to increased temperature, along with modifications in cell walls occurring at extremes of both hot and cold, but by different mechanisms. Perhaps the most striking feature of this study was the apparent metabolic 'panic response' of cells that was triggered between 36˚C and 44˚C. In a drought time-course study in six week old rice seedlings we observed a tightly coordinated response whereby aquaporins and VTPases were reduced in expression at the onset of drought and then greatly increased in expression as the drought cycle progressed. Additionally, in a complex study of split-rooted plants under drought stress we found evidence of directional long distance signaling between roots in different environments but attached to the same shoot. The availability of a complete sequenced and annotated genome for *Vitis vinifera* has allowed us to move into working in grapevines, which are a vitally important crop plant. We have undertaken a detailed proteomic analysis of water deficit stress in 2 year old Cabernet Sauvignon grapevines. One intriguing finding from this work is the detection of protein expression changes in response to water stress, before any phenotypic changes are seen at the whole plant level.
We will also present initial results from detailed studies of temperature stress in Chardonnay grape cells and photoperiodsensitivity in bud tissues from *Vitis riparia*.
Keywords: label-free quantitation, agricultural proteomics, abiotic stress

PS31-02 Green Systems Biology - From Single Genomes, Proteomes and Metabolomes to Ecosystems Research and Biotechnological Applications

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Systems biology is the approach to combine molecular data, genetic evolution, environment and species-interaction with the computer-assisted understanding, modeling and prediction of active biochemical networks. The idea relies strongly on the existence of complete genome sequences and the development of new technologies for the analysis of molecular data. Here, projection of metabolomics and proteomics data into genome-wide metabolic networks combined with metabolic modeling emerge as important technologies for improving gene annotation processes [1, 2]. Using quantitative proteomics and metabolomics we begin to investigate the genome-scale molecular phenotype and the interrelation of the metabolome, the proteome and its environment [1]. Genome-, proteome- and metabolome- as well as anatomical/morphological/physiological- data integration strategies and modeling approaches will be discussed for systems such as Arabidopsis, Tomato and *Chlamydomonas reinhardtii*, the "green yeast" as a model system for third-generation biofuels. For these approaches an extended proteomics and metabolomics platform is presented [3-6]. However, before data reveal their interrelation, extended statistical and mathematical concepts are required for the integrative analysis of multifactorial phenomena [7]. The detection of significant correlations between the different components based on principal components analysis or related techniques is the basis for biological interpretation [8-11]. We have extended this idea and developed an approach which connects systematically the predicted genotype with the statistical features of metabolomics and proteomics data [12]. By using this approach, recently, we were able to calculate the differential biochemical Jacobian from perturbed metabolomics data for the first time [5]. Results and implications of this approach will be discussed.

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PS31-03 Golgi Proteomics in Plants and Application for Biofuel Plant Development

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The production of cellulosic biofuels utilizes the energy reserves contained in plant cell walls. Plant cell walls are costly to deconstruct, contain inhibitors, have non-optimal sugar profile and are a low density material. Nonetheless, incremental modifications to this biomass can result in significant economic improvements. The plant Golgi apparatus synthesizes a significant proportion of matrix polysaccharides destined for incorporation into the plant cell wall. Recently, we developed the first plant Golgi purification strategy based on a combination of density centrifugation and charge based separation using free-flow electrophoresis. The application of this technique to the model plant Arabidopsis enabled a proteomic survey of this organelle which consistently identified around 400 proteins. This collection of Golgi resident proteins provided a list of targets for the development of engineering strategies for the manipulation of the plant cell walls. Recently, in an attempt to identify more subtle approaches for cell wall engineering, we examined the possibility that sub-compartment charge differences of *cis*- *medial*- and *trans*-Golgi components may separate during FFE. We have employed immunoblots, single fraction resolution shotgun mass spectrometry and MRM to support the hypothesis of *cis*- *medial*- and *trans* separation of plant Golgi by free-flow electrophoresis. These approaches have enabled the grouping of functional partners at the sub-Golgi level. These findings should eventually enable more subtle engineering of matrix polysaccharide biosynthesis for optimal cell wall profiles.

PS32-01 Quantitative Proteomics of Lysine Acetylation, Succinylation, and Ubiquitylation

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A great majority of proteins in eukaryotic cells are modified by different posttranslational modification (PTMs). PTMs are key constituents of cell signaling networks, comprising some of the most sophisticated control switches in the cell. Given the vast complexity and their all essential role in controlling biology of living cells, analysis of proteins and PTMs is one of the most exiting areas of research in biology.
Recent developments in high-resolution mass spectrometry (MS), computational proteomics, and optimized PTM-enrichment strategies have greatly facilitated unbiased (non-hypothesis driven) analysis of posttranslational modifications. We have applied these approaches to investigate dynamics of the three major lysine PTMs – acetylation, succinylation and ubiquitylation. Large-scale analysis of these PTMs provided systems view of signaling networks, and revealed their extensive involvement in diverse biological processes. I will discuss these recent developments and highlight applications of high resolution MS for analysis of lysine acetylation and ubiquitylation dynamics in response to cellular perturbations.

PS32-02 Comprehensive Identification of Human N-Myristoylated Proteins Using cDNA Resource and Cell-Free Protein Synthesis System

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Protein N-myristoylation is a well-recognized form of lipid modification that occurs in eukaryotic and viral proteins. Many N-myristoylated proteins play key roles in regulating cellular structure and function. However, comprehensive identification of human N-myristoylated proteins has not been accomplished. The analysis of protein N-myristoylation has been performed mainly by the cell-based strategy such as the MS analyses of proteins extracted from cells or tissues, or the metabolic labeling of the cells transfected with a particular cDNA. One possible alternative method to analyze protein N-myristoylation is the use of cell-free protein synthesis system. Recent development of novel eukaryotic cell-free protein synthesis system and improvement of mass spectrometric analysis system, coupled with the enlargement of the available well-characterized cDNA resources, enables genome-wide analyses of protein N-myristoylation. In this study, in order to establish the strategy for comprehensive identification of human N-myristoylated proteins, the susceptibility of human cDNA clones in human cDNA resources to protein N-myristoylation was evaluated by metabolic labeling in an insect cell-free protein synthesis system and in transfected mammalian cells.
As a result, the products of 35 out of ~6300 human cDNA clones (Kazusa ORFeome project (KOP) human cDNA clones) were found to be novel human N-myristoylated proteins. These novel N-myristoylated proteins contain not only physiologically important cytoplasmic proteins such as protein kinases, phosphatases, E3-ubiquitin ligases, cytoskeletal regulating proteins, apoptosis related proteins, disease related proteins, but also many integral transmembrane proteins that play critical roles in various cellular functions. Functional analyses of non-myristoylated mutants of these proteins revealed that protein N-myristoylation plays critical roles in the expression of their physiological functions. These results indicate that the strategy proposed in this study is useful for the comprehensive identification of human N-myristoylated proteins from human cDNA resources.

PS32-03 Identification and Characterization of the Posttranslational Modifications of Yeast 26S Proteasome

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The 26S proteasome is a multisubunit protease involved in the proteolysis of various proteins tagged with polyubiquitin chains and controls the expression of their functions at appropriate times and locations. Posttranslational modifications (PTMs) are expected to be involved in the dynamic regulation of the proteasome activity.
We have identified more than 110 PTM sites in the 34 subunits of the yeast 26S proteasome. For the N-terminal peptides, 21 N-acetylation sites and one N-methylation site were identified. In addition, we also found one N-myristoylation site, a lipid anchor modification on N-terminal glycine, in Rpt2 subunit. It is widely accepted that the N-myristoylation is implicated in the intracellular localization or intermolecular interaction of modified proteins. For the functional characterization of the N-myristoylation on Rpt2, we constructed the yeast strains carrying site-specific substitution/deletion mutation. Both mutants showed abnormal sensitivity to the protein misfolding and increased accumulation of polyubiquitinated proteins. Fluorescence microscopy of the GFP-tagged proteasomes showed the nuclear localization of proteasome in normal cells, while the mutation resulted in the diffusion of the nuclear proteasome into the cytoplasm, where it formed aggregates. In contrast, assembly and peptidase activity of the 26S proteasome were totally unchanged, as revealed by the MS analysis of the TAP-purified proteasome and in-gel peptidase assay. This is the first report indicating that PTM on a single subunit influences the subcellular localization of a huge protein complex unless disturbing its assembly or activity.

Keywords: Posttranslational modification, Myristoylation, Proteasome

PS32-04 Mapping the Human Methyltransferasome Reveals the Existence of a Posttranslational Modification Code that Targets Molecular Chaperones to Regulate Functional Organization of the Human Proteome

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In efforts aimed at identifying and characterizing protein substrates and regulators of all known and putative human methyltransferases (the methyltransferasome) using multiple cell compartment affinity purification coupled with mass spectrometry (MCC-AP-MS), our laboratory has discovered a novel family of lysine methyltransferases that preferentially target and regulate molecular chaperones. Our results indicate that methylation of molecular chaperones VCP by METTL21D and Hsp70 by METTL21A regulates the activity of both chaperones. In the case of VCP, trimethylation of lysine 315 by METTL21D is stimulated by the addition of the UBX cofactor ASPSCR1, which we show directly interacts with the methyltransferase. This stimulatory effect was lost when we used VCP mutants (R155H, R159G and R191Q) known to cause Inclusion Body Myopathy with Paget' s disease of bone and Fronto-temporal Dementia (IBMPFD) and/or some familial forms of Amyotrophic Lateral Sclerosis (ALS). Lysine 315 falls in proximity to the Walker B motif of VCP's first ATPase/D1 domain. Our results indicate that methylation of this site negatively impacts the ATPase activity of this molecular chaperone. In the case of Hsp70, methylation of lysine 561 by METTL21A was shown to decrease formation of stress granules in response to arsenite treatment, a model that is widely used to study improper protein folding in degenerative disorders. In addition to having implications for the development of therapeutics for degenerative neuromuscular disorders such as ALS and IBMPFD, the discovery of a family of chaperone-targeting methyltransferases led us to propose the existence of a chaperone posttranslational modification code, that we termed the chaperone code, which is at play to orchestrate the proper folding and assembly of protein complexes that make up the human proteome. We will report on our most recent results relating to decryption of the chaperone code and its role in diseases.

Keywords: Protein-protein interactions, Methylation, Molecular chaperones

PS32-05 A Proteomic Investigation of Proteasome Malfunctioning

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Inhibition of the proteasome is pro-apoptotic in most cell types. In cancer therapeutics, the induction of apoptosis using chemical agents is widely used, for instance for the treatment of myeloma patients. Here, we aim to dissect the functional modules of the proteasome by profiling the dynamic proteome and ubiquitinome as a result of proteasome dysfunctioning. We study the effect of proteasome malfunctioning on the global cellular *Drosophila* S2 cellular proteome using a SILAC-based nanoLC-MS/MS (Q Exactive) approach. Inhibition of the proteasome is accomplished by either using chemical agents (MG132 and lactacystin) or by using selective RNAi knockdown constructs against different proteasomal subunits. For the identification of the dynamic pool of ubiquitinated proteins we use a recently developed protocol based on immunoprecipitation of peptides derived from ubiquitinated proteins. Roughly 5,000 proteins are identified and quantified routinely in these SILAC screens. After relatively short incubation times with drugs, approx. 100 proteins are specifically found to be upregulated and/or accumulated. After longer incubation times and upon RNAi of knockdown of proteasomal subunits, the abundances of several hundreds of proteins are altered. Proteins that show severe and relatively fast upregulation and/or accumulation are associated with functional categories such as stress response, cell cycle regulation, apoptosis and the ubiquitin-proteasome system. In addition, the pool of ubiquitinated proteins is upregulated after proteasome inactivation. Strikingly, there is little overlap between the sets of proteins with increased abundances and proteins showing increased ubiquitination ratios. Currently, we are investigating the target specificity of various proteasome-bound deubiquitinating enzymes by analysis of the dynamic ubiquitinome. Results are expected to give more insight into the mechanism and specificity of the proteasome functioning.

Keywords: Proteasome, Ubiquitinome, SILAC

PS33-01 Tracking Protein Turnover in Plant Cells and Intact Plants: A New Frontier is Understanding the Energy Efficiency of Plant Growth

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Shotgun approaches or targeted SRM analyses now dominate proteome studies as tools to find changes in the proteome. Key limitations, however, are that these approaches focus just on the proteins that are changing in abundance to find biological insights and they require statistically significant changes in the total accumulated protein pool size in order to identify that anything has occurred. Analysing protein synthesis and degradation rates with progressive stable isotope labelling provides a new window on the control of protein abundance as we seek to determine the 'relative age' of the proteins that we see. Through progressive ^{15}N labelling of plant cells from nitrate and ammonia salts and modelling incorporation fits, we can calculate the rate at which proteins which are static in abundance in the proteome are turning over, and thus provide an extra dimension to proteome analysis by an assessment of what is controlling protein abundance in cells. Through combining such labelling with separation of protein complex and subcomplexes by native electrophoresis, we can observe the *in vivo* turnover rate of assembly intermediates of protein complexes. We have developed pipelines to undertake these studies in plant cells, plant leaves and in whole plants through the use of hydroponics. Projects assessing the impact of phosphate limitation, groundwater salinity and nitrogen fertilization on protein turnover changes in plants will be discussed. Combined there approaches provide new avenues for peptide mass spectrometry to provide answers to a wide range of questions in plant biology, and allows researchers to assess the cost of environmental factors on protein turnover and plant growth efficiency.

Keywords: plant proteomics, protein turnover, protein complexes

PS33-02 Functional Proteomics of Plant Signaling and Metabolic Networks

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Environmental stresses affect plant growth, development, yield and bioenergy production. Cellular molecular networks connect environment signals to phenotypes. Our knowledge about how different molecules and pathways connect to each other is very limited. Here we implement functional proteomics approaches to tackle plant molecular networks. We make use of stomatal movement and glucosinolate metabolism as model systems. Stomatal movement is essential for plant growth, yield, and interaction with the environment. Quantitative proteomics of stomatal guard cells under different hormone treatments has revealed novel redox responsive proteins, which not only highlight crosstalk between hormone signaling at the posttranslational level, but also provide nodes and edges for biotechnological applications. Glucosinolates constitute a large group of plant specialized metabolites in Brassica crops and vegetables. In addition to anticarcinogenic activities, glucosinolates play important roles in plant defense against herbivores and pathogens. Here we have implemented a systems biology approach by perturbing the expression of key genes, followed by examining changes in metabolite and proteins levels in other pathways. In this presentation, our recent progress on developing and implementing proteomics and metabolomics tools to elucidate plant molecular networks will be reported.

Keywords: Quantitative proteomics, Molecular networks, Brassica plants

PS33-03 Comparative Analysis of the Proteomes of *Ricinus communis* and *Jatropha curcas* Seeds

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The seeds of *Ricinus communis* and *Jatropha curcas* are regarded as a potential source of raw material for the production of biodiesel, but exploitation of this potential is hampered by a lack of understanding regarding key aspects of seed development and biochemistry. We have performed a detailed histological analysis of seed development in these two species and used it as a guide to undertake an in-depth proteome analysis of the inner integument, nucellus and endosperm of developing seeds, as well as of plastids isolated from these tissues. In this presentation, the many commonalities unraveled by our proteome analysis in the biochemical machinery of seeds from these species will be discussed, particularly in relation to carbon and nitrogen flow. We will also discuss results pertaining to the pattern of deposition and synthesis of ricin and phorbol esters in maternal (inner integument and nucellus) and embryo (endosperm) tissues.

PS33-04 Proteomic Analysis Showing the Features of Metabolism Regulation During Rice Seed Germination

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Rice seeds are not only the staple food resource of the world population, but also an essential material for agricultural production. Successful germination of rice seeds is a prerequisite for its cultivation. In order to uncover the mechanisms underlying rice seed germination, systematic proteomic analyses were conducted on rice seeds during this process. Metabolic pathways were constructed based on the proteome profiling data. Along with the comparative proteomic analyses, we found that the enhancement of anaerobic respiration including glycolysis and fermentation might be the main source of ATPs at the early stage of germination, and the aerobic respiration was only enhanced at the late stage. Starch granules were quickly degraded in the endosperm into glucose 6-phosphate, which was transferred into embryos either for further degradation or for biosynthesis of starch. The newly biosynthesized starches were mainly accumulated in the tissues around the bundle sheath. The genetic information for germination were programmed during seed maturation, so the initiation of transcription is unnecessary, but it can help to ensure the rapidity and efficiency of the germination. Unlike transcription, translation is absolutely needed. Furthermore, we also found that there were several GA and ABA responsive elements in the promoters of most of the genes encoding the up and down-regulated proteins respectively, which suggested that the germination of rice seed might also be regulated by GA and ABA. These studies bring us some new insights into the rice seed germination.

This work was supported by the 100 talents project of Chinese Academy of Sciences. The author gratefully acknowledges the support of K.C. Wong Education Foundation, Hong Kong

Keyword: rice, proteomics, germination

PS33-05 Involvement of the Plasma Membrane in Plant Cold Adaptation: A Protomics Perspective

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The plasma membrane (PM) plays a number of important roles in determining plant survival under adverse environmental conditions. We have revealed that the PM is the primary site of freezing injury due to its central role in a freeze-thaw cycle and cold acclimation (CA) results in changes in its compositions dynamically to increase the cryostability of the PM. Responses of PM proteome to CA are well corresponded to the increase of freezing tolerance and, in fact, we demonstrated that a few PM proteins directly affect freezing tolerance during CA. We have been studying cold-induced PM proteome changes in plants using oat, rye and *Brachypodium*, all in Family Gramineae, to explore possibilities to increase agricultural productivity of the crops in cold-temperature regions. Using purified PM fractions and nano-LC-MS/MS analysis, we comprehensively catalogued cold-responsive PM proteins and subsequently categorized them according to proposed functions. *Brachypodium* PM proteome was quite similar to that of oat and rye but a few differences were apparent. Furthermore, PM proteome changes during CA is somewhat species-specific, which may be associated with differences in freezing tolerance development of the three plant species during CA. We will present profiles of PM proteome of three Gramineae plant species before and after CA and discuss how we can use PM proteome profiles to understand plant adaptation to cold stress conditions. (Supported in part by grants-in-aid from MEXT and JSPS, Japan, #22120003, #24370018 and #24-7373.)

Keywords: Plants, Plasma membrane proteome, Stress adaptation

PS33-06 Characterization of Muscadine Berry Proteome Using Label and Label Free Mass Spectrometry Approaches

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Muscadine grapes (*Vitis rotundifolia*) are well known for variety of nutraceutical and enological characteristics. Our earlier results indicated potential anticancer activity of berry extracts on human cancer cell lines. These extracts contained variety of phenolics associated with anticancer activity. Until recently, most studies were focused on viniferas grapes, while little information is available on muscadine grape berries. Advances in mass spectrometry enhanced the ability to reveal more identified proteins in tissues. The objective of this research was to investigate the proteome profile of muscadine berry using gel based and gel free separation methods. Total proteins of pericarp were extracted from different stages of berry development and ripening. Two-dimensional electrophoresis resolved approximately 350 proteins. Gel free iTRAQ label followed by mass spectrometry identified 600 proteins, while label free mass spectrometry revealed over 1600 proteins. Over 150 proteins showed differential expression during the berry development. We contemplate that, gel free and label free method of protein identification revealed more proteins in the pericarp tissue. Functional annotation revealed the proteins involved in pathways related to defense and secondary metabolite synthesis. Protein-interaction studies of these differentially expressed proteins revealed several orthologous proteins showing interactions in *Arabidopsis* interactome database. Further investigation on interaction network will determine the role of differentially expressed proteins associated with the biosynthesis of nutraceutical compounds.

Keywords: Grape berry, iTRAQ label, Mass spectrometry

PS33-07 Quantitative MSE Proteomics as a Tool for the Determination of Clinically Relevant Proteins in Wheat Grain

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The amounts of clinically relevant disease-related proteins in wheat grain are largely unknown. Developing the methods for quantitative measurement of clinically relevant proteins could support advancements in understanding exposure thresholds and clinical study design. The aim of our study was to use a data-independent mass spectrometry (MS^E) approach for quantifying gliadin and glutenin proteins in wheat grain. The biological replicated analysis yielded concentrations for 34 gliadin and 22 glutenin proteins and detected several peptides carrying four previously discovered epitopes that belong to gamma gliadin B precursor. The technical coefficients of variation ranged from 0.12 to 1.39 and indicates that MS^E proteomics is a reproducible quantitative method for the determination of gliadin and glutenin content in the highly complex matrix of protein extracts from wheat grain.

Keywords: wheat, grain, allergy

SS-02 (Keynote) The Strategy, Organization, and Progress of the Human Proteome Project

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The global Human Proteome Project aims to identify and characterize at least one protein product and many PTM, SNP, and splice variant isoforms from all 20,300 human protein-coding genes [www.thehpp.org]. The deliverables are an extensive parts list and an array of technology platforms, reagents, spectral libraries, and linked knowledge bases that advance the field and facilitate the use of proteomics by a much wider community of life scientists. Such enablement will help address the Grand Challenge of using proteomics to bridge major gaps between evidence of genomic variation and diverse phenotypes.

SS-03 (Keynote) Genome-wide Proteomics: The Role the C-HPP Initiative to Study Cancer Gene Amplicons and Proteomic Signatures

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The development of a genome-wide proteomic strategy has been elucidated by the Human Proteome Project (HPP) and promotes the integrating transcriptomic and proteomic data in the context of biology and disease studies. Also the proteomic researcher will better understand the genomic context of their observations. This lecture will illustrate this approach with a study of breast and gastric cancer driven by the oncogene ERBB2, a member of epidermal growth factor receptor (EGFR) superfamily of tyrosine kinase receptors. In this study the gastric cancer were of the intestinal type and consisted of two cancer sample sets, ERBB2 positive and negative. For both sets the control was normal tissue adjacent to the tumor. In the analysis proteins were extracted from the tissue samples separated by SDS-PAGE, then analyzed by nanoLC coupled to an LTQ-Orbitrap mass spectrometer. We also examined transcriptomic data from ERBB2 expressing cancer cell lines, SNU16 and KATOIII (gastric) and SKBR3, SUM 149 and 190 (breast cancer). While the proteomic study was of lesser depth (approximately 3,000 proteins identified) than the RNA-Seq analysis that identified 11,000 transcripts (100 million reads) proteomics can aid the identification of significant pathways and expression events identified by the transcriptome analysis. The study identified the ERBB2 amplicon, which contains a group of genes co-amplified with the oncogene as well as other pathways of potential significance such as the MEK-ERK and the proteasome degradation pathway.

SS-04 Protein Post-Translational Modifications and their Regulation of Cardiovascular Disease

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To improve our understanding of cardiovascular biology, we investigate the role of proteins under defined disease conditions via the use of model systems. Heart disease is the leading cause of mortality and morbidity worldwide, with coronary heart disease (CHD) accounting for 52% of attributable deaths. Acute myocardial infarction (AMI) is directly linked to CHD and results from myocardial ischemia, where the heart receives an inadequate supply of oxygen. The extent of damage to the heart is proportionate to the duration of ischemia, whereby transient episodes are protective, brief periods cause reversible contractile dysfunction and extended insults result in cellular necrosis and ultimately organ failure. Given the broad range of functional responses resolvable on a relatively sort time scale (1-60 minutes), we hypothesize that subtle protein post-translational modifications play an essential role in these outcomes. Proteomic technologies have facilitated our understanding of the role of protein degradation, phosphorylation, acetylation, glycosylation and oxidation in response to myocardial ischemia. To model disease, we utilise ex-vivo induction of ischemia in rat (*Rattus norvegicus*) and rabbit (*Oryctolagus cuniculus*) myocardium as model organisms. This permits investigation of the cellular events, under otherwise ideal conditions prior to validation in clinical cohorts, which are ultimately more challenging given the heterogeneous nature of AMI patients whom present with diverse co-morbidities including diabetes. An essential component of proteomic PTM investigations are the observation of non-modified counterparts to ensure that changes in the modified species are independent of changes at the protein level and to improve our coverage of the cardiac proteome. We have identified over 4,000 unique myocardial proteins of which nearly 50% are modified at one point during the progression from protection to necrosis using our ex-vivo model. In our experience, to improve our understanding of myocardial I/R injury, model systems provide the ideal biological situations from which to generate hypotheses for testing in more complex clinical situations.

JHP-01 Molecularly-informed Discovery of Proteomic Biomarkers for Cancer Detection

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Development of proteomic biomarkers, in part, has been driven by intuition and serendipity. This type of approach has led to discovery and development of biomarkers, which were later found to be compromised by the lack of reproducibility, generalizability and usefulness in clinical application. This is despite the fact that over the years the molecular knowledge of cancer initiation, causation and progression has made remarkable progress. The Whole Genome Sequencing (WGS) is rapidly generating data on gene expression profiling, copy number variation profiling, SNP genotyping, genome wide DNA methylation profiling, microRNA profiling, and exon sequencing of at least 6,000 genes, including microRNA. These efforts have led to identification of several important cancer networks and pathways that are becoming useful tools for discovering genomic biomarkers. However, proteomics offers more phenotypically-related biomarkers than genomically-related biomarkers and are more amenable to clinical applications. The Speaker will discuss the importance of genetically driven knowledge in developing strategies for protein-based biomarkers in cancer detection. For example, the NCI's Early Detection Research Network (EDRN; www.cancer.gov/edrn) investigators are proactively analyzing The Cancer Genome Atlas (TCGA) data along with their own expression data to guide the discovery of ovarian cancer-related secretome genes that can be further prioritized and subjected to scrutiny for clinical validation. The secretome will be used to interrogate large numbers of microarrays performed on a variety of cancer and control tissues including TCGA. For example, all 480 serous ovarian cancers (within the TCGA), which have mRNA expression data, will be utilized to identify differentially expressed transcripts between cancer and normal tissue. Lists of genes with significant differential expression will then be filtered using a variety of expression profiling databases of microdissected ovarian cancers and normal control tissues. Any gene found in multiple datasets will be considered high priority epithelial biomarkers. The final product will be a highly filtered list of differentially expressed genes (not found to be highly expressed in other tissues) that potentially encode proteins found in the serum. Such approaches are being employed in discovering biomarkers for ovarian, pancreatic and other types of cancers.

JHP-02 A Proteomic Approach to Decipher the Molecular Link between HIV-1 Gag and Host Proteins

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Background & Purpose: The process of assembly and release of Human Immunodeficiency Virus type1 (HIV-1) is driven by the active trafficking and assembly of Gag precursor protein (p55) in infected host cells. Although the involvement of host proteins in the regulation of Gag during the virus particle production has been proposed, the detailed molecular mechanisms are still not yet well characterized. The discovery of cellular factors that participate in this process can provide further insights into the nature of HIV-1 replication pathways and assist with identifying new targets for anti-viral therapies.
Methods & Results: In our current study, we utilized the amplified luminescent proximity homogenous assay (AlphaScreen) as an *in vitro* protein-protein interaction analysis in an attempt to identify host factor(s) that directs Gag to promote virus replication. Using full-length HIV-1 Gag and host proteins synthesized by the wheat cell-free protein production system, we found that the atypical protein kinase C (aPKC) can bind HIV-1 Gag. LC-MS/MS and immunoblotting analysis with a phospho-specific antibody confirmed both *in vitro* and *in vivo* that aPKC phosphorylates HIV-1 Gag at Ser-487. Computer-assisted structural modeling and a subsequent cell-based assay revealed that this phosphorylation event is necessary for the interaction between Gag and HIV-1 accessory protein Vpr (viral protein R), and results in the incorporation of Vpr into virus particles. Moreover, the inhibition of aPKC activity reduced the Vpr levels in virus particles and impaired HIV-1 infectivity in human macrophages.
Conclusions: Our current study sheds new light on the molecular link between Gag phosphorylation by aPKC and viral infectivity through the incorporation of Vpr into virions. The targeting of aPKC activity could be a potential option as a novel therapeutic intervention against HIV-1 infection.
Keywords: HIV-1, host factor, phosphorylation

JHP-03 Phosphoproteomics of Human Liver Cancer Analyzed by 2-Dimensional Image-Converted Analysis of Liquid Chromatography and Mass Spectrometry (2DICAL)

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Two-dimensional image-converted analysis of liquid chromatography and mass spectrometry (2DICAL), which we developed originally, is a proteomic analysis system that compares individual peptide peaks of multiple samples and selects significant peptide peaks with statistical analyses in a label-free method. We applied 2DICAL in analyses of the phosphoproteomics of human liver cancer to search for specific phosphorylation changes in human liver cancer.

106 samples were collected from patients with liver cancer in an ethically approved process. Deep-frozen raw tissue (1mg) was fixed in 100% methanol and trypsinized in 1% sodium deoxycholate solution. Phosphopeptides were extracted by the HAMMOC method and desalted. Liquid chromotography-mass spectrometry and tandem mass spectrometry (MS-MS) spectra were acquired by Triple-TOF 5600. LC-MS-MS data (n = 212) were analyzed by 2DICAL, and the peptide peaks with fragment expectation below 0.05 by Mascot analysis were statistically analyzed in liver cancer and noncancerous liver tissue. The significant phosphopeptide peaks were selected and applied to the informatics investigation.

In the detected 44,990 peptide, 2,390 were phosphopeptides with fragment expectations below 0.05 by Mascot analysis. In the differential analysis between the liver cancer and noncancerous liver tissues, 173 phosphopeptides were increased more than two-fold and 145 were decreased by less than half in liver cancer tissue compared to noncancerous liver tissue. The altered phosphopeptides were referred to the derived proteins and classified by the Gene Ontology terms. There were several phosphopeptides that were not reported to change in human liver cancer. They were considered to be not only candidate biomarkers but also potential therapeutic targets for liver cancer. A phosphoproteomics analysis of 106 human liver tissues by 2DICAL revealed specific and systemic phosphorylation changes in liver cancer.

Keywords: 2DICAL, Liver cancer, Phosphoproteomics

YI01-01 Role of CYLD Deubiquitinase in EGF Signaling Pathway

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The tumor suppressor for human cylindromatosis, CYLD, is an ubiquitiously expressed deubiquitinase that specifically hydrolyzes K63-linked polyubiquitin chains. CYLD was initially described as an inhibitor of the TNF-activated NFkB pathway through deubiquitination and subsequent inactivation of key adaptor molecules such as NEMO, TRAF2, TRAF6 or Bcl-3. Accordingly, CYLD down-regulation has been reported in several types of tumors including lung, liver or colon cancer, whereas cyld-/- mice show increased susceptibility to develop colon or skin tumors. Nevertheless, recent evidences suggest its spectrum of biological activities is not limited to the NFkB pathways. Our earlier studies revealed that CYLD could be involved in the signaling cascade initiated by the epidermal growth factor (EGF), as it was found to be strongly tyrosine phosphorylated upon EGF stimulation. The engagement of CYLD in EGF signaling has also been documented in several very recent studies. However, the functional meaning and impact of the novel role attributed to CYLD still needs to be elucidated. In order to decipher the implication of CYLD in EGF signaling, we applied SILAC-based quantitative proteomics to compare the phosphoproteome as well as the CYLD-interacting protein network in wild type- and CYLD- silenced cells. These unbiased analyses may provide better understanding of the role of the CYLD deubiquitinase in the signal transduction initiated by EGF.

Keywords: EGF signaling, CYLD deubiquitinase, Quantitative proteomics

YI01-02 ATP Accessibility Screening (AAS), A High-Throughput and High-Resolution Kinase Analysis Platform for Signaling Research

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Phosphoproteome analysis is now widely used for various signaling research. It is possible to quantitate phosphorylation level of thousands or more phosphosites. However, deep phosphoproteome analysis is low-throughput. Due to this disadvantage, it is very difficult to use phosphoproteome analysis for experiments which need high-throughput fashion, such as chemical library screening or genome-wide knockdown experiment. Phosphoproteome analysis is useful to monitor kinase activity, when target kinase has a phosphorylation site which represents its kinase activity. However, a kinase which activity is not regulated by phosphorylation, such as constitutive active kinase, cannot be monitored by phosphoproteome analysis. In that case, we might predict kinase activity from phosphosites (substrate) data using bioinformatics analysis, but such kind of analysis is low-resolution (for example, it is difficult to distinguish AKT1 and AKT2 activation). In order to overcome these challenges, we developed a high-throughput and high-resolution proteomic analysis platform, ATP Accessibility Screening (AAS). AAS is a kind of activity-based proteomic analysis targeted on ATP accessibility of kinases. We employed amine-reactive ATP-biotin tag to label lysines conserved in ATP binding pocket. After labeling, protein was digested, enriched by streptavidin beads and applied to LC-MS/MS. About 15% of identified proteins were kinases. Thus we constructed an inclusion list contains *m/z* and retention time information on peptides from 249 kinases. Using this inclusion list, we can quantified ~130 kinases in 1 hour run from 4 mg SILAC labeled Hela-S3 lysate. We will also report the effects of ionized radiation at different doses and at different time after irradiation on ATP accessibility.

Keywords: ATP accesibility, kinase, screening

YI01-03 Hidden Proteome: Multiplex Quantitation of Low- and Ultralow-Copy Number Proteins in HepG2 Cells and Human Plasma

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Using ordinary SRM approach we have registered almost complete chromosome 18th proteome in human plasma and HepG2 cells. Still, there is some portion of proteins that remaine undetectable due to their low concentration. To discover and quantitate such "hidden" proteins we designed an experiment comprising reducing sample complexity, increasing the sensitivity and simultaneous qualification and quantitation using stable isotope dilution in QED-SRM mode. The problem of complexity of biological sample was resolved by fractionation. The resulting fractions were enriched by irreversible covalent binding, which allowed proteins immobilization and enhancement of digestion with trypsin. Quantitative analysis was performed in QED-SRM mode using multiplex calibration approach with stable isotope dilution. The defined attitude permitted simultaneous verification of the targeted peptides by full MS and following progressive MS/MS scanning of the isolated precursor ions overlaying SRM transitions. Quantitative analysis in reduction energy ramping manner was performed in the case where SRM transitions match the defined criteria after dd-MS/MS scanning. The correctness of the isolated and quantified peptides was verified by,firstly,MASCOT processing of the accumulated MS/MS data and matching them to the registered peptides; co-elution of native peptides with heavy internal standard peptides; and assay of the transitions stability and conformity using MLD (Mean Logariphmed Derivative) function. Thus, we registered up to 90% of proteins after fractionation and enrichment with sensitivity of 10^{-17} M. Only 70% of the verified proteins were quantified while the remaining proteins were registered with the signal outside of the LLOQ. Bioinformatic processing of the registered and quantified proteins interaction and molecular functions has been applied in order to eunsure the possibility of their adequate presence and quntitation in HepG2 cells and human plasma.

Keywords: quantitation, QED-SRM, low-copy number

YI01-04 Targeted Proteomics to Validate and Quantify One-Hit Wonders Proteins in Human Liver

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In shot-gun proteomics, protein identification and quantification based on peptide fragment sequences commonly exclude single hit protein identifications. Although rigid guidelines ensure high quality of the reported identifications and avoid the inflation of identification lists with erroneous entries, exclusion of single hit wonders may result in the loss of potentially valuable meta-data. Since the concept of proteotypic peptides are widely used in quantitative proteomics, retrial of these one-hit wonders will replenish our knowledge in gene-centric proteomics. We employed SRM to verify the one-hit wonders proteins in Chinese Human Liver Proteome Project dataset. Crude peptides were synthesized and used to develope SRM assays for target peptides. Proteins extracted from normal human liver were separated in SDS-PAGE and digested in split gel slice. Then the digests were subjected to LC- scheldued SRM analysis. Totally, 184 SRM assays were developed and expression of 57 target proteins were confirmed in normal human liver tissues. Among the proved 57 one-hit wonders, 48 proteins are of minimally redundant set in the PeptideAtlas data base, 8 proteins even have none MS-based information before.

Keyword: SRM, one-hit wonders,human liver

YI01-05 Basic Fetoprotein is Homologous with G6PI/AMF/NLK/MF/PGI/PHI/SA-36 by the Identification of 99% AA Sequence for BFP Using MALDI-MS

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Basic fetoprotein (BFP), which is found in serum, gastro-intestinal tract and brain tissue of human fetuses, is widely used as a serum and urinary tumor marker, but its structure has not yet been investigated. Hence, we investigated the amino acid (AA) sequence of BFP, derived from the human hepatocellular carcinoma tissues grafted into nude mouse, using matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS)(AXIMA Performance and AXIMA Resonance; Shimadzu/Kratos). By employing a wide variety of protease digestion (Trypsin, Lys-C, Lys-N, Asp-N, Arg-C, Trypsin & V8, Trypsin & Asp-N, Lys-C & Asp-N), selection of matrix system (3AQ/CHCA as matrix, MDPNA as additive), and MALDI-MS, we have successfully sequenced up to 99% of BFP without pre-fractionation. As a result, BFP tumor marker was found to be homologous to glucose-6-phosphate isomerase (G6PI), which revealed that BFP is also homologous to autocrine motility factor (AMF) / neuroleukin (NLK) /maturation factor (MF) /phosphoglucose isomerase (PGI) / phosphohexose isomerase (PHI) /sperm antigen (SA-36).

In conclusion, we showed that the BFP tumor marker is homologous to human G6PI protein by the identification of 99% AA sequence of BFP using MALDI-MS. Results demonstrated that the analytical system using MALDI-MS can serve as a very powerful tool for protein sequencing. Here in this session we describe details of this work.

Keywords: sequence coverage, biomarker, PTMs analysis

YI01-06 Systematic Characterization of Human Platelets in Arterial Vascular Disorders by Quantitative Proteomics

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Anti-platelet treatment is of fundamental importance in combatting functions/dysfunction of platelets in the pathogenesis of cardiovascular and inflammatory diseases. Dysfunction of anucleate platelets is likely to be completely attributable to alterations in protein expression patterns and post-translational modifications. Combining elaborate protocols for platelet isolation from fresh blood donations in conjunction with quantitative mass spectrometry, we created the first comprehensive and quantitative proteome of highly pure human platelets, comprising almost 4,000 unique proteins with copy number estimates for ~3,700 of those and relatively quantified ~1,900 proteins between four different healthy donors - with negligible contamination by leukocytes, erythrocytes and plasma, respectively. For the first time, our data allow for a systematic and weighted appraisal of protein networks and pathways in human platelets, and indicate the feasibility of differential and comprehensive proteome analysis from small blood donations. Since 85% of the platelet proteome show no variation between healthy donors, this study represents the starting point for disease-driven platelet proteomics. These findings allow for correlation to genome-wide association studies which identified in a retrospective manner a set of chromosomal regions affecting the risk of cardiovascular diseases. While respective gene products could be identified in platelets, a comprehensive and quantitative comparison of protein patterns between patients and relevant controls such as relatives and spouses to validate risk factors is still missing. In order to improve cardiovascular risk management, genomic and proteomic analyses of respective corresponding gene loci and proteins using next generation sequencing and targeted MS strategies are applied with the final goal to characterize valuable biomarkers for biomedical screenings.

Keywords: platelets, quantitative proteomics, posttranslational modification

YI01-07 Secretome Analysis of Three-Dimensional in Vitro Model Cholangiocarcinoma

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Cholangiocarcinomas (CCA) is a malignant neoplasm of biliary tract epithelium with an incidence and mortality progressively increase over the past decades. Although the occurrences of this cancer are variable amongst countries, Thailand has the highest incidence rate in the world. As this aggressive and poorly understood malignancy remains largely incurable, developing more effective biomarkers and therapeutic modalities could significantly extend survival rate for these patients. Nowadays, study of cancer cell lines secretome as a means to identify diagnostic and prognostic markers has been widely performed. However, conventional method is to collect conditioned media obtained from monolayer culture that may not be wholly representative of dynamic features in which tumors exist in vivo. At present, three-dimensional (3D) culture has been extensively used since it provides more realistic microenvironment in natural physiology than routine method. Therefore, scaffold-based 3D culture of human intrahepatic cholangiocarcinoma isolated from Thai patient (HuCCA-1) was established and differentially secreted proteins between 3D and monolayer cultures were identified. In total, 25 distinct proteins which belong to categories of metabolic enzymes, signal transduction, stress response, cytoskeleton, and protein synthesis and degradation upregulated in 3D culture. These altered proteins might indicate similarity between this model and in vivo secretion from solid tumor and would provide more valuable data about bile duct cancer pathogenesis that could improve future diagnosis and therapeutic strategies.

Keywords: three-dimensional culture, cholangiocarcinoma, secretome

YI02-01 Applying SWATH-MS to Dissect the Variability and Heritability of the Human Plasma Proteome

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The plasma proteome has attracted a lot of attention in translational medicine and biomarker discovery studies. However, fundamental questions such as the variability of the plasma proteins in a population, their inheritability and their longitudinal pattern over years remain unexplored. This is due to the lack of suitable analytical methods that can consistently identify and quantify a large number of proteins among individuals in large sample cohort. We demonstrate that our newly developed Data independent Acquisition (DIA) method, SWATH-MS [1], provides the unique and unprecedented chance to address these difficulties [2] for population proteomic studies.
Using 232 plasma samples from monozygotic (mz) and dizygotic (dz) twins that were collected with 2-7 year intervals, we aimed to systematically investigate and decompose the technical (peptide- and protein-level) and biological variance (such as genetic and individual-/common-environmental and longitudinally unstable factors) in human plasma proteome profiles. The results indicate that SWATH-MS identified and quantified at least 2500 unique stripped peptides (regardless of charge-state and modification) at an FDR of 1%, corresponding to more than 400 proteins at a high degree of reproducibility in the plasma samples. The data showed an unprecedented degree of reproducibly, with the median CV of 11.2% for technical replicates. Overall, monozygoctic twins showed significantly higher proteomic concordance in plasma compared to di-zygotic twins (P=2.97E-37). An accurate estimation protein heritability allowed by twin strategy is currently underway.

References:
[1] Gillet, L. C., et al, Mol Cell Proteomics. 2012, 11(6): O111.016717.
[2] Liu, Y. S., et al., Proteomics. 2013 Apr;13(8):1247-56.
Keywords: Plasma Proteome, SWATH-MS, Population proteomics

YI02-02 Plasma Proteome Analysis Using LC-MS/MS with Travelling Wave Ion Mobility and an Alternative Computational Solution to Protein Quantitation

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Biomarker discovery involves the analysis of highly complex biological samples. Even using chromatography coupled with mass spectrometry, many species still co-elute, causing masking of ion signals and challenging protein identification and quantitation. Many techniques for incorporating additional separation, such as pre-fractionation and electrophoresis, decrease sample throughput and involve additional sample preparation which can contribute to preanalytical variation. Travelling wave ion mobility coupled with label free data independent acquisition (DIA) requires no additional sample preparation, yet confers significant advantages in proteomic analysis. The inclusion of an ion mobility step into the workflow also allows the number of identified proteins to be significantly increased whilst precursor and product mass accuracies are maintained between the modalities The ion packeting behaviour of travelling wave ion mobility (TWIMS) affords improved and less interfered detection of lower abundant species, however, one obstacle encountered with the analysis of high dynamic range proteomic samples is signal saturation of high abundant ions, causing issues in quantitating the most abundant proteins. This abstract presents an alternative bioinformatic approach which overcomes this by calculating protein quantities from product ion data. Protein quantities calculated with this method are more in line with the widely accepted label free data independent acquisition quantities calculated from precursor ion data. It is thus shown that ion mobility enhances proteome coverage, and with an adapted data processing method, it can be reliably used for quantitation as part of a biomarker discovery pathway.
Keywords: Plasma, Ion mobility, Absolute quantitation

YI02-03 Quantifying the Dynamics of a 14-3-3 Protein Interaction Network by Affinity Purification and SWATH Mass Spectrometry

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Protein complexes and protein interaction networks are essential mediators of most biological functions. Complexes supporting transient functions such as signal transduction processes are frequently subject to dynamic remodeling. Currently, the majority of studies into the composition of protein complexes are carried out by affinity purification and mass spectrometry and present a static view of the system. To move toward a better understanding of inherently dynamic biological processes, methods which can reliably quantify temporal changes of protein interaction networks are essential. In this study we determined the capability of affinity purification combined with SWATH mass spectrometry (AP-SWATH) to quantify the reorganization of protein-protein interactions in time resolved perturbation experiments. We chose to study the dynamics of the 14-3-3β scaffold protein interactome after stimulation of the insulin/PI3K/AKT pathway. The analysis, performed using the open source software OpenSWATH, provided a complete quantitative data matrix highlighting patterns of dynamic regulation in 567 14-3-3β interacting proteins with respect to IGF1 stimulation. Quantitative changes in 14-3-3β interacting proteins clustered in to 5 distinct time profiles, 2 of which were strongly related to the activity of basophilic kinases such as AKT. To our knowledge this study represents the largest reported interactome for a single bait indicating that at least 2.8 % of the proteome is engaged by 14-3-3β containing scaffold dimers, and that a substantial portion of these are regulated after IGF1 stimulation. We therefore establish AP-SWATH as a tool to sensitively quantify dynamic changes in protein complexes and interaction networks in perturbed systems.
Keywords: quantitative interaction proteomics, data independent analysis, systems biology

YI02-04 Investigation of Time Dependent Competitive Protein Adsorption to Surfaces Using Mass Spectrometry

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Introduction
Surfaces in a complex protein solution will adsorb proteins. This event is fast and dynamic and triggers a biological response against the inserted/implanted biomaterial that eventually will lead to biofouling and encapsulation. This affects the properties of the inserted devices, such as hampered membrane functions of microdialysis (MD) probes or distortion in response of biosensors.
Methods
Untreated and coated filtration membranes were used as adsorption templates for human ventricular cerebrospinal fluid (vCSF). After adsorption in an incubation chamber, the membranes were washed, dried and the proteins were reduced, alkylated and digested. The sample preparation procedure was conducted according to an on-surface enzymatic digestion (oSED) protocol previously described by our group. The oSED digests were analyzed by nanoLC ESI-MS/MS using a 7T hybrid LTQ FT and Velos pro orbitrap mass spectrometer.
Preliminary Data
In this study, we present a time resolved map of protein adsorption. Non-coated and tri-block polymer coated, polycarbonate membranes was used as templates. As expected, a time and surface property dependent protein adsorption relationship was observed. It is not surprising that the degree of protein binding onto modified and non-modified surfaces was dependent on the properties of the protein as well as the properties of the surface. The process of biofouling for in vivo inserted materials can be postponed and thereby increasing the lifetime and use of e.g. microdialysis probes for patient monitoring. The preliminary data are very promising making it possible to identify a spectra of adsorbed proteins on different surfaces in a time dependent way.
Keywords: Competitive, Surface, Adsorption

YI02-05 Establishment and Application of a High-Quality Comparative Analysis Strategy of Low-Abundance Biomarker Peptide in Serum Based on Optimized Novel Peptide Extraction Method

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Low-abundance native peptides are an attractive target for the discovery of disease biomarkers. However, validating candidate peptides is difficult due to challenges associated with precise peptide identification and development of high-throughput assays using specific antibodies. Therefore, a highly reproducible and sensitive strategy based on effective peptide enrichment methods is needed to identify clinically useful biomarkers. We optimized our novel differential solubilization (DS) method [1] to selectively enrich peptides less than 6,000 Da, using tricine-SDS-PAGE to evaluate the optimization. The modified DS method was combined with LC-MS using conventional HPLC. The reproducibility and sensitivity of the proposed strategy were sufficient to enable discovery of low-abundance (ng/mL range) candidate biomarker peptides. A total of 40 serum samples collected pre- and post-surgery from renal cell carcinoma (RCC) patients were analyzed, resulting in discovery of 2 peptides that are upregulated and one peptide that is downregulated in pre-surgery RCC patients. These peptides were validated using 40 serum samples collected pre- and post-surgery from bladder tumor (BT) patients. Two candidate peptides that were upregulated in pre-surgery RCC patients were not upregulated in the sera of the pre-surgery BT patients. Finally, we propose 2 candidate marker peptides that could be used to detect RCC.

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Keywords: biomarker, peptide, serum

YI02-06 Quantitative Proteomic Approach to Identify Proteins Signalling Pathways in Human Cerebral Microvascular Endothelial Cells Modified with 17β-Estradiol

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Oestrogen has been shown to facilitate multi actions in regulating downstream cellular signals such as transcription, proliferation, and differentiation processes. However, the complete molecular mechanism involved in oestrogen modulation in human cerebral microvascular endothelial cells (HCMEC) is still unclear. Identification and quantification of proteomic changes will allow targeted research into protein networks associated with cerebrovascular disease related to oestrogen deficiency. In this study, interaction between 17β-estradiol and oestrogen receptors was investigated by incubating HCMEC for 24 hours with 17β-estradiol followed by Proximity Ligation Assay (PLA). Interactions were recognized as red dot formations on the cells. In order to systematically profile the changes in protein expression, the cells were fractionated into three different components; 1) membrane proteins 2) cytoplasm proteins, and 3) nuclear proteins respectively. The protein extracts were tagged using iTRAQ labeling and analyzed by LC ESI MS/MS. A total of 2350 unique proteins were identified and the expressions of 317 proteins were significantly altered ($p<0.05$) following treatment with 17β-estradiol. Ingenuity pathway analysis was performed for the regulated proteins and the signalling events directed to *Eukaryotic Initiation Factor 2 (eIF2)* signalling was identified as the major pathway. In addition, 2 other pathways were identified in membrane and cytoplasm components, i) *hypoxia signalling* and ii) *glycolysis signalling*. In nuclear fraction, i) *granzyme signalling* and ii) *Eukaryotic Initiation Factor 4 (eIF4) & p70S6K signalling* are highly stimulated. Thus, a combination of high throughput technique together with bioinformatics analysis is valuable in providing insights of molecular signalling events of 17β-oestradiol modulations in HCMEC.

Keywords: oestrogen, protein signalling pathways, protein interactions

YI02-07 Defining the Structure of Mitotic Chromosomes Using Multi-Classifier Combinatorial Proteomics Together with DT40 Genetics

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Packaging of DNA into condensed chromosomes during mitosis is essential for the faithful segregation of the genome into daughter nuclei. Although studied for over 100 years, mitotic chromosome structure and composition is yet to be fully elucidated. A novel approach called multi-classifier combinatorial proteomics (MCCP) has revealed the protein composition of mitotic chromosomes purified from chicken DT40 cells. One of the main advantages of MCCP is that it can be combined with SILAC to quantitatively compare chromosomal proteomes from different genetic knock-out cell lines. Here, we apply this method to compare chromosomal proteomes in the presence or absence of individual SMC complexes; key components that have been shown to play a crucial role in mitotic chromosome structure. While SMC1/3 and SMC2/4 form part of Cohesin and Condensin, respectively, the SMC5/6 complex is thought to be involved in DNA repair. Mitotic chromosomes were isolated from conditional genetic knockouts of Scc1, SMC2 or SMC5 cultured under SILAC conditions. Using quantitative Mass Spectrometry we were then able to rank >3,000 proteins based on whether their chromosomal association was dependent on individual SMC complex components. MCCP with using machine learning, Random Forest, will enable the first attempt at mapping the entire proteome regulation networks associated with SMC complexes, which in turn will explain how these complexes are coordinating chromosome structure.

Keywords: mitosis, chromosome, SMC

YI02-08 Targeted Analysis of *Salmonella* Effector Proteins Using Multiple Reaction Monitoring

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Salmonella enterica is a versatile pathogen capable of infecting diverse hosts and causing different diseases. In humans, *S. enterica* strains can cause typhoid or gastroenteritis, resulting in much morbidity and mortality throughout the world. Upon encountering a human cell, *Salmonella* delivers a choreographed series of virulence factors (also known as 'effector' proteins) into the cells. These effector proteins initially enable the bacteria to enter the host cell and then to target various natural processes within that host cell. To develop a clearer picture of the global effects of *Salmonella* on host cell processes, multiple reaction monitoring (MRM) assays for all known and potential new *Salmonella* effectors have been constructed by synthesizing at least two unique peptides per protein. Transitions were optimized by characterizing the synthesized peptides on a triple quadrupole mass spectrometer mainly focusing on optimizing collision energies. The three most intense fragment ions for each peptide were selected and used as transitions to detect the peptides in biological samples. A baseline level of effector proteins was established by analyzing *Salmonella* bacteria grown under effector secreting conditions. Finally, effector protein dynamics were investigated by analyzing *Salmonella* infected HeLa cells. This data shows how *Salmonella* takes control of its host cell by secreting specialized proteins at specific time points.

Keywords: Multiple Reaction Monitoring, Salmonella, Quantitative proteomics

YI03-01 Differentially Glycosylated Circulating Protein Biomarker Discovery for Barretts Esophagus and Esophageal Adenocarcinoma

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Esophageal adenocarcinoma (EAC) arises from precursor metaplastic condition Barrett's Esophagus (BE). BE patients are frequently screened using endoscopy-biopsy for early neoplastic changes. However, being an asymptomatic condition, it is very difficult to identify BE patients and to recruit them for screening. Also endoscopy is not suitable for large scale population screening due to high cost and patient non-compliance. Taken together, majority of EAC cases are diagnosed very late during pathogenesis and showed high mortality.
To facilitate early diagnosis, we focused on alterations in circulatory protein glycosylation, using a panel of 20 lectins to isolate different glycan structures on serum glycoproteins. Serum samples from control (n=9), BE (n=10) and EAC (n=10) patient groups were analyzed by lectin magnetic bead array-coupled mass spectrometry [1]. Customized database ""GlycoSelect"" was developed which incorporates outlier detection and sparse Partial Least Squares regression Discriminant Analysis[2]. We identified a ranked list of candidate glycobiomarkers that distinguish a) EAC from BE and b) BE from control group. In general, glycoproteins bound several lectins, reflecting heterogeneity and multiplicity of glycosylation. Specific glycan structure changes were observed as loss and gain of binding to a single lectin while maintaining binding to other lectins. Future work will validate the candidate protein-lectin pairs using a customized lectin-affinity array-coupled with quantitative mass spectrometry using independent cohort of 100+ patients. The specificity and sensitivity of panels of glycobiomarkers will be determined to develop a serum screening test for BE/EAC.

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[2] Lê Cao et al., BMC Bioinformatics 12, 253-268 (2011)

Keywords: Esophageal Adenocarcinoma (EAC), Barrett's Esophagus (BE), Biomarker

YI03-02 A Novel Titanium Dioxide Plate (TiO_2 Plate) for Phosphopeptide Enrichment and On-Target MALDI-TOF Analysis

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Protein phosphorylation is a major protein post-translational modification that regulates many cellular processes and activities. Because mass spectrometer (MS) signals of low abundance of phosphorylated peptides are commonly suppressed by the presence of abundant non-phosphorylated peptides, one of the major challenges in the detection of phosphopepdies is the enrichment of low-abundant phosphopeptides from complex peptide mixtures. Titanium dioxide (TiO_2) has been proven to be a highly efficient approach and is widely applied for phopshopeptide enrichment. In this study, a novel TiO_2 plate was proposed by coating TiO_2 particles onto MALDI plates, glass or plastic substrates with a simple and rapid approach. The TiO_2 plate can be used for on-target MALDI-TOF analysis or as a purification plate, on which phosphopeptides were eluted out and subjected to MALDI-TOF or nanoLC-MS/MS analysis. The detection limit of the TiO_2 plate is 10 folds lower than TiO_2-packed tips approach. The capacity of the 2.5 mm TiO_2 spot was estimated to be about 10 μg of β-casein. With TiO_2 plate enrichment of SCC4 cell lysate digests and nanoLC-MS/MS analysis, 82% of the detected proteins were phosphorylated, illustrating the TiO_2 plate is practical and effective to enrichment phosphopeptides from complex samples.

Keywords: phosphorylation, on-plate, MALDI-TOF

YI03-03 Site-Specific N-linked Glycosylation Analysis by Nano-LC Tandem Mass Spectrometry Coupled with a Spectral Library Searching Approach

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Protein glycosylation is one of the most common forms of post-translational modification in eukaryotic proteins, playing critical roles in protein structures and functions. The most common approach of protein glycosylation analysis by mass spectrometry involves chemical or enzymatic release of the glycans from glycoproteins, followed by LC-MS analyses of the glycans and the deglycosylated peptides separately. However, the glycan heterogeneity on each glycosylation site is lost in this approach. In our work, protein N-linked glycosylation is characterized at the glycopeptide level to reveal the microheterogeneity on each glycosylation site. Glycopeptides were enriched by hydrophilic affinity interaction using cellulose microcrystalline, analyzed by nano-LC/MS, and fragmented by collision-induced dissociation in data-dependent acquisition mode. For automated identification, the resulting spectra are searched against a library of reference spectra of *N*-linked glycopeptide predicted by MassAnalyzer [Zhang et al, *Anal. Chem.* **2010,** *82*, 10194-10202], using SpectraST [Lam et al, *Nat. Methods* **2008**, *5*, 873-875]. Both the m/z and the intensity information of the possible fragmentation ions are taken into consideration in our search algorithm to achieve a more reliable glycopeptide identification. We validated our method using purified glycoprotein standards, and evaluated its potential to be used in automated profiling of complex samples.

Keywords: site-specific N-glycosylation analysis, automatic glycopeptide identification and profiling, spectral library searching

YI03-04 Proteome-Wide Identification of Poly (ADP-Ribosyl)ation Targets in Different Genotoxic Stress Responses

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Poly(ADP-ribos)ylation (PARylation) is a reversible post-translational protein modification found in higher eukaryotes synthesized by the catalytic activity of poly(ADP-ribosyl)transferases (ARTDs/PARPs). PARylation is involved in various cellular processes such as DNA damage response, transcription, energy metabolism and cell death. Supporting its role in DNA repair, specific inhibition of ADP-ribosyltransferase activity has recently been shown to constitute an effective target in treatment of several types of cancer. However, despite this recent scientific progress only little is known about the actual acceptor proteins of PARylation and how the modification regulates the functional role of these target proteins in mammalian cells. We performed a sensitive proteomics approach based upon quantitative mass spectrometry (SILAC) for macrodomain-based enrichment and identification of PARylated proteins that become covalently modified under different conditions of genotoxic stress. Our screen identified novel candidates not previously reported to be targets of PARylation, while confirming the majority of known PARylated proteins. Biochemical *in vitro* and *in vivo* validation of novel acceptor proteins confirmed that our methodology targets covalently PARylated proteins. Nuclear proteins encompassing nucleic binding properties were most prominently found to be PARylated upon genotoxic stress, in agreement with the nuclear functions ascribed to ARTD1/PARP1 and ARTD2/PARP2. Distinct differences in proteins becoming PARylated upon various genotoxic insults were observed. Most significantly, proteins involved in RNA metabolism are PARylated upon oxidative and alkylation induced stress, demonstrating that post-transcriptional processes are readily controlled through specific genotoxic stress-induced PARylation.

Keywords: poly (ADP-ribos) ylation, PARylation, genotoxic stress

YI03-05 Synthesis of Galactose-Deficient IgA1 O-glycans by GalNAc-transferases: Implications for the Pathogenesis of IgA Nephropathy

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IgA1 with galactose (Gal)-deficient hinge-region (HR) *O*-glycans (Gd-IgA1) plays a key role in the pathogenesis of IgA nephropathy (IgAN). IgA1 HR has up to 6 of the 9 potential *O*-glycosylation sites occupied; some Gal-deficient glycans consist of terminal *N*-acetylgalactosamine (GalNAc). IgA1-producing cells derived from IgAN patients secrete more IgA1 with Gal-deficient *O*-glycans and higher content of GalNAc compared to IgA1 from cells of healthy controls (HC). IgA1 *O*-glycosylation is thought to be initiated by GalNAc-T2, but the expression of GalNAc-T2 does not differ between the cells from IgAN patients and HC. In contrast, expression of GalNAc-T14, a GalNAc-T with high structural similarity to GalNAc-T2, is elevated in the cells from patients. To determine potential contribution of these enzymes to Gd-IgA1 formation, we analyzed kinetics and site-specificities of GalNAc-T2 and -T14 for IgA1 HR using high-resolution mass spectrometry. A synthetic IgA1 HR peptide (sHR) and a panel of synthetic IgA1 HR glycopeptides (sGP) with a single GalNAc residue at different sites were used as acceptors. GalNAc-T2 had higher activity, *i.e.*, faster rate of glycosylation of sHR, than did GalNAc-T14. The sites of glycosylation in sHR catalyzed by GalNAc-T2 and -T14 were the same for the variants with up to 5 sites and appeared in a predominantly ordered fashion. Localization of GalNAc on sGP did not affect the kinetics of GalNAc-T2, but GalNAc-T14 more effectively glycosylated the sGP variant with a GalNAc at S9. In summary, GalNAc-T2 and -T14 have similar site-specificity for IgA1 HR, but differ in kinetics and in how their activity is affected by preexisting glycosylation. We speculate that the elevated expression of GalNAc-T14 could contribute to the production of Gd-IgA1 in IgAN.

Keywords: IgA nephropathy, IgA1 glycosylation, O-glycopeptides

YI03-06 A SILAC-based Approach Defines an Angiotensin II- Regulated Proteome in Primary Human Kidney Cells

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Angiotensin II (AngII), the major effector of the renin-angiotensin system, mediates kidney disease progression by signalling through AT-1 receptor (AT-1R), but there are no specific measures of renal AngII activity. Accordingly, we sought to define an AngII-regulated proteome in primary human proximal tubular cells (PTEC) in order to identify potential markers of AngII activity in the kidney. We utilized stable isotope labelling with amino acids (SILAC) in PTECs to compare proteomes of AngII-treated and control cells. Of 4618 quantified proteins, 83 were differentially regulated in 4 replicates. SILAC ratios for 18 candidates were confirmed by Selected Reaction Monitoring (SRM) assays. Both SILAC and SRM revealed heme oxygenase-1 (HO-1) as the most significantly upregulated protein in response to AngII stimulation. AngII-dependent regulation of HO-1 gene and protein was further confirmed by qRT-PCR and ELISA in PTECs. In order to extend these *in vitro* observations, we overlaid a network of significantly enriched gene ontology (GO) terms from our AngII-regulated proteins with a dataset of differentially expressed kidney genes from AngII-treated wild type mice and AT-1R knock-out mice. Five GO terms were enriched in both datasets and included HO-1. Furthermore, HO-1 kidney expression and urinary excretion were reduced in AngII-treated mice with PTEC-specific AT-1R deletion compared to AngII-treated wild-type mice, thus confirming AT-1R-mediated regulation of HO-1. In summary, our *in vitro* approach identified novel molecular markers of AngII activity and the animal studies demonstrated that these markers are relevant *in vivo*. These interesting proteins hold promise as specific markers of renal AngII activity in patients and in experimental models.

Keywords: renin angiotensin system, proximal tubular kidney cells, SILAC

YI03-07 An Improved Protocol for the Enrichment of Plasma Membrane Proteins Allows the Identification of Accessible Antibody Targets on Trastuzumab-Resistant Breast Cancer Cells

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Characterization of the cell surface proteome is of fundamental importance for the development of antibody-based therapies, as well as for the understanding of how cells interact with their environment. Biotinylation of cell surface proteins is one of the most frequently used procedures for their mass spectrometry-based characterization. Here, we present a study investigating the enrichment of membrane proteins of SK-BR-3 breast cancer cells by surface biotinylation performed alone or in combination with either ultracentrifugation or detergent-based fractionation. SK-BR-3 cells are a model system for HER2-positive breast cancer, clinically characterized by high metastatic potential and dismal prognosis. The anti-HER2 antibody Trastuzumab is frequently used for the treatment of these patients, but its clinical efficacy is limited by the emergence of resistance. To understand the changes in cell surface proteome, we have developed an SK-BR-3 cell line resistant to Trastuzumab-mediated growth inhibition and we have studied proteome changes by cell surface biotinylation and detergent-based fractionation. Thirteen membrane-associated proteins were found to be regulated between resistant and parental cell lines. Human monoclonal antibodies against most promising antigens, isolated from phage display libraries and conjugated to a highly potent cytotoxic agent, mediated cancer cell killing *in vitro*. In summary, the procedure described in this article allowed an efficient characterization of the cell surface proteome of closely related SK-BR-3 cell lines and led to the identification of three tumor-associated antigens which could be considered for the development of anti-breast cancer antibody products.

Keywords: cell-surface biotinylation, Trastuzumab resistance, antibody drug conjugates

YI03-08 Mass Isotopomer Analysis of Metabolically Labeled Nucleotide Sugars and N- and O-glycans for Tracing Nucleotide Sugar Metabolisms

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Nucleotide sugars are the donor substrates of various glycosyltransferases, and an important building block in N- and O-glycan biosynthesis. Their intercellular concentrations are regulated by cellular metabolic states including diseases such as cancer and diabetes. In order to investigate the fate of UDP-GlcNAc, we developed a tracing method for UDP-GlcNAc synthesis and utilization, and GlcNAc utilization using $^{13}C_6$-glucose and $^{13}C_2$-glucosamine, respectively, followed by the analysis of mass isotopomers using liquid chromatography-mass spectrometry.

Metabolic labeling of cultured cells with $^{13}C_6$-glucose and the analysis of isotopomers of UDP-HexNAc (UDP-GlcNAc plus UDP-GalNAc) and CMP-NeuAc revealed the relative contributions of metabolic pathways leading to UDP-GlcNAc synthesis and utilization. In pancreatic insulinoma cells, the labeling efficiency of a $^{13}C_6$-glucose motif in CMP-NeuAc was lower compared with that in hepatoma cells.

Using $^{13}C_2$-glucosamine, the diversity of the labeling efficiency was observed in each sugar residue of N- and O-glycans on the basis of isotopomer analysis. In the insulinoma cells, the low labeling efficiencies were found for sialic acids as well as tri- and tetra-sialo N-glycans, whereas asialo N-glycans were found to be abundant. Essentially no significant difference in secreted hyaluronic acids was found among hepatoma and insulinoma cell lines. This indicates that metabolic flows are responsible for the low sialylation in the insulinoma cells. Our strategy would be useful for systematically tracing each stage of cellular GlcNAc metabolism.

Keywords: mass spectrometry, glycosylation, glucose metabolism

CL-01 Translation of Clinical Proteomics: Opportunities and Challenges

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Most human diseases, such as cancer, are often diagnosed either in their late stages when the chance of cure is relatively low or in the form of diseases that might not have to be treated. What we need is to be able to detect lethal diseases in their early stages. Proteomic biomarkers offer the best opportunity for making significant impacts in the flights against lethal diseases. During the last decade of proteomic research, significant progress has been made in the advancement of new technologies and the discovery of potential biomarkers. However, limited successes have been shown in the translation of proteomic discovery into clinical practice. I believe that the time has come for us to focus on the translation of clinical proteomics.

In my presentation, I will discuss the opportunities and challenges for biomarker discovery, validation and translation. Case studies will be presented. To be successful, we need to develop a roadmap and identify several key steps that are critical in this process. I will discuss the 4Bs, the 4Gs and 4Ps for proteomics translation. (1) To define clearly a specific "intended use" for unmet clinical needs, (2) to generate sufficient evidence in preliminary studies to support the investment for a large-scale validation study, (3) to select and develop assays with analytical performance suitable for clinical laboratory and (4) to conduct clinical trial to demonstrate clinical utilities in order to obtain regulatory approval and gain acceptance by the clinical community. The successful translation of clinical proteomics into clinical practice will require close collaboration between researcher, industry, regulator and clinician/ clinical laboratory.

CL-02 Clinical Proteomics for Microbiology

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Until recently, bacterial identification in clinical laboratories has relied on conventional and time-consuming phenotyping. In the last few years, MALDI TOF MS has been widely applied as an identification procedure because of its diagnostic and economical benefits.
Judging from the final program of the MSACL (The Association for Mass Spectrometry: Applications to the Clinical Lab) 2013 (San Diego), two commercial systems including commercial databases are available; the Bruker Biotyper (Bruker-Daltonics) and the VITEK MS (bioMerieux). Generated unique spectra of intact cells are compared with previously collected fingerprint libraries that are commercially available. Reports from around the world have indicated genus-level identifications of 97%-99% and species level identifications of 85%-97% when testing routinely isolated bacteria and yeast using Bruker Biotyper MALDI-TOF MS.
MALDI-TOF MS identification of bacterial at species level remains unsatisfactory. One of the reasons is an incomplete database that still needs refinement and expansion. Augmentation of the commercial database by incorporating mass spectra obtained in-house from clinical isolates may increase the identification rate. Recent studies have shown that this technology can be applied to accurately identify filamentous fungi and *Mycobacterium* species providing that effective sample preparation methods are established for these microorganisms.
A rapid identification of microorganisms growing in blood culture will have a great impact on the management of bloodstream infections. In terms of detection of antibiotic resistance, MALDI-TOF MS may be a promising tool, but this technology is not mature enough to provide a whole picture of complex process of antibiotic resistance.
Keyword: Clinical Microbiology

CL-03 Proteomic Investigations of Heart and Lung Diseases

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Our research aims to understand health and disease at the molecular level. Meeting these goals requires new energy and insight, close interactions among chemists, biologists and clinicians, and the continuous evolution of technologies and tools for data handling and interpretation. Dynamic post-translational modifications of proteins, *e.g.*, glycosylation, phosphorylation, acylation, oxidative modifications, and their specific position(s), site occupancy, co-occurrence and kinetics, affect the properties of proteins and whole cells, their interactions, transport, activity, and lifetimes. Mass Spectrometry-based approaches that drive novel, emerging capabilities are essential for investigation of the healthy state and aberrations. This lecture will focus on strategies developed to facilitate investigations of metabolic causes underlying cardiovascular disease and changes that occur during development of pulmonary arterial hypertension. These examples, chosen from projects now underway in our laboratories, will illustrate promising approaches, interesting results and remaining challenges.
Acknowledgements: This research is supported by NIH/GM P41 GM104603,NIH/NHLBI HHSN268201000031C, S10 RR020946, S10 RR025082 and S10 OD010724.

CL-04 Challenges and Opportunities in Biomarker Discovery by Comparative Proteomic Analysis of Blood Circulating Proteins - Biological and Statistical Concerns

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Over the past 10 years, large amount of global efforts have been made to discover serum/plasma protein biomarkers by comparative proteomic analysis of blood circulating proteins. The most commonly used approach was the single-center case-control design. In case-control proteomic studies, quantitative profiles of serum/plasma proteins were first obtained in an untargeted manner, and then compared to identify the differences as individual potential biomarkers or a combination of differential features as diagnostic/prognostic disease-associated fingerprints. In spite of advantages of case-control design such as time-efficiency and cost-effectiveness, there are many pitfalls. Surface-enhanced laser desorption/ionization (SELDI) TOF mass spectrometry (MS) (or called ProteinChip SELDI technology) is the first high-throughput technology that allows comparison of plasma/serum proteome contents in a large number of subject samples within a short period of time. Using this technology, numerous case-control studies found serum/plasma proteomic fingerprints with over 90% accuracy in the diagnosis or prognosis of various diseases. However, criticisms and hesitations on this approach have been appearing all over the world. After accumulating more research experiences, researchers now have better understandings of characteristics and limitations of applying comparative proteomic analysis of blood circulating proteins to biomarker discovery. By using our MS-based biomarker discovery studies as examples, opportunities as well as biological and statistical concerns on applications of case-control comparative proteomic analysis to biomarker discovery will be discussed in this lecture. With rapid advancement of MS technologies and proper clinical study designs, discoveries of clinically useful biomarkers should be forthcoming.

CL-05 How Useful is Proteomics in the Clinic? A Case Study of Breast Cancer

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Breast cancer is a complex and heterogeneous disease that is usually characterized by histological parameters such as tumour size, cellular (re-)arrangements, necrosis, nuclear grade and the mitotic index leading to a set of around twenty subtypes. Together with clinical markers such as hormone receptor status, this classification has considerable prognostic value but there is a large variation in patient response to therapy. Gene expression profiling has provided molecular profiles characteristic for distinct subtypes of breast cancer that reflect the divergent cellular origins and degree of progression. Here we present a large-scale proteomic profiling study of 483 sporadic and hereditary breast cancer tumours with matching mRNA expression analysis. The subgroups formed upon unsupervised clustering agree very well with groups found on transcriptional level however the classifiers (genes or their respective protein products) differ almost entirely between the two data sets. We have also carried out an in-depth quantitative proteomic survey of five breast cancer cell lines as well as two breast derived cell lines representing fibroblasts and adipose cells. We show a poor correlation between transcriptomics and proteomics data as well as a low degree of similarity between the proteomes on samples obtained *in vivo* and *in vitro*.
The protein data can be transferred into a rapid highly multiplexed assay that is easily implemented in standard clinical chemistry practice, allowing a rapid and cheap characterisation of tumour tissue suitable for directing choice of treatment. We have studied the response to both chemo- and radiotherapy treatments. The response to DNA damage by alkylation and DNA topoisomerase inhibition was studied in two breast cancer cells lines as was the effect of ionising radiation. We present data from both a shotgun and a targeted, pathway-centric approach to highlight the different DNA repair pathway modulation in the cell lines and the correlation with viability and DNA damage assays. This type of focussed profiling may be of utility in rapidly defining non-responders undergoing systemic neoadjuvant therapy. These assays, together with the molecular classifiers and hormone receptor readouts are now being established into the clinic in a first evaluation phase.

CL-06 Mass Spectrometric Molecular Phenotyping of Tissues and Bodyfluids

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Systems biology is focused on the study of dynamic networks of interacting molecules and places such networks between genotype and phenotype. It is assumed that a specific genome encodes the molecules that constitute such a network and that the network is modulated by perturbing effects such as environmental factors. The properties emerging from the network as a whole determine observable phenotypes. Many of the molecular networks of the cell consist of or involve proteins. Therefore, the precise determination of the acute state of protein networks is highly informative as an acute phenotypic readout.

Mass spectrometry based proteomics is a central life science technology that has realized great progress towards the identification, quantification and characterization of the proteins that constitute a proteome. In this presentation we will discuss how mass spectrometry based proteomics has been applied to network biology to identify the nodes and edges of biological networks, to detect and quantify disease related network changes and to correlate dynamic network rewiring with a disease phenotype. We will also discuss future directions for mass spectrometry based proteomics within the network biology paradigm and their significance for the study of networks perturbed in human disease.

ED-01 Integrating Proteomics, Transcriptomics and miRNAs for Biomarker Discovery

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In recent years, Omics approaches have been in the frontier of biomarker discovery, although the clinical outcome of these efforts is yet to be fully realized. One of the major challenges in cancer biomarker discovery has been to successfully translate potential candidates from the discovery phase to clinical validation, on account of large heterogeneity that exists among individual tumor cases. Therefore generating robust discovery panels that carry analytical rigour as well as relate in the biological and regulatory context would be important for translation to the clinic. Integration of multiomics data and deciphering their relationships and the key pathways will not only enhance our understanding of the tumor but is also crucial for improved outcomes. However, despite advances in analytical platforms, there are still limitations to achieve linear correlation between transcripts and proteins, in sufficient numbers. Although in a limited way, we have attempted integration of altered miRNAs and their mRNA and protein targets for Glioblastoma multiforme (GBM), using transcriptomics study carried out by the Cancer Genome Atlas (TCGA) group and differential proteomics data generated from our lab. Transcriptomics analysis by TCGA group has revealed a large number of altered miRNAs associated with these tumors. When we examined the presence of predicted targets of these miRNAs in the mRNA (TCGA) and protein (our lab) datasets, we observed interesting correlations consistent with vertical regulatory linkage. Extension of this to multiple miRNAs would generate large portfolio of the target molecules with a second horizontal linkage in terms of their biological function and pathways. Such 2 Dimensional molecular maps – with a. regulatory linkage in one dimension and b. biological/functional linkage in the second dimension, would form strong panels to be integrated into clinical experimental designs. They also offer the plausibility of developing clinical assay methods at three different levels of gene expression - regulatory miRNA or target mRNA or protein, two of these are also accessible as circulatory molecules in body fluids.

ED-02 Construction and Analysis of Protein-Protein Interaction Networks: A Tutorial

Marc Wilkins

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Most proteins in the cell interact with other proteins to deliver their function. To define these interactions, large-scale studies of protein-protein interactions have been recently undertaken using two-hybrid techniques or the affinity purification of complexes followed by mass spectrometry-based protein identification. These approaches have been applied to a number of species; the best studied species is *Saccharomyces cerevisiae* (baker's yeast), however bacterial and mammalian species have also been analysed. Interaction networks can be built using software tools and interaction data. These networks can be co-analysed with other data types, for example protein expression or functional data. This can provide numerous novel insights into the function of the cell. This tutorial will introduce protein-protein interactions, relevant databases, and discuss case studies to illustrate the construction and analysis of protein-protein interaction networks.

ED-03 Adventures in Personal Genomics and Whole Omics Profiling

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Personalized medicine is expected to benefit from the combination of genomic information with the global monitoring of molecular components and physiological states. To ascertain whether this can be achieved, we determined the whole genome sequence of an individual at high accuracy and performed an integrated Personal Omics Profiling (iPOP) analysis, combining genomic, transcriptomic, proteomic, metabolomic, DNA methylomic, and autoantibodyomic information, over a 38-month period that included healthy and six virally infected states. Our iPOP analysis of blood components revealed extensive, dynamic and broad changes in diverse molecular components and biological pathways across healthy and disease conditions. Importantly, genomic information was also used to estimate medical risks, including Type 2 Diabetes, whose onset was observed during the course of our study. Our study demonstrates that longitudinal personal omics profiling can relate genomic information to global functional omics activity for physiological and medical interpretation of healthy and disease states.

ED-04 Significance of Secretome Analyses

Richard J. Simpson

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The identification of secreted proteins (secretome) in the tumour microenvironment and a detailed knowledge of their interplay with tumour cells and stroma are of critical importance for improving our understanding of fundamental tumour biology. Such an understanding of secreted '*cancer signatures*' will greatly enhance prospects of improved early diagnostic biomarkers and therapeutics. Although the term 'secretome' was introduced to define proteins released from cells grown in culture, the composition of this sub-proteome should be extended to include not only classically-secreted proteins (i.e., endoplasmic reticulum and Golgi-dependent) and non-classically secreted proteins, but also proteins released through secretion of membranous vesicles (Extracellular Vesicles, EVs) such as shed microvesicles (SMVs) and exosomes. In this lecture I will focus on methods for preparing both secreted proteins from cell lines and tumour interstitial fluid, and released EVs, discuss associated technical challenges, and also update recent efforts at delineating cancer cell associated secretomes. Finally, I will appraise recent outcomes from cancer secretome studies, particularly the exciting advances within the exosome proteomics field which highlight their pivotal role in preparing metastatic niche formation.

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ED-05 Proteomic Strategy for Development of Clinical Approach

Siqi Liu

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Protein biomarkers represent an enormous advance to our understanding of the clinical diagnosis and treatment to disease. With a great achievement in molecular pathology, we realize that most diseases are not directly resulted from or indicated as a single element, but are closely related with multiple factors, genes, proteins or metabolites. Considerable effort therefore has been expended to characterize the disease genomes, proteomes and metablomes, especially for detection of the biomarkers at early stage. Similar to the traditional approach of clinical biochemistry, the new generation of clinical methods based upon proteomics are still focused on the measurement to the samples derived from body fluids, particularly serum. The number of disease biomarkers measured by the updated techniques, however, is significantly enlarged. As regards the protein candidates in serum, the potential biomarkers are generally divided into two sets, serum proteins and autoantibodies, while in technical consideration, the updated methods are largely antibody- and mass spectrometry-based. With careful selection of immuno-signals, array-ELISA and protein chip have become the feasible approaches in clinical practices. On the basis of large screening of proteomic analysis and the resolution improvement in mass spectrometry, the approach of target proteomics upon multiple reaction monitoring (MRM) has emerged as a powerful means in clinical application.

ED-06 A New Genome-Wide Proteome Project for the Future Biomedical Sciences

Young-Ki Paik

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Proteomics is well positioned to address the gap between the genome and phenome, and complement data from the reference human genome sequence and gene expression studies. HUPO has recently initiated a new genome-wide proteome project termed "Chromosome-Centric Human Proteome Project (C-HPP) (1). The initial goal of the C-HPP is to identify at least one representative protein encoded by each of the approximately 20,300 human genes and match it with its tissue localization and major isoforms including post-translational modification (PTM) based on quantitative mass spectrometry complemented with antibody reagents (1). Throughout this 10-year project (2012-2022), C-HPP will generate information useful for the search for new diagnostic biomarkers and drug targets and also study disease gene families clustered in each chromosome (2). Human genome studies (e.g, ENCODE), as well as transcriptome sequencing provides a basis for identification of protein isoforms generated by alternative splicing transcripts (AST) and by nsSNP, creating transformative advances for use in the proteomics community (3). Likely results of the C-HPP are: i) integrated transcriptomics/proteomic measurements, ii) a paradigm shift from individual laboratories to international research alliances, iii) the development of informatics systems and associated interfaces, and iv) powerful new MS for applications (e.g., intact protein variant analysis for biomarker discovery) (1, 3). Currently more than 25 countries participate in this C-HPP initiative by taking each chromosome and set the guidelines for data collection, collaboration and operation of the consortium (2). Special issue on the C-HPP has been published in 2013, setting a major milestone of this global project (4). We believe this new initiative will provide not only a new paradigm of education in integrated omics field but also fresh view on the genome-wide protein resources for biomedical societies in the future. I will present the ongoing exploration of the biological resources of the C-HPP for study of preeclampsia disease.

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HA-A-01 Exposing the Proteome in Full Glory Through Advances in Enabling Technologies

Albert J. R. Heck

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Over the past decades we have learned that the proteome is extremely multifaceted due to splicing and protein modifications, further amplified by the interconnectivity of proteins into complexes and signaling networks that are highly divergent in time and space. Proteome analysis heavily relies on a broad mixture of analytical techniques, starting from sample preparation, to separation and enrichment and last but certainly not in the least mass spectrometry. Through developments in these enabling technologies MS-based proteomics has matured and start to deliver biological relevant information. Here I will portray this emerging next-generation of proteomics describing recent applications; highlighting especially some of the enabling technologies our laboratory has contributed in e.g. phosphoproteomics, quantitative proteomics and analysis of intact proteins and protein complexes.

JHA-A-01 Development and Application of Analytical Technologies for Proteomics and Phosphoproteomics

Yasushi Ishihama

Graduate School of Pharmaceutical Sciences, Kyoto University, Japan

Rapid progress has been made in identifying cellular phosphoproteins by shotgun phosphoproteomics using nanoLC-MS combined with phosphopeptide enrichment techniques. Because samples for shotgun proteomics exhibit extremely high complexity with a wide dynamic range of concentration, fractionation approaches before/after phosphopeptide enrichment prior to nanoLC-MS are often necessary to widen the phosphoproteome coverage. Recently, by using one-dimensional nanoLC-MS with meter-long, monolithic silica-C18 capillary column, we successfully identified the proteome expressed in *E. coli* cells [Iwasaki et al, Anal Chem 2010] and human induced pluripotent stem cells [Yamana et al, JPR 2013]. We also developed a highly efficient phosphopeptide enrichment protocol based on aliphatic hydroxyl acid-modified metal oxide chromatography (HAMMOC) using lactic acid-modified titania [Sugiyama et al, MCP 2007]. We combined the 'one-shot' approach with HAMMOC, and applied to STY phosphoproteomics without any fractionation, identifying more than 12,000 phosphosites (3,700 phosphoproteins) from 0.25 mg HeLa lysate. We also employed sequential enrichment of tyrosine phosphopeptides using lactic acid-modified titania chromatography followed by immunoprecipitation using pY antibody cocktails. Furthermore, we minimized the required samples down to 10,000 cells (approx. 1 microgram of proteins) by miniaturizing LC, skipping autosampler and optimizing protocol based on phase-transfer surfactants [Masuda et al, JPR 2008], resulting in identification of more than 1,000 phosphopeptides [Masuda et al, Anal Chem 2011]. Based on these approaches, phosphorylation dynamics induced by kinase inhibitors were quantitatively analyzed to elucidate the mechanism of actions of these drugs [Imami et al, MCP 2012].

JHA-A-02 Snapshot Analysis of Protein Complexes Using Proteomic Technology

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Proteomic technologies allow a comprehensive study of multi-protein complexes that carry out many cellular functions in a higher-order network in the cell. The use of tagged proteins as affinity bait, coupled with mass spectrometric identification, enables us to isolate almost any functional protein complex or its synthetic intermediates that might represent snapshots of nascent functional protein complexes at particular stages of its biogenesis and to identify their constituents-some of which show dynamic changes for association with the intermediates at various stages of the biogenesis. The idea behind this snapshot analysis is that some of the associated proteins in one initially isolated complex can also be present in other precursor complexes, and thus would allow the purification of intermediates from different stages of biogenesis. Initially, we had applied this approach to the analysis of protein constituents of pre-ribosomes, and is now expanded this to the analysis of not only protein but also RNA constituents of small nuclear ribonucleoprotein (snRNP) intermediates formed during spliceosome biogenesis in cooperation with Dr. Isobe of Tokyo Metropolitan University. In this presentation, I would like to talk about mainly current status of the snapshot analysis of snRNP complexes.

Keywords: protein-protein interaction, biogenesis of protein machinery, ribonucleoproteomics

JHA-A-03 A Success in a Diagnosis Kit for Liver Fibrosis Using Multiple Novel Technologies of Glycoproteomics

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One methodology for biomarker discovery of chronic diseases exploits the fact that glycoproteins produced by impaired cells have altered glycan structures although the proteins themselves are common, ubiquitous, abundant and familiar. Here, we describe our strategy to approach the detection of these low-level glycoproteins as serum biomarkers: 1) A quantitative real-time PCR array for glycogenes to predict the glycan structures of secreted glycoproteins; 2) Analysis by lectin microarray to select lectins which distinguish disease-related glycan structures on secreted glycoproteins; 3) An isotope-coded glycosylation-site-specific tagging (IGOT) high-throughput method to identify carrier proteins having the specific lectin epitope. 4) Selection of candidate molecules by skillful bioinformatics technology. 5) Re-selection of the best lectin distinguishing the glycan-alteration in the patient serum. 6) Final verification using more than 100 patient sera.

Using this strategy, we have identified many glycoproteins containing glycan structures altered in impaired cells. These candidate glycoproteins were immunoprecipitated from serum using commercially available antibodies, and their glycan alteration was examined by a lectin microarray. Finally they were analyzed by multistage tandem mass spectrometry (MS^n).

According to this protocol, we succeeded in establishment of a diagnosis kit for liver fibrosis. A novel marker hyperglycosylated *Wisteria floribunda* agglutinin-positive Mac-2 binding protein (WFA^+-M2BP) was developed for liver fibrosis using the glycan "sugar chain"-based immunoassay. This kit was named FastLec-Hepa, and automatically detects unique fibrosis-related glyco-alteration in serum M2BP within 20 min. FastLec-Hepa is the only assay currently available for clinically beneficial therapy evaluation through quantitation of disease severity.

JHA-A-04 The Analysis of Protein Post-Translational Modifications

Yayoi Kimura

Advanced Medical Research Center, Yokohama City University, Japan

Post-translational modifications (PTMs) frequently play a key role in regulating protein function. The proteasome is composed of at least 33 different subunits and is a multi-catalytic protease complex that degrades ubiquitinated proteins in eukaryotic cells. The comprehensive PTM analysis of proteasome subunits using proteomic techniques indicated that 21, 1, 1 and 28 subunits were N-acetylated, N-myristoylated, N-methylated and phosphorylated, respectively. Furthermore, the study using the yeast mutants showed that N-acetylation might be involved in the chymotrypsin-like activity and accumulation level of the 20S proteasome and that N-methylation of Rpt1 might be essential for cell growth or stress tolerance in yeast. Like this, it is evident that the PTMs significantly affect the function of the proteasome. The phosphorylation of heterogeneous nuclear ribonucleoprotein K (hnRNP K) is also thought to play an important role in cell regulation and signal transduction. However, the relationship between hnRNP K phosphorylation and cellular events has only been indirectly examined, and the phosphorylated forms of endogenous hnRNP K have not been biochemically characterized in detail. The PTM analysis using 2-D Phos-tag affinity electrophoresis was successful to characterize multiple forms of hnRNP K produced by alternative splicing of the single hnRNP K gene and phosphorylation of Ser116 and/or Ser284. Furthermore, this analysis demonstrated that each form of hnRNP K was differentially modulated in response to external stimulation with bacterial lipopolysaccharide or serum. Like this, the PTM analysis is also crucial for a better understanding of the functional properties of proteins.

JHA-A-05 Development of Glycoproteomic Technologies and Identification of Glycan-Targeting Tumor Markers

Koji Ueda

Laboratory for Genome Sequencing Analysis, Center for Integrative Medical Sciences, RIKEN, Japan

Protein glycosylation is one of the most complicated post transcriptional modifications, while it plays diverse physiological functions by controlling protein folding, charge state, ligand-receptor interactions, or immunogenicity. To date, we have focused on developing novel glycoproteomic technologies which allowed rapid and comprehensive profiling of clinically-important glycan structure disorders on both targeted glycoproteins and even whole serum/plasma glycoproteins.

Isotopic glycosidase elution and labeling on lectin-column chromatography (IGEL) technology (Mol Cell Proteomics, 2010, 9(9):1819) enabled us not only to identify N-glycosylation sites comprehensively but also to compare glycan structures on each glycosylation site quantitatively in a single LC/MS/MS analysis. Using this technology, we revealed that glycans on A2GL_Asn151, A2GL_Asn290, CD14_Asn132, CO8A_Asn417, C163A_Asn64, TIMP1_Asn30, and TSP1_Asn1049 demonstrated lung cancer-specific alterations.

Recently we developed Energy resolved oxonium ion monitoring (Erexim) technology to evaluate glycan microheterogeneities on therapeutic protein drugs (Anal Chem, 2012, 84(22):9655). The Erexim method can quantify ~50 glycan structure variations on a therapeutic antibody molecule according to energy-resolved MRM for oligosaccharide-derived oxonium ions in only 10 minutes. We discovered significant lot-to-lot variations of glycosylation profiles on Herceptin and Avastin. The existence of anaphylaxis-inducible glycan structures on Erbitux were also quantitatively determined by Erexim. This technology has already been put to practical use in R&D and CMC areas by a contract research organization (CRO).

Thus I'd like to continue facilitating the life innovation by creation of further sophisticated MS-based glycoproteomic technologies in the future.

JHA-A-06 Rapid Discrimination between Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus* Using MALDI-TOF Mass Spectrometry

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens responsible for nosocomial infection. The presence of MRSA in a hospital is very detrimental to patients and to hospital management. Thus, rapid identification of MRSA is needed. This study performed a prospective study of rapid discrimination of MSSA from MRSA using the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) system. We evaluated 305 clinical isolates of *S. aureus* using the MALDI-TOF MS system and support vector machine. The predictive model was trained using 100 *S. aureus* isolates (50 MSSA and 50 MRSA). The identification rates were 90.2% for MSSA and 78.6% for MRSA following the 10-fold cross-validation SVM. In the blind test sets, 205 *S. aureus* isolates (95 MSSA and 110 MRSA) were correctly classified. The identification rates were 95.8% for MSSA and 81.8% for MRSA. In conclusion, the method proposed in this study using a predictive model enables detection from one colony in 5 minutes, and thus is useful at clinical sites at which rapid discrimination of MRSA from MSSA is required.

JHA-A-07 Industrialization of Clinical Proteomics Using Mass Spectrometry-Based Technologies

Takao Kawakami, Hiroshi Gushima

Medical ProteoScope Co., Ltd., Japan

Medical ProteoScope (MPS) is the company of proteomics excellence towards the better quality of life. In clinical practice, there is a need for biomarkers to help improve treatment outcomes. Based on integration of the state-of-the-art proteomics technology, we have focused on development of protein biomarkers. Label-free comparative LC-MS/MS is our core analytical platform, associated with the self-made algorithms, i-OPAL and i-RUBY, for data alignment of non-linearly fluctuated LC elution time. Under quality control (QC) operations of these technologies, collaborative studies with academic institutions and drug companies led to identification of proteins related closely to drug response, current state of the disease, progression of the disease and metastatic risk in the case of cancer patients. Posttranslational modifications (PTMs) of proteins are the promising targets for understanding disease mechanisms. Since 2008, MPS participates in the coordination funds presided by Yokohama City University. We developed differential analysis systems of PTMs including reversible phosphorylation. Formalin-fixed paraffin-embedded (FFPE) tissues archived in hospitals have a potential for discovery of the therapeutic targets as well as proteomic biomarkers. We have optimized protocols to dissect minute lesions from the FFPE tissues. Proteomic data of the collected specimens enabled to evaluate more accurately the disease state of the tissues. Thus, as a proteomics leading venture, MPS is continuously contributing to the fields of medicine.

JHA-A-08 A Novel Approach for Discovering Proteins at Extremely Low-Abundance in Serum

Giman Jung, Michimoto Kobayashi, Yoshinori Tanaka, Kazuhiro Tanahashi, Hideo Akiyama

Toray Industries, Inc., Japan

The proteomic analysis of serum (plasma) has been a major approach to determining biomarkers essential for early disease diagnoses and drug discoveries. The determination of these biomarkers, however, is analytically challenging since the dynamic concentration range of serum proteins/peptides is extremely wide (more than 10 orders of magnitude). Thus, the reduction in sample complexity prior to proteomic analyses is essential, particularly in analyzing lowly abundant protein biomarkers.
Here, we demonstrate a novel approach to the proteomic analyses of human serum that uses an originally developed serum protein separation device and a subsequently linked high-performance mass spectrometer system. The hollow-fiber membrane based serum pre-treatment device we developed can efficiently deplete high-molecular-weight proteins and concentrate low-molecular-weight proteins/peptides automatically within an hour. The proteomic analysis of healthy human serum pre-treated using the device, followed by the mass spectrometer, successfully identified about 2,000 proteins. According to the concept of the pre-treatment device, 64% of the identified proteins were smaller proteins than the human serum albumin. We believe this unique serum pre-treatment device and the proteomic analysis protocol allow high-throughput and efficient discovery of serum disease biomarkers.

Sunday, September 15, 8:15 - 9:30 am

PSI/ProteomeXchange/KBpC + C-HPP (Part 1)

Room 303-304

The HUPO Proteomics Standards Initiative Standard Formats and Their Use in Current Software

Eric Deutsch

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The HUPO Proteomics Standards Initiative (PSI) defines community standards for data representation in proteomics to facilitate data comparison, exchange and verification. We will present the recent activities of the PSI, including the development of the TraML, mzIdentML, mzQuantML, and mzTab formats. We will describe the new minimum information specification MIAPE - Quant, as well as updates for other MIAPE modules. For most of the community, these formats and specifications are only as useful as the software that supports the formats. Therefore, we will discuss the implementation of these formats in currently available software and software in progress. We will conclude with current and future projects of the PSI and how you can become involved.

Update on ProteomeXchange

Henning Hermjakob

European Bioinformatics Institute, Cambridge, UK

The ProteomeXchange consortium (PX) provides a globally coordinated infrastructure for the deposition and public dissemination of MS-based proteomics data. For each dataset, PX aims to capture raw data, metadata, and processed results, to allow different views of the submitted data, from the original author analysis and interpretation in e.g. PRIDE to re-processed views in e.g. PeptideAtlas to higher abstraction levels in molecular biology resources like UniProtKB.

For all types of PX datasets, all the data remains private by default and each submission becomes publicly available only on author instruction or publication of the manuscript supported by the dataset. When this happens, a short summary announcement is released though a public announcement system, as a RSS feed. All the PX announcement messages are stored and searchable in ProteomeCentral. This resource generates a unique identifier and constitutes a registry for each PX data dataset. In addition, it provides the users with an efficient way to identify datasets of interest.

ProteomeXchange has been in production mode since summer 2012. As of July 2013, 310 submissions with a total volume of 20 TB from 28 countries have been received, ranging from a few hundred spectra to 4 TB in a single submission. A significant fraction of depositions stems from HPP participants. ProteomeXchange submission and dissemination sites are accessible from www.proteomexchange.org/

Integration of Proteomics Data in neXtProt

Lydie Lane

Swiss Institute of Bioinformatics, Geneva, Switzerland

neXtProt (www.nextprot.org/) is a web-based knowledge platform dedicated to human proteins. It is built upon the corpus of data from Swiss-Prot, to which are added selected high quality data originating from a variety of high-throughput approaches. In addition to mass-spectrometry-based protein and post-translational modification (PTM) site identifications, those data include coding polymorphisms, splice variants, as well as information about protein interactions, structure, localization and function.

We have established a pipeline to integrate all the human peptide identifications from PeptideAtlas. Other large-scale proteomics datasets are integrated from direct submissions and original papers. Each annotated peptide or PTM site identification is accompanied by metadata that provides details on the experimental setup using controlled vocabularies, and by a description of their confidence (Gold meaning <1% error rate, Silver meaning <5% error rate). We have integrated 32521 PTM sites (N-glycosylation, phosphorylation, S-nitrosylation, ubiquitination and sumoylation), which corresponds to 17410 new PTM annotations. We have also mapped 279,933 identified peptides to 14,131 proteins.

neXtProt data is available in XML and PEFF formats ("PSI extended FASTA format"), as well as through an API (www.nextprot.org/rest/) that allows users to retrieve the complete set of PTMs or variants for all isoforms of a protein, along with their experimental evidence and Gold/Silver data confidence assessment.

Sunday, September 15, 8:15 - 9:30 am

EyeOME

Room 301

Introduction to the Human Eye Proteome Project

Richard Semba[1]; Jan J. Enghild[2]; Vidya Venkatraman[3]; Thomas F. Dyrlund[2]; Jennifer E. Van Eyk[3]

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There are an estimated 285 million people with visual impairment worldwide, of whom 39 million are blind. The pathogenesis of many eye diseases remains poorly understood. The human eye is currently an emerging proteome that may provide key insight into the biological pathways of disease. We review proteomic investigations of the human eye and present a catalogue of 4842 non-redundant proteins identified in human eye tissues and biofluids to date. We highlight the need to identify new biomarkers for eye diseases using proteomics. Recent advances in proteomics now allow the identification of hundreds to thousands of proteins in tissues and fluids, characterization of various post-translational modifications, and simultaneous quantification of multiple proteins. To facilitate proteomic studies of the eye, the Human Eye Proteome Project (HEPP) was organized in September 2012. The HEPP is one of the most recent components of the Biology/Disease-driven Human Proteome Project (B/D-HPP) whose overarching goal is to support the broad application of state-of-the-art measurements of proteins and proteomes by life scientists studying the molecular mechanisms of biological processes and human disease. The large repertoire of investigative proteomic tools has great potential to transform vision science and enhance understanding of physiology and disease processes that affect sight.

Proteome of Human Tears and the Mouse Retina

Zhou Lei[1,2,4], Roger Beuerman[1-4]

[1]Singapore Eye Research Institute, [2]Duke-NUS, SRP Neuroscience and Behavioral Disorders, [3]Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, [4]Ophthalmology, University of Tampere Medical Center, Tampere, Finland

Current mass spectrometry technologies have significantly improved the coverage of the proteome, which provides more systemic insights into the pathogenesis of the disease. Here, we gave two examples of such in-depth analysis of human tear proteome and mouse retina proteome. Tears, an easily accessible body fluid covering the ocular surface, provide valuable information of different ocular diseases. Recently, we showed that more than 1500 proteins could be identified from normal human tears. A quick 1D-RPLC-MS/MS using a 90-mins gradient was able to identify around 500~700 tear proteins using 4 mg of total tear proteins equivalent to about 0.5 ml of tears. MS/MSALL with SWATH acquisition enables MRM-based quantitation (high reproducibility with CV% < 20%) of around 500 tear proteins using the same 1D LC-MS/MS setup. In another study, iTRAQ-based quantitative proteomics was utilized to investigate the changes of the mouse retina proteome in a lens-induced myopia model and in response to atropine treatment. Approximately 4000 proteins were identified with high confidence (<1% FDR) from mouse retina tissue and 3300 of them had quantitative information. Pathway analysis revealed that GABA transporters (GATs) that were upregulated in experimentally-induced myopic mouse retina and chronic administration of atropine were found to restore these levels back to normal. This study indicated that GATs could be a potential target for regulating the anti-myopic effects of atropine in mouse eyes. In conclusion, the ability for in-depth and quantitative analysis of the proteome will provide more information for understanding the disease and useful for biomarker research.

The Proteome of the Anterior Segment in Relation to Glaucoma

Deepak Edward[1,2]; Rachida Bouhenni[3]

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Glaucoma, a heterogeneous group of diseases involving impairment of aqueous humor (AH) outflow from the anterior chamber, includes alterations in AH, ciliary body, iris, and trabecular meshwork (TM). The AH proteome of control and primary congenital glaucoma (PCG) patients and iris proteome of normal eyes were examined using liquid chromatography followed by tandem mass spectrometry (LC-MS/MS). Proteomic alterations in anterior segment tissues in primary open angle glaucoma (POAG) were reviewed. The proteomes of AH in PCG and control revealed differential expression of 7 proteins, including apolipoprotein A-IV (ApoA-IV), albumin (Alb), and antithrombin III (ANT3), which were detected at significantly higher levels in PCG AH compared to control, and transthyretin (TTR), prostaglandin-H2 D-isomerase (PTGDS), opticin (OPT), and interphotoreceptor retinoid binding protein (IRBP), which were detected at significantly lower levels in PCG. Normal iris proteome included 550 proteins that consisted of cytoskeleton and extracellular matrix (ECM) proteins (10%), development (25%), adhesion (4%), antioxidant activity (2%) and other cellular processes such as growth. Proteins previously implicated in glaucoma such as PTGDS, OPT, TTR were detected. In POAG, elevated levels of transforming growth factor beta 2, abnormal accumulation of ECM proteins such as cochlin and secreted protein acidic and rich in cysteine, oxidative stress proteins, and abnormal protein expression and changes in cytoskeletal interactions with the TM have been described in the literature. Establishment of the normal proteome of AH and iris and alterations of the proteome of AH and TM will help in further understanding of the pathophysiological processes and pathways involved in glaucoma.

Vitreous Proteome in Diabetic Retinopathy

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1Joslin Diabetes Center and Departments of 2Medicine and 3Ophthalmology, Harvard Medical School, Boston, MA, USA

Diabetic retinopathy (DR) a leading causes of vision loss worldwide. DR includes nonproliferative diabetic retinopathy and can progress to proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME). Proteomic analyses of vitreous have begun to characterize the vitreous proteome in normal subjects and identify changes in vitreous protein abundance associated with PDR and/or DME. The gel-like composition of the vitreous is derived mainly from a hydrated network of fibular macromolecules, including glycosaminoglycans, proteoglycans, and collagen fibrils. Proteomics has identified several hundred soluble proteins that are reproducibly detected within this vitreous fluid/matrix and many more that are detected randomly or in a small subset of vitreous samples, suggesting that levels of certain proteins can markedly differ among vitreous samples. Vitreous proteins have been annotated to mediate signaling, transport, chaperone, and proteolytic functions. These findings suggest potential roles for vitreous proteins in the maintenance of vitreous structure, intraocular metabolism, complement activation, redox balance, inflammation, coagulation, and cell survival and growth. Several groups have shown the vitreous proteome is markedly altered in advanced stages of DR and is often accompanied by a 3-4 fold increase in total vitreous protein. Changes in the DR vitreous proteome appear to be mediated by a number of factors including the breakdown of blood retinal barrier function and extravasation of circulating proteins, intraocular hemorrhage, cell lysis, and alterations in the production and/or clearance of locally expressed proteins. Functional studies are underway to identify vitreous proteins that may contribute to, or protect against, the incidence and/or progression of DR.

Personalized Proteomics for Inflammatory Retinal Disease Therapy

Vinit B. Mahajan[1,2]; Jessica M. Skeie[1,2]

[1]Omics Laboratory, [2]Department of Ophthalmology and Visual Sciences, University of Iowa, Iowa City, IA, USA

The causative molecules for inflammatory vitreoretinal disease are not known. We applied proteomic strategies to identify biomarkers and therapeutic targets in patients with genetically identical and genetically heterogeneous proliferative vitreoretinopathy (PVR). After establishing a surgical vitreous biorepository, we used proteomic profiling to identify biomarkers and therapeutic targets in patients with inherited and non-inherited proliferative vitreoretinopathy (PVR). The clinical phenotype of patients was ascertained using standardized grading methods. Vitreous cytokine expression was analyzed using antibody arrays and label-free proteomics. Pathway analysis was performed using MetaCore software. The clinical response of patients treated with anti-cytokine therapy was studied. Bioinformatic analysis revealed statistically significant, stage-specific cytokines in the inherited CAPN5-associated vitreoretinal disease. Following personalized therapeutic targeting, phenotypic ascertainment and proteomic profiles revealed drug responsive and nonresponsive pathways. For example, anti-VEGF therapy was highly effective in controlling neovascularization as was anti-IL-17 therapy for vitreous inflammation. The absence of TNFalpha and B-cell markers correlated with treatment failure using infliximab and B-cell immunosuppression. Failure to control pathologic fibrosis was associated with persistent expression of specific cytokines that escaped steroid therapy, which were also found in comparative profiles of non-inherited PVR. Personalized proteomic profiling of surgical tissue can identify the specific proteins and pathways involved in the progression of PVR. This data can be used to select rational therapies, avoid drugs where the target is nonexistent, and overcome the limitations of large clinical trials. Studies that take advantage of inherited disease may help convert biomarkers into therapeutic targets for common non-inherited disease.

The Tears as a Source for Proteomic Biomarkers of Human Eye Diseases

Roger Beuerman[1-4]; Zhou Lei[1,2,4]

1Singapore Eye Research Institute, 2Duke-NUS, SRP Neuroscience and Behavioral Disorders, 3Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, 4Ophthalmology, University of Tampere Medical Center, Tampere, Finland

Tears reflect the health of the ocular surface and can be easily collected for evaluation and screening. Proteomic studies of tears have identified key molecules associated with inflammatory diseases such as dry eye, Meibomium gland disease (MGD), and pterygium. Upregulated proteins include S100 proteins such as alpha-enolase, alpha-1-acid glycoprotein 1, S100 A8 (calgranulin A), S100 A9 (calgranulin B), S100 A4 and S100 A11 (calgizzarin) and down-regulated proteins, include prolactin-inducible protein (PIP), lipocalin-1, lactoferrin and lysozyme. Receiver operating curves (ROC) were evaluated for individual biomarker candidates and a biomarker panel, which had a diagnostic accuracy of 96% (sensitivity, 91.0%; specificity, 90.0%). The clinical classification of the severity of the dry eye condition was successfully correlated to the proteomics by using three proteins that are associated with inflammation, alpha1-acid glycoprotein 1, S100 A8, and S100 A9. In MGD, levels of S100A8 and S100A9 were correlated to disease severity and levels of S100A8 protein were significantly correlated to sensations of grittiness, whereas S100A8 and S100A9 were correlated to symptoms of redness and transient blurring. Higher levels of S100A6, S100A8, and S100A9 expressions were found in pterygium relative to normal conjunctiva. In addition, a distinct alteration of localization of S100A11 expression was observed in pterygium epithelium compared to the conjunctiva. In each disease, severity of disease was reflected in expression of proteomic biomarkers. Proteomic biomarkers must undergo thorough clinical trials for application, but in the future could replace lengthy and often inconclusive clinical observations and be applied in general clinical use.

Sunday, September 15, 8:15 - 9:30 am

Proteome Biology of Stem Cells

Room 311-312

Deep Subcellular Proteome Profiling of Human Induced Pluripotent Stem Cell by One-Shot Nanolc-MS/MS Analyses with Meter-Scale Monolithic Silica Columns

Mio Iwasaki[1], Masato Nakagawa[1], Yasushi Ishihama[2], Shinya Yamanaka[1,3]

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The molecular mechanisms for self-renewal and pluripotency of human induced pluripotent stem cell (hiPSC) still remain unclear. Previously, some researchers have been performed to characterize hiPSCs using high-resolution

MS-based proteome analyses. However, the technology of proteome analysis is still immature to uncover the real proteome state in the cells including low expressing proteins. On the other hand, proteome profile in each subcellular location is a valuable knowledge of cellular functions. We thought fractionation of cellular organelle is effective approach to increase the proteome coverage and to get the knowledge of localization.

In this study, we improved the identification efficiency of low expressing proteins using subcellular fractionation technique combined with high sensitive one-shot nanoLC-MS/MS analyses. Cellular organelle of nucleus, cytoplasm and membrane were isolated and analyzed by nanoLC-MS/MS using meter-scale monolithic silica columns. Finally, we successfully identified more than 6,000 unique proteins from the nuclear fraction including 400 transcription factors which are known as very low expression levels. This deep subcellular proteome analysis data disclosed more detailed differences between iPSC and fibroblast cells and elucidated the key components governing the cellular functions of iPSC.

Proteomic Analysis of LGR5+ve Intestinal Adult Stem Cells and Their Immediate Undifferentiated Daughters

Javier Muñoz[1,2], Daniel E. Stange[3], Marc van de Wetering[3], Shabaz Mohammed[1,2], Hans Clevers[3], Albert J. R. Heck[1,2]

[1]Biomolecular Mass Spectrometry and Proteomics Group, Utrecht University, The Netherlands; [2]Netherlands Proteomics Center, Utrecht, The Netherlands; [3]Hubrecht Institute, KNAW, Utrecht, The Netherlands

The identification of Lgr5 as an intestinal stem cell marker has made it possible to isolate and study modest amounts of primary intestinal stem cells. Here, we show that starting with only 300,000 cells quantitative mass spectrometry, using a chemical isotope labeling approach at the peptide level, can be used to profile in-depth directly at the protein level changes between FACS-sorted Lgr5+ve stem cells and their immediate undifferentiated daughter cells, enabling quantitation of 3,224 proteins. The data revealed the increased presence in Lgr5+ve stem cells of several previously reported stem cell markers including Ascl2 and Olfm4. The overall comparison of mRNA and protein levels indicated a high level of correlation, authenticating the novel proteomics approach and implying that the initial control of intestinal stem cell biology occurs largely at the mRNA level. Our study presents a valuable resource for the study of intestinal stem cell biology.

Stem Cells and Neural Development

Akhilesh Pandey

Johns Hopkins University, Baltimore, USA

The ability to derive neural progenitors, differentiated neurons and glial cells from human embryonic stem cells (hESCs) with high efficiency holds promise for a number of clinical applications. However, investigating the temporal events is crucial for defining the underlying mechanisms that drive this process of differentiation along different lineages. I will discuss our efforts using quantitative proteomics to monitor the temporal dynamics of protein abundance as human embryonic stem cells differentiate into motor neurons and astrocytes. We identified a number of proteins whose expression was largely confined to specific cell types, embryonic stem cells, embryoid bodies and differentiating motor neurons. I will also describe our proteomic analysis of ESCs differentiating into oligodendrocytes, which are glial cells of the central nervous system that produce myelin. This is exciting because cultured oligodendrocytes provide immense therapeutic opportunities for treating a variety of neurological conditions.

Sunday, September 15, 8:15 - 9:30 am

Human Brain Proteome Project (HBPP)

Room 313-314

The Role of Neuroproteomics to Elucidate Neurodegenerative Disease Mechanisms

Lea T. Grinberg

University of Sao Paulo (Brazil) and University of California, San Francisco (USA)

Neurodegenerative diseases demonstrate lesions of opposite nature. Abnomal protein deposits are known as positive lesions, and neuronal loss comprise the negative lesions. Proteomics methods were instrumental to identify the pivotal proteins that compose inclusions in frontotemporal lobar degeneration: TDP-43 and FUS. In this lecture, we will discuss which fundamental questions on neurodegenerative diseases are still open and how proteomics tools may help to find the answers

Biomarker Discovery for Alzheimer and Parkinson Disease

Helmut E. Meyer[1], Caroline May[1], Andreas Schrötter[1], Michael Turewitz[1], Martin Eisenacher[1], Dirk Woitalla[2], Helmut Heinsen[3], Jens Wiltfang[4], Renata Leite[5] and Lea T. Grinberg[5]

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Besides known molecular markers for Alzheimer's disease (AD) and Parkinson's disease (PD) like Aß-peptides, tau, phospho-tau and α -synuclein, which are used to diagnose dementia patients already affected or in an early stage of the respective disease, new validated biomarkers for AD or PD are urgently needed.

In an approach to identify new biomarker candidates we started a collaboration with the brain bank in São Paulo, Brazil, and the University Clinic, Würzburg, Germany, to focus our proteomic strategies on those brain regions of interest which are usually affected by AD or PD. Data from literature suggest that autoantibodies from sera of affected individuals showed immuno-reactivity in neurons in disease specific brain regions. Especially membrane proteins are of great interest, since they may represent first targets of autoimmune reactivity.

Therefore, in case of AD the raphe nucleus, the entorhinal region, the hippocampus and cortex layer 3b, in case of PD the enteric neuronal system, the dorsal motor nucleus of the vagal nerve, the locus coeruleus and the substantia nigra pars compacta and in case of ALS primary and secondary motor neurons are of primary interest. Different types of neurons will be isolated from post mortem human control brain by laser microdissection. These samples will then be analyzed by proteomic technologies, i.e. 1D-SDS/LC-MSMS and Western blot analysis. We are focusing on the identification of auto-antigen targets in AD and PD verifying the presence of pathologically effective autoantibodies in the sera of affected patients.

This work was supported by the European Regional Development Fond (ERDF) of the European Union and the Ministerium für Innovation, Wissenschaft und Forschung des Landes Nordrhein-Westfalen (ParkChip, FZ 280381102 and PURE, Az. 131/1.08-031).

Monday, September 16, 8:15 - 9:30 am

Liver (HLPP and B/D-HPP-Liver)

Room 303-304

Urine as a Source of Liver Disease Biomarkers and Proteomics Studies with Human Hepatoblastoma Samples

Felix Elortza

CIC bioGUNE, CIBERehd, ProteoRed-ISCIII, Technology Park of Bizkaia, Derio, Spain

Liver proteomic research keeps being one of the most active topics at CIC bioGUNE´s proteomics platform. We keep involved in the liver disease biomarker discovery in urine by analyzing urine peptides and exosome proteomes. Urine samples are ideal for diagnostic purposes because of the ease of sample collection using standardized protocols without the need for invasive methods, manipulation and storage. We explored this biofluid as a source to identify makers for liver pathologies by using two different approaches: exosome proteomics and natural occurring peptidome profiling.

Besides, we are involved in a collaboration with Dra. Carolina Armengol at IGTP-CIBERehd, and we have performed differential proteomics experiments

with hepatoblastoma human samples. Hepatoblastoma is a malignant embryonal tumor of the liver usually diagnosed in children younger than 3 years of age. We have combined DIGE based analysis together with the nLC MS/MS label free quantitation approach searching for new prognostic biomarkers. The results obtained from both techniques are complementary and show interesting aspects when comparing with previously published related studies in liver cancer context.

Identification of Proteins Driving the Progression of Liver Injury and Potential Biomarkers

Fernando J. Corrales

Division of Hepatology and Gene Therapy, CIMA, University of Navarra, Pamplona, Spain

The liver is one of the main topics in the B/D-SpHPP as there is an urgent need for novel clinical strategies to improve the management of liver diseased patients. In our lab, studies have been performed to identify mechanisms of liver disease progression. Proteomic analyses lead us to the identification of mitochondrial Prohibitin 1 as a central protein to preserve liver homeostasis and its down-regulation correlates with the development of non-alcoholic steatohepatitis. Defective prohibitin 1 induces apoptosis in human hepatoma cells and its partial deletion in mice induces liver damage and an exacerbated inflammatory response. The use of proteomic approaches led us to define a collection of proteins that might be relevant in the progression of human hepatocellular carcinoma (HCC). VASP has emerged as a biomarker candidate for the early detection of HCC as its plasma levels increase significantly and this change can be detected in cirrhosis, which is considered as a pretumoral condition. Preliminary results will be also presented relative to the follow-up of a group of HCC patients subjected to radioembolization.

Towards the System Medicine of Non-Alcoholic Fatty Liver Disease

Tommy Nilsson

The Research Institute of the McGill University Health Centre, the McGill University Health Centre & McGill University, Montreal, Quebec, Canada

Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of disease states ranging from benign non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) associated with increased mortality rates. As a chronic polygenic and complex disease, it places a large portion of the general population at risk of developing terminal cirrhosis, hepatocellular carcinoma and/or hepatic decompensation. Significantly, between 3-19% of the general population have some form of NASH (inflammation) depending on their body mass index and when coupled with increased fibrosis, over time, are at high risk of advancing towards terminal liver disease. Through funds obtained from FRQ-S (lead PI-T. Nilsson), The Research Institute of the McGill University Health Centre (RI-MUHC), the McGill University Health Centre & McGill University (McGill), we have undertaken a translational study to delineate underlying pathophysiologies of involved disease states associated with NAFLD. Central to the project has been the establishment of a liver biobank (lead PI-P. Metrakos) designed such that it enables immediate processing of procured samples (from patients and donors) through sub-cellular fractionation to enrich for organelles (lead PI-J. Bergeron) followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) and advanced bioinformatics (lead PI-T. Nilsson) to assign hepatic proteins to their cognate organelles and to map how these change in relation to NAFL and NASH. In addition, to compare at the genomic and transcriptional level, changes with respect to disease (lead PI-R. Sladek). The aim of the project is to develop non-invasive patient-centric approaches to diagnose and monitor disease progression and to highlight avenues for early intervention. To date, following 18 months of analysis, we have identified 6,053 proteins (FDR below 0.1% as deduced through Protein Prophet/Scaffold) each corresponding to a unique gene based on a minimum of two independent and unique peptides (threshold 95%). In this, we assigned a total of 106,530 unique peptides based on 7,278,890 assigned spectra. An additional 3,556 proteins were detected with one unique peptide (threshold 95%) based on 6,579 peptides and 227,403 spectra. A large portion of identified proteins has been assigned to their cognate organelles through hierarchal clustering (an approach we term "The Protein Microscope") awaiting confirmation through antibody-based methods. High fidelity peptide and protein assignment was made possible through the use of the human centric database NeXtProt combined with high mass accuracy/high resolution LC-MS/MS. A complete progress report with selected highlights will be presented. Additional PIs involved in the projects are: Rima Rozen (McGill); Vassilios Papadopoulos (RI-MUHC); Silvia Vidal (McGill); Brian Gilfix (RI-MUHC); Barry Posner (McGill); Bartha-Maria Knoppers (McGill); Yann Yoly (McGill).

Monday, September 16, 8:15 - 9:30 am

Cancer

Room 302

CPTAC - a Proteogenomics Network for Cancer

Christopher Kinsinger

Clinical Proteomic Tumor Analysis Consortium (CPTAC), National Cancer Institute, USA

Molecular characterization of human cancers has generated large volumes of genomic data through comprehensive and coordinated efforts such as The Cancer Genome Atlas (TCGA) project. However, the mechanisms by which cancer genes singly or cooperatively transform cells remain poorly understood. Proteomic characterization of cancer tissues with genomic data represents a key step in not only verifying the genomic alterations at the protein level, but also allowing for the analysis of unique features that are inherent to proteins including post-translational modifications. The proteomic characterization of cancer tissues is therefore essential for the development of a successful strategy to reduce cancer mortality. The Clinical Proteomic Tumor Analysis Consortium (CPTAC) was launched by the National Cancer Institute (NCI) as a comprehensive and coordinated effort to accelerate the understanding of the molecular basis of cancer through the application of robust and quantitative proteomic technologies and workflows. CPTAC aims to systematically identify proteins that derive from alterations in cancer genomes and related biological processes, and provides this data with accompanying assays and protocols to the public. In this initiative session for the Cancer Proteome-Human Proteome Project (CP-HPP), we will describe the specimens, proteomic technologies, workflows, and public data releases of the CPTAC program.

Analysis of Tissue Biopsies by PCT-SWATH

Guo Tiannan

ETH Zurich, Institute of Molecular Systems Biology, Switzerland

Fast, quantitatively accurate, deep and reproducible proteomic analysis of complex biological samples remains a major hurdle in systems biology and systems medicine. Here we propose a method to convert the proteome contained in small (biopsy) cell or tissue sample into a digital data set that contains signature fragment ion patterns for all peptides derived from the sample. These datasets can be then perpetually mined in silico. In essence, the method converts the physical proteome in a (clinical) sample into a permanent digital representation. The method is built on pressure-cycling technology and SWATH-MS. The entire workflow can be completed within 12 hours. Subsequent targeted data analysis offers unprecedented performance in identifying and quantifying low-abundance signaling protein networks in human tissues in an extendable manner. We have applied this method to a kidney cancer cohort, and uncovered dis-regulated signaling protein networks.

Proteomics-Based Studies on Colorectal Cancer

Edouard Nice

Clinical Biomarker Discovery and Validation, Monash Antibody Technologies Facility, Monash University, Australia

Colorectal cancer (CRC) is currently the third most common cause of cancer death worldwide. If detected early while the disease is localised, 5 year survival following simple surgical resection is greater than 90%. By contract, if the cancer has metastasised, 5 year survival is less than 10%. Improved early detection and understanding of the metastatic process is clearly urgently required.

In this presentation I will describe some of our recent proteomics-based studies to both identify novel faecal biomarkers for detection and surveillance of the disease and also describe how we have we have integrated shotgun proteomics approach with a genome-wide transcriptomic approach (RNA-Seq) on a set of human colon cancer cell lines (LIM1215, LIM1899 and LIM2405) to both identify cancer associated proteins with differential expression patterns as well as protein networks and pathways which appear to be deregulated in these cell lines.

Integrative Analysis - Bridging the Gaps in Genetic Information Flow between Genomic and Proteomic Data

Zhen Zhang

Biomarker Discovery and Translation, Johns Hopkins University, USA

Molecular characterization of human cancers has generated large volumes of genomic and proteomic data through comprehensive and coordinated efforts such as TCGA and CPTAC. However, analyses of such genomic and proteomic data, even when comparing the same phenotypic differences, often yield results that do not overlap much in terms of the corresponding genes. We will present our effort in developing an integrative analysis approach and associated bioinformatics tools to take advantage of existing knowledge and databases such as pathways and interactomes, to search for the missing layer of molecular entities that could provide the most plausible linkage between the observed phenotype-dependent genomic alterations and proteomic expression changes. Results from such tools will not only provide plausible interpretations of the analysis results but also generate meaningful hypotheses for further verification.

Breast Cell Index and Atlas Projects

Peter James

Department of Immunotechnology, Lund University, Sweden

We have analysed over 450 primary human breast tumours and seven cells lines that represent the most common types of breast cells. These include model human cell lines representing the main molecular portraits of breast cancer as defined by mRNA expression (Luminal A and B, Basal, ErbB2, and normal-like) as well as a breast adipocyte and fibroblast cell line. In addition to cataloguing protein expression levels and modifications, we have been examining the intracellular distribution of proteins amongst the organelles. We have built up a large spectral library that enables us to create and validate rapid SRM assays for various aspects of breast cancer development and therapy choice and response monitoring. For example we have developed SRM assays for the majority of the proteins involved in DNA repair to allow monitoring of patient response to chemo- and radiotherapy. SRM analysis also allows a rapid determination of the levels of the hormone receptors (Estrogen, Progesterone and HER receptors) as well as known proteins involved in therapy resistance. We have been analysing the changes in protein expression during cancer advance using paired sets of primary tumour and local recurrence and comparing this to primary tumour and distal metastasis. This will enable us to more accurately define the degree of aggressiveness of a tumour and to develop a method to test for the presence of cancer cells in the margins of the primary tumour and in the sentinel node.

Human Proteome Knowledge Discovery Gateway：Progress and Perspective

Dong Li and Fuchu He

State Key Laboratory of Proteomics, Beijing Proteome Research Center, Beijing Institute of Radiation Medicine, National Center for Protein Sciences, National Engineering Research Center for Protein Drugs, China

High-throughput proteomic research approaches, such as mass spectrometry and antibody-based experiments, have generated a great amount of heterogeneous datasets. Mining knowledge from these datasets presents significant challenges, including the quality control, storing, processing, integrating and visualization of various types of proteomic data.

To address these challenges, we are constructing a human proteome knowledge discovery gateway. First, an interactive, configurable and extensible, configurable and interactive workflow-based platform will be constructed to analyze the proteome datasets based on the open-source galaxy framework on a cloud infrastructure. And now, this platform has integrated the analyses tools for identifying missing proteins, mapping peptides onto chromosomes, integrating peptide with ENCODE datasets, protein annotation, Gene Ontology enrichment analyses, protein biological network/pathway analyses, fundamental statistical analyses and machine learning. More importantly, this platform also integrates a configurable workflow system to combine these software tools, supporting workflow construction, editing, running, sharing and viewing. Second, bioinformatics browsers will be integrated into this gateway including the chromosome-based proteome browser (CAPER) and pathway-based proteome browser (PAPER). And now CAPER has been available online as a web-based, user-friendly web service, which can display proteomic data sets and related annotations comprehensively, CAPER employs two distinct visualization strategies: track-view for the sequence/site information and the correspondence between proteome, transcriptome, genome, and chromosome and heatmap-view for the qualitative and quantitative functional annotations. The PAPER will assign the proteome datasets to the human biological pathways, and this service will be online as soon as possible. Third, application software will be developed to annotate the human genome, such as finding novel protein-coding genes and presenting the correlation between the chromosome to genes, epigenome, proteomes, and phenotype, and also to find the key regulatory molecules/modules, the potential drug target and biomarkers by mapping proteome profile to human pathways.

Taken together, the human proteome knowledge discovery gateway will greatly facilitate the complete annotation and functional interpretation of the human genome by proteomic approaches, thereby making a significant contribution to the Human Proteome Project and even the human physiology/pathology research.

Monday, September 16, 8:15 - 9:30 am

HKUPP+HPPP

Room 311-312

Proteomic Analysis of Nephron Segments of Formalin-Fixed Paraffin-Embedded Human Kidney Tissues

Tadashi Yamamoto, Keiko Yamamoto, Yutaka Yoshida, Bo Xu, Ying Zhang, Sameh Magdeldin

HKUPP

Nephrons are the functional units of the kidneys and consist of several parts (glomerulus, proximal tubule, descending and ascending loop of Henle and distal tubule) connected to collecting ducts. Knowing of proteome profile of each nephron segment will contribute to understand characteristics and functions of the segment. Nephron segments (glomeruli, proximal tubules, descending and ascending loops of Henle and distal tubules) and collecting ducts were separated from formalin-fixed paraffin-embedded human kidneys by laser microdissection after immune-labeling of each part with antibodies against unique proteins in each part. Tissue sections of total area of ~1 mm2 each were digested with trypsin after autoclave treatment (On-site direct digestion, OSDD method). Peptides were collected from the segments (three samples each) and purified by C-18 Stage-Tip and analyzed by LC-MS/MS (Thermo Orbitrap LTQ) and proteins were identified by Mascot with FDR less than 1%.

More than 1000 proteins were identified by a single MS analysis of each sample and proteins uniquely expressed in each nephron segment were selected in glomerulus; 153, proximal tubule; 306, distal tubule; 58, and collecting duct; 40. Gene Ontology annotation analysis showed that the most enriched cellular components were cytoskeleton proteins in the glomerulus, cytoplasm proteins in the proximal tubule, mitochondrion proteins in the distal tubule, and cytoplasmic part proteins in the collecting duct.

The proteome analysis of nephron segments demonstrated their significant features and provided useful knowledge of their functions.

A Collective Analysis of Three Human Subproteomes using PeptideAtlas

Eric Deutsch, Terry Farrah, Tadashi Yamamoto, Julian Watts, Micheleen Harris, Zhi Sun, Gil Omenn

HPPP

The kidney, urine, and plasma subproteomes are intimately related: waste products are filtered from the plasma by the kidney and excreted via the urine, while some kidney proteins pass directly into the urine. Human kidney, urine, and plasma samples were measured using shotgun proteomics and all peptides were mapped to neXtProt. A software tool was developed within PeptideAtlas to show which neXtProt entries were unique to, or highly enriched in, each of the subproteomes relative to the others. The tool also performed detailed comparisons to reveal which urine proteins were likely derived from kidney, which were likely derived from plasma, and which were likely not derived from either. Gene Ontology analysis confirmed what is already known about the relationships among these subproteomes and revealed some new findings.

Different Levels of Variability in the Human Plasma Proteome

Yansheng Liu, Ben Collins, Ludovic CJ Gillet, Alfonso Buil, Emmanouil T. Dermitzakis, Lin-Yang Cheng, Olga Vitek, Ruedi Aebersold

Institute of Molecular Systems Biology, ETH Zurich

Human plasma is an appealing sample for translational medicine and biomarker discovery studies. However, fundamental questions of the variability of the plasma proteins in a population and their longitudinal pattern over years remain poorly characterized. This is due to the lack of suitable analytical methods that can consistently identify and quantify a large number of proteins among individuals in large sample cohort. We demonstrate that our newly developed Data independent Acquisition (DIA) method, SWATH MS [1], provides the unique and unprecedented chance to address these difficulties [2] for population proteomic studies. Using this technology we analyzed above 200 healthy plasma samples from monozygotic (mz) and dizygotic (dz) twins that were collected with 2-7 year intervals. The results indicate that SWATH-MS identified and quantified at least 2500 unique stripped peptides (regardless of charge-state and modification) at an FDR of 1%, corresponding to more than 400 proteins at a high degree of reproducibility in the plasma samples. Using the set of linear mixed effects models provided by MSstats software, we aim to systematically investigate and decompose the technical (transition-, peptide- and protein-level) and biological variance in human plasma proteome profiles. The data shows an unprecedented degree of reproducibly, with the median peptide CV of 11.2% for technical replicates. We estimate that the stable variation, comprising familial and individual-environmental factors, accounts in general for considerable fractions of biological variation in plasma protein concentrations.

Quantification of Peptides in Clinical Samples Based on High-Resolution Mass Measurements

Bruno Domon

Luxembourg Clinical Proteomics Center, Luxembourg

New hybrid mass spectrometers with high resolution and accurate mass (HR/AM) capabilities have opened new avenues in quantitative proteomics. Targeted analyses, routinely performed on triple quadrupole mass spectrometers using the selected reaction monitoring (SRM) mode, were replicated on a high-resolution quadrupole-orbitrap instrument (Q-Exactive) to improve the selectivity of the measurements. In this context, targeted measurements benefit from a narrow mass filtering window of the precursor ions together with the orbitrap HR/AM measurement of the fragment ions, while the multiplexing capability of the instrument was leveraged to measure precisely, using internal standards, a large number of peptides in a single LC-MS run. The quantification of peptides was performed in this parallel reaction monitoring mode (PRM), by extracting post-acquisition ion traces of specific fragment ions. The performance of the PRM technique was benchmarked against that of the reference SRM approach, and more specifically, the trapping capability proved beneficial for the enrichment of precursor ions of peptides in very tiny amounts (sub-amol level) and thus dramatically increase the signal-to-noise ratio. The PRM technique was applied to the analysis of clinical samples with a complex background (plasma and urine) to demonstrate the benefits of the gain in selectivity and the identification of the fragments through accurate mass, to increase confidence in the measurements. More specifically, lung cancer candidate markers were analyzed by this technique to differentiate the disease stages and subtypes. The results obtained by this technique allowed a clear discrimination of the different disease stages.

Monday, September 16, 8:15 - 9:30 am

Human Diabetes Proteome Project (HDPP)

Room 313-314

The Human Diabetes Proteome Project (HDPP)

D. Schvartz[1], F. Topf[1,2], P. Gaudet[3], F. Priego-Capote[4,5], A. Zufferey[1], N. Turck[1], PA. Binz[2,6], P. Fontana[1,7], A. Wiederkehr[8], F. Finamore[1], I. Xenarios[2,6], D. Goodlett[9,10], M. Kussmann[8,11,12], P. Bergsten[13], J-C. Sanchez[1]

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Diabetes mellitus is a complex multifactorial disease characterized by hyperglycemia and deranged lipids, which have been linked to diabetes-related complications. The Human Diabetes Proteome Project (HDPP) consortium was created at HUPO 2012 to unravel molecular mechanisms leading to diabetes and to understand the dysfunctions induced by glucose and free fatty acids. During the first year, the partners of HDPP identified the short-to-mid term objectives of the project. Various omics datasets will be collected, mainly by analyzing insulin producing cell lines, islets, and human blood from different conditions. Data integration as well as network biology approaches will be applied to enhance our knowledge of pathways centrally involved and deregulated in diabetes. Existing projects from the partners are already delivering omics data on human islets, rodent beta-cells, mitochondria, glycation in human blood as well as key results on modifications associated to beta-cell dysfunction. Based on the three pillars of the B/D-HPP projects, HDPP has already made publicly available (www.hdpp.info) three key protein resources [1]: (1) the 1' 000 diabetes-associated protein (the 1000-HDPP) database with links to their neXtProt, Peptide Atlas and Human Protein Atlas references; (2) a list of 5' 300 human islet proteins; and (3) a list of 2' 500 rodent beta-cell proteins. All results obtained so far through the HDPP initiative will be presented in the HDPP workshop held at the 12th HUPO world congress.

[1] Topf F., Schvartz D. et al. The Human Diabetes Proteome Project (HDPP): From Network Biology to Targets for Therapies and Prevention. Translational Proteomics 2013, in press.

Tuesday, September 17, 8:15 - 9:30 am

HGPI Glycoproteomics

Room 301

Summary of the First to Third Pilot Studies of Human Disease Glycomics/Proteome Initiative (HGPI)

Hiromi Ito

Department of Biochemistry, Fukushima Medical University

The Human Disease Glycomics/Proteome Initiative (HGPI) aims to standardize the data presentation in glycomics associated with diseases and promote data exchange, comparison, and verification among researchers. We have done three analytical pilot studies to date. The first and second studies were analyzed N- and O-linked glycans using standard glycoproteins (i.e. 1st: IgG and transferrin, 2nd: IgA), respectively. The purpose of these pilot studies was verification of comparing different methods (e.g LC-based and MS-based technologies) for quantitation of N- and O-linked glycans [1,2]. Then, the third study was attempted the following two analyses under the theme of 'Glyco-Biomarker Discovery'. One is the structural analyses of glycans derived from three different cancer cell lines (i.e. L428: Hodgkin's Lymphoma cell, U937: Lymphoma cell, and SK-N-SH: Neuroblastoma cell). Second is the identification of carbohydrate antigen-carrier proteins which is based on interest of each participant. The comprehensive analysis of glycans derived from cancer cells is helpful for understanding the activity of the glycosylation machinery of the cell. Here, we summarized the results of the third pilot study in addition to the first and second study.

[1] Comparison of the methods for profiling glycoprotein glycans – HUPO Human Disease Glycomics/Proteome Initiative multi-institutional study. Glycobiology 2007, 17, 411 - 422.
[2] Comparison of Methods for Profiling O-glycosylation: HUPO Human Disease Glycomics/Proteome Initiative Multi-Institutional Study of IgA1. Mol. Cell. Proteomics 2010, 9, 719 - 727.

Proposal of a New International Collaboration under the HPP: Biology/Disease-driven Glycoproteome Project (B/D-GPP) and Current Resources

Hisashi Narimatsu

Research Center for Medical Glycoscience (RCMG), National Institute of Advanced Industrial Science and Technology (AIST)

In Boston Congress 2012, I have proposed a new international collaboration for systematic uncovering of protein glycosylation as a part of B/D-HPP under the HPP of HUPO to expand the past studies in HGPI. This activity is named as Biology/Disease-driven Glycoproteome Project (B/D-GPP) aiming at construction of the open-access Glycoproteome Atlas that contains following information. First, we would like to collect data on mouse as a model organism, because common sources are available worldwide and a wide range of gene-deleted mice would be useful to reveal the structures and functions of glycans. Next or in parallel, human cell lines would be appropriate for the project. We should establish the Glycoproteome Atlas as a database, and collect the data for each of the tissues, body fluids, and cells. The Glycoproteome Atlas should encompass: 1) glycan profiles by lectin microarray, 2) mass spectra of glycans (MS and MSn), 3) expression profile of glycogenes (glycan biosynthesis-related genes), 4) tissue images stained with a series of lectins, 5) LC and CE (capillary electrophoresis) profiles of glycans, and 6) N- and O-glycoproteome profiles that provide protein ID and actual glycosylated sites. In this talk, the basic plan and current data resources such as N-glycoproteome profiles by the IGOT-LC/MS method and glycan profiles by lectin array analyses for mouse tissues are presented to call for the participation to this project.

Dissection of the Human GalNAc O-glycoproteome? Mapping Specific Functions of Individual Polypeptide GalNAc-transferase Isoforms by Zinc-finger Gene Engineering of Human Cells

Katrine ter-Borch Gram Schjoldager

Department of Cellular and Molecular Medicine, University of Copenhagen

Posttranslational modifications (PTMs) greatly expand the function and regulation of proteins, and glycosylation is the most abundant and diverse PTM. Deficiencies in protein glycosylation lead to a number of severe diseases and multisystemic disorders. Recently we have begun to uncover more subtle disease phenotypes associated with deficiencies in glycosyltransferase genes that are members of large homologous gene families with partial redundancies. Thus deficiencies in protein O-GalNAc glycosylation, where the first initiation step is regulated by 20 distinct GalNAc-transferases, produce cell and protein specific effects and subtle distinct phenotypes such as hyperphosphatemia with hyperostosis and dysregulated high density lipoprotein cholesterol (HDL-C) and lipid metabolism. We uncovered a possible mechanism by which one of the GalNAc-transferases, GalNAc-T2, co-regulates proprotein convertase (PC) processing and activation of the lipase inhibitor ANGPTL3, which can affect HDL-C and lipid plasma levels, both of which are heritable risk factors for coronary artery disease (CAD). More recently we have used the zinc-finger nuclease (ZFN) gene targeting of the human C1GalT1 chaperone COSMC to generate stable HepG2 SimpleCells with homogenous truncated GalNAc O-glycans in order to characterize the simplified O-glycoproteome. We have now applied this strategy to show non-redundant O-glycosylation performed by a single polypeptide GalNAc-T using differential analysis of O-glycoproteomes produced in an isogenic cell model with and without knock-out or knock-in of GalNAc-transferases. We have found several isoform specific substrates for GalNAc-T1, -T2 and -T3 that serve as potential biomarkers for disease caused by dysfunctional O-GalNAc-glycosylation and demonstrated that the human O-glycoproteome is differential and dynamic.

Tuesday, September 17, 8:15 - 9:30 am

iMOP - Initiative on Model Organism Proteomes

Room 302

iMOP – Initiative on Model Organism Proteomes

Sabine P. Schrimpf[1], Andreas Tholey[2], Emøke Bendixen[3], Michael O. Hengartner[1]

[1]Institute of Molecular Life Sciences, University of Zurich, Switzerland, [2]AG Systematic Proteome Research and Bioanalytics, Christian-Albrechts-Universität zu Kiel, Germany, [3]Department of Molecular Biology and Genetics, Aarhus University, Denmark

Fundamental pathways and biological processes are conserved across species, and studies of model organisms play an important role for understanding human biology and health. The continuous improvement and use of mass spectrometry have led to a dramatic increase in proteome coverage, and to the inclusion of an increasingly broad range of model organisms over the recent years. However, new model organism species are typically supported by only small communities. To form a broader model organism proteomics community, the initiative on model organism proteomes (iMOP; www.imop.uzh.ch) was integrated into HUPO. The research interest of the iMOP community is already quite diverse; however, we still encourage more research groups to join iMOP. Current iMOP members either focus on the interaction between humans and other organisms at the proteome level or and they are interested in the relevance of model organism proteomes to the human proteome. They run inter- and intra-species proteome and transcriptome comparisons to address evolutionary aspects and pathway development. iMOP members also focus on species that are important for food production and on host-pathogen-interactions. The iMOP initiative distributes proteomics knowledge to a wide range of model organism communities that are otherwise not closely linked. iMOP will contribute to the human proteome project (HPP) by providing comparative studies across species.

The Methylproteome Network of *Saccharomyces cerevisiae*

Marc R. Wilkins

University of New South Wales, Australia

Methylation of proteins occurs predominantly on arginine and lysine residues in the eukaryotic cell. Until recently, its predominance was unknown and its role obscure. This presentation outlines our efforts to construct the first 'methylproteome network' for a eukaryotic cell and provides evidence that arginine methylation modulates protein-protein interactions in this network. We analysed the yeast methylproteome to identify methylated proteins and precise modification sites. Immonium ion-based scanning and targeted data acquisition - electron transfer dissociation MS/MS was used, as were yeast proteome arrays (containing 4,400 chips spotted onto microscope slides). This showed that protein methylation is widespread in the eukaryotic cell. To build the intracellular methylation network, all known and putative

methyltransferases in yeast were knocked out and the methylproteome re-analysed to determine which enzyme was responsible for which methylation event. This led to the discovery of a new lysine methyltransferase, we named Efm2. Enzyme-substrate links were further investigated by the analysis of recombinant substrate proteins methylated by recombinant enzymes, by in vivo methylation assays and/or the incubation of proteome arrays with recombinant enzymes. Validated enzyme-substrate links were integrated with the yeast protein-protein interaction network to generate the first 'methylproteome network'. Interestingly, this suggested that many protein-protein interactions could be controlled by protein methylation. To test this, we constructed a new 'conditional two-hybrid' (C2H) system. Interactions of proteins were tested in the presence of a methyltransferase or in the presence of the same enzyme with active site knocked out. Of the protein-protein pairs involving arginine methylated proteins, half of those tested to date have shown increases in interaction in association with methylation.

Secretome Protein Profiling of Growth Interaction between *Listeria monocytogenes* and *Lactobacillus lactis* subsp. *lactis*

Paola Roncada

Lazzaro Spallanzani, Italian Experimental Institute, Milan, Italy

It is well known that starter cultures produce a wide range of anti-microbial metabolites which include organic acids, diacetyl, acetoin, hydrogen peroxide, antibiotics and bacteriocins. This anti-microbial activity can contribute in a number of ways towards improving the quality of fermented foods, for example, through the control of pathogens, extending shelf life and improving sensory qualities. The aim of this work is to explore the inhibition power of lactic acid bacteria in vitro against a pathogen, Listeria monocytogenes, that can occur on the rear of some type of cheese.

It has already been documented how bacterial competition can be used to overcome the growth of foodborne pathogens. As inter-cell communication can occur among bacteria for quorum sensing mechanisms, the secretion of extracellular proteins for other bacterial-growth inhibition has been as well documented.

In this project it has been decided to study the putative mechanisms of *Lactobacillus lactis* subsp. lactis in the inhibition of *Lysteria monocytogenes* growth. In particular it is already been documented that some species of lactic acid bacteria (LAB) are able to produce bacteriocins responsible for listeria monocytogenes growth inhibition. Briefly, cell culture filtrate of LAB, *Listeria monocytogenes*, and of LAB growing together with listeria have been analyzed from the peptidomic and proteomic point of view. Image analysis interestinguily revealed the presence of some specific proteins that are secreted only from one or both bacteria just when they are growing in the same medium. This demonstrates that inter-specific cell-cell communication is occurring for bacterial competition mechanisms. The bands and the spots of interest have been excised from the gels to be analyzed and identified through mass spectrometry.

Acetyl-phosphate Links Metabolism to Global Acetylation Dynamics in *E. coli*

Brian Tate Weinert

University of Copenhagen, Denmark

Recent proteomic studies identified thousands of lysine acetylation sites in diverse organisms from bacteria to humans. However, little is known about their origin and regulation. We used a quantitative mass spectrometry-based quantitative proteomics approach to study acetylation dynamics at thousands of sites in the model bacterium Escherichia coli (E. coli). We identified more than 8,000 unique acetylation sites and found that acetylation occurred at a low level and accumulated in growth-arrested cells in a manner that depended on the formation of acetyl-phosphate (AcP) through glycolysis. Mutant cells unable to produce AcP had significantly reduced acetylation levels while mutant cells unable to convert AcP to acetate had significantly elevated acetylation levels. We showed that AcP can chemically acetylate lysine residues in vitro and that AcP levels are correlated with acetylation levels in vivo, suggesting that AcP acetylates proteins nonenzymatically in cells. Most acetylation occurred independently of the YfiQ acetyltransferase and the CobB deacetylase suppressed acetylation at ~10% of sites. CobB-regulated acetylation sites were more sensitive to increased acetylation in growth-arrested cells and to AcP in vitro, suggesting that CobB suppresses acetylation at these sites to maintain very low levels of acetylation. These results uncover a critical role for AcP in bacterial acetylation and indicate that most acetylation in E. coli occurs at a low-level and is dynamically affected by metabolism and cell proliferation in a global, uniform manner.

Luncheon Seminar 1 Sunday, September 15 13:00-14:00 Room 303+304 *Sponsored by Bruker Daltonics Inc.*	LS-01 **Quantitative and Targeted Proteomics: MRM or Accurate Mass?** LS-01-1 **The New EVOQ Elite ER: Highest Sensitivity and Selectivity for Absolute Quantitation and Targeted Proteomics** Rohan Thakur Bruker Corporation, USA LS-01-2 **Proteomic Profiling and Imaging for the Studies of Neurological Diseases** Professor Masaya Ikegawa Doshisya University, Japan LS-01-3 **The Power of Accurate Mass Screening and DIA in Quantitative Proteomics** Pierre-Olivier Schmit Bruker Daltonique S.A., France
Luncheon Seminar 2 Sunday, September 15 13:00-14:00 Room 301 *Sponsored by* *GE Healthcare Japan Corporation*	LS-02 **Fluorescent Detection Overcomes the Challenges in Quantitative Western Blotting (tentative)** Susanne Grimsby, B.S. Senior Research Engineer, GE Healthcare Life Sciences To overcome the challenges in quantitative Western blotting, we report on; - Ways to use chemiluminescent and fluorescent Western blotting for confirmatory and quantitative detection - What the prerequisites are for achieving quantitative results, and how those can be fulfilled using fluorescent Western blotting detection - Tips on how to overcome common challenges in Western blotting, in order to achieve consistent results
Luncheon Seminar 3 Sunday, September 15 13:00-14:00 Room 302 *Sponsored by Thermo Fisher Scientific*	LS-03 **HRAM Quantification Using Orbitrap Technology** Thermo Fisher Scientific
Luncheon Seminar 4 Sunday, September 15 13:00-14:00 Room 311+312 *Sponsored by* *KIKO TECH CO., LTD./ ProteinSimple*	LS-04 **The Simple Western: A Fully Automated and Quantitative Tool for Protein, Biotherapeutic, and Vaccine Research** John Proctor, Ph.D. Director of Corporate Development, ProteinSimple The Simple Western is a fully automated walk-away solution that is a gel-free and blot-free reinvention of the traditional Western blot for protein separation and characterization. As an automated instrument, it drastically reduces the hands-on time traditionally required when performing an immunoassay. It delivers reproducibility and true quantitation while addressing the major challenges that researchers face on a daily basis-unreliable data, delayed time to results, reduced productivity and, ultimately, more costly research programs. In this seminar, I will illustrate examples of how the Simple Western has been applied to protein quantitation, cell signaling analysis, biotherapeutic characterization, and vaccine research. This sensitive technology measures either protein molecular weight or charge in complex samples and provides this critical information without the need for sample purification. Whether you' re doing protein research in biotech, pharma, or academia there is a Simple Western application for you.

Luncheon Seminar 5 Monday, September 16 13:00-14:00 Room 303+304 *Sponsored by AB SCIEX*	LS-05 **Deep and Efficient Acquisition of Human Proteome/Phosphoproteome Data by One-Shot Proteomic LC-MS with Long Monolithic Columns** Yasushi Ishihama Kyoto University, Kyoto, Japan Because peptide samples for shotgun proteomics exhibit extremely high complexity with a wide dynamic range of concentration, the separation capability of the current nanoLC is not sufficient enough to reduce the complexity. Recently, by using one-dimensional nanoLC-MS with meter-long, monolithic silica-C18 capillary column, we successfully identified 9,510 proteins in human induced pluripotent stem cells and fibroblasts [Yamana et al, JPR2013]. In this presentation, we will also present deep and efficient acquisition of phosphoproteomes by one-shot proteomic LC-MS approach.
Luncheon Seminar 6 Monday, September 16 13:00-14:00 Room 301 *Sponsored by Bruker Daltonics Inc.*	LS-06 **Top-Down Sequencing for Full Protein Characterization in Proteomics and Biopharma** LS-06-1 **A paradigm Change in Mass Resolution - The New solariX XR** Mike Easterling Bruker Daltonics Inc., USA LS-06-2 **Top-down Proteomics with Ultra-high Resolution QTOF Instruments** Peter Brechlin Bruker Daltonik GmbH, Germany LS-06-3 **MALDI-TDS of the Biooriginator Cetuximab Providing 100% Sequence Coverage and Complete Glycosylation Elucidation** Detlev Suckau Bruker Daltonik GmbH, Germany
Luncheon Seminar 7 Monday, September 16 13:00-14:00 Room 302 *Sponsored by SHIMADZU CORPORATION*	LS-07 **Quantitative Analysis by LC/MS/MS of Progranulin as a Marker for Insulin Resistance** Susumu Seino Division of Molecular and Metabolic Medicine, and The Integrated Center for Mass Spectrometry, Kobe University, Graduate School of Medicine Obesity and diabetes are diseases that afflict enormous populations in the 21st century. Insulin resistance is a characteristic feature in both obesity and diabetes. Adipose tissue secretes various cytokines or hormone-like substances called "adipokines". We have recently identified progranulin as a key adipokine that links high fat diet to obesity, suggesting that progranulin can be a biomarker for insulin resistance as well as a therapeutic target of obesity. Progranulin has been shown to be processed to granulin peptides (GRNs). In this lecture, we will discuss a novel method for selective quantification of GRNs in the blood, based on our recent data.
Luncheon Seminar 8 Monday, September 16 13:00-14:00 Room 311+312 *Sponsored by Bio-Rad Laboratories K.K.*	LS-08 **Innovative Technologies for Increased Confidence in Proteomic and Protein Analysis Workflows** Anton Posch Staff Scientist, Bio-Rad Laboratories, Hercules, USA Proteomic analysis of biological systems requires the integration of multiple technologies into robust work-flows for protein separation, analysis, and detection. Gel-based protein separations are widely used in these workflows and frequently followed by protein visualization/staining, mass spectrometry and/or immunodetection applications. Currently, these applications suffer from at least two important limitations: First, standard methods of protein visualization/staining are time-consuming and the associated manual handling adds to overall variability between experiments. Second, the quality of antibodies used for immunodetections are often unsatisfactory, affecting confidence in final outcome. Here, we present innovative technologies to address each of these limitations. To address the first limitation, we present TGX Stain-Free SDS-PAGE gels which contain unique trihalo compounds formulated into the gel chemistry for immediate visualization of proteins across the entire gel without staining. When activated, these compounds covalently bind to tryptophan residues in proteins and emit a fluorescent signal that is easily detectable with suitably matched imager systems. We will illustrate benefits of stain-free technology for increased confidence in two dimensional electrophoresis, western blotting, mass spectrometry (MS), and protein purification/visualization applications. To address the second limitation, we introduce HuCAL - a novel technology for high quality antibody generation for western blotting, immuno-MS and diagnostics.

Luncheon Seminar 9

Tuesday, September 17
13:00-14:00
Room 303+304

Sponsored by Thermo Fisher Scientific

LS-09

Advanced Proteomic Discovery Workflows Using Novel Multiplexing Methods

Thermo Fisher Scientific

If cannot be prepared by deadline, then will not put abstract.

Luncheon Seminar 10

Tuesday, September 17
13:00-14:00
Room 301

Sponsored by GlycoTechnica Ltd. / Sysmex Corporation

LS-10-1

Introduction of a Powerful Lectin Microarray Platform for Biomarker Discovery and Screening

Masao Yamada, Ph.D.
Cheif Exective Scientific Officer, GlycoTechnica Ltd.

LS-10-2

Development of Quantitative Glyco-indices for Hepatic Diseases

Atsushi Kuno, Ph.D.
Team Leader, Glycodiagnosis Translation Team, Research Center for Medical Glycomics, National Institute of Industrial Science and Technology

LS-10-3

Introduction of HISCL Automated Analysis System

Tomoyuki Nishida
Manager, Product Development, Immunology & Chemical Product Engineering, ICH Business Unit, Sysmex Corporation

Glycomics opens up a novel strategy for searching new biomarkers, screening the candidates, and ending up to developing clinical inspection assays. A lectin microarray platform presented here named "GlycoStation" (GlycoTechnica Ltd.) is very powerful in biomarker development, and has the highest sensitivity and quantitative feature comparing with any other similar systems. In this seminar, new biomarker developments for human liver fibrosis and cholangiocarcinoma are highlighted as typical examples of this success (National Institute of Advanced Industrial Science and Technology). Finally, practical clinical inspection assay designed on HISCL Automated Analysis System (Sysmex Corp.) is introduced as one the most successful results of this strategy.

Luncheon Seminar 11

Tuesday, September 17
13:00-14:00
Room 302

Sponsored by Waters Corporation

LS-11

Innovations in High-resolution Mass Spectrometry for Protein Quantification

James Langridge
Waters Corporation, Manchester, UK

Multiple Reaction Monitoring (MRM) mass spectrometry has emerged as a sensitive and robust method for the quantification of target analytes, particularly those in biological matrices. However, for the quantification of putative protein biomarkers sensitivity and specificity are paramount. For this reason increasing the selectivity of the MRM assay is crucial and high-resolution approaches have been suggested.

We will compare and contrast results from a new high resolution MRM approach (high-definition MRM) on a Synapt G2-Si mass spectrometer with the classical tandem quadrupole approach.

Luncheon Seminar 12

Tuesday, September 17
13:00-14:00
Room 311+312

Sponsored by Funakoshi Co., Ltd.

LS-12-1

Absolute Quantification of Human Proteome by Large-Scale Targeted Proteomics

Professor Keiichi Nakayama
Kyushu University

We have developed a new technology termed information-based multiple reaction monitoring (iMRM) to measure the absolute abundance of all human proteins. With the use of iMRM system, we have now measured the absolute abundance of all metabolic enzymes in normal and cancer cells and uncovered the secret underlying "Warburg effect."

LS-12-2

Products for MRM

Dr Akihiro Yoshida
Funakoshi Co.,Ltd.

- Peptide probes for Human protein
- Proteomics Sample preparation Kit
- Retention Time Markers for iMRM Analysis

Luncheon Seminar 13 Tuesday, September 17 12:00-13:00 Room 303+304 *Sponsored by* *Wako Pure Chemical Industries, Ltd.*	LS-13 **Phos-tag-Based Technological Advances for Studies on Signal Transduction in the Coming Generation** Eiji Kinoshita Hiroshima University, Japan We have been involved in developing a technology known as Phos-tag to permit the analysis of phosphorylated biomolecules. The Phos-tag technology has made contributions to the development of several procedures for research on the phosphoproteome, including a phosphate-affinity chromatography technique for the separation and enrichment of phosphopeptides and phosphoproteins, a phosphate-affinity electrophoresis technique for the detection of shifts in the mobilities of phosphoproteins, and microarray techniques for the detection of protein phosphorylation multiplexes. In this seminar, I discuss the impact of Phos-tag-based technological advances for studies on signal transduction in the coming generation.
Luncheon Seminar 14 Tuesday, September 17 12:00-13:00 Room 302 *Sponsored by Nihon Pall Ltd.*	LS-14 **Hit Identification and Lead Confirmation in the Discovery of Drugs Targeting Bromodomain Proteins** Liu Liu, PhD Comprehensive Cancer Center, University of Michigan
Luncheon Seminar 15 Tuesday, September 17 12:00-13:00 Room 311+312 *Sponsored by* *Agilent Technologies, Inc.*	LS-15-1 **QC or Bust: Performance Assessment for Quantitative Plasma Proteomics by MRM** Christoph Borchers, Ph.D. University of Victoria - Genome BC Proteomics Centre, Victoria, BC, Canada LS-15-2 **Defining the Next Experiment: Pathway-directed Analysis from Protein Discovery Data** Christine Miller Senior Application Scientist, Agilent Technologies Learn how pathway knowledge can be used to create targeted proteomics experiments to verify and validate candidate disease biomarkers in complex matrices. Examples are shown in the seminar. For some targeted assays, absolute quantitation is key to finding the true biological differences. In order to enable the acquisition of more precise and accurate quantitative data, two reference kits were developed by MRM Proteomics using SIS peptide approach for undepleted and non-enriched human plasma.

Evening Seminar 1

Sunday, September 15
18:00-19:30
Exhibition Hall Session Space

Sponsored by Tanaka ms3d Project

ES-01

Development of the Next Generation Mass Spectrometry System, and Contribution Toward Drug Discovery and Diagnostics - Focus on Software Development

ES-01-1

FIRST ms3d Project

Koichi Tanaka
Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation

"FIRST" <http://first-pg.jp/english/> is a major project funded by the Cabinet Office of Japan (100 billion JPY). One of the 30 "FIRST" projects is "ms3d project" (core-researcher: Koichi Tanaka) <http://www.first-ms3d.jp/> whose objective is to develop all MS systems from "Sample Preparation", "Ionization", "MS Hardware" up to "Software" mainly for Drug Discovery and Diagnostics. One of the foremost achievement is "Improved selectivity and sensitivity by >10,000 folds".

ES-01-2

Introduction of MS Analysis Software Mass++

Satoshi Tanaka
Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation

Mass++ is freeware for viewing and manipulating various types of mass spectrometric data. Its primary objectives are: 1. To provide essential functionality mass for proteomics and metabolomics analysis. 2. To support a wide range of vendors' data file formats. 3. To be easily extendible using plug-in technology. In this section, we will introduce and demonstrate some functions of Mass++ such as identification and quantitation.

ES-01-3

Biomarker Discovery Using Mass++ Software

Ken Aoshima
Eisai Co., Ltd Biomarkers and Personalized Medicine

Mass++ is an universal mass spectrometry data analysis software, which allows to develop plug-ins for different types of research needs. Recently we have developed a label free quantitation algorithm called AB3D as a new plug-in of Mass++, and we have successfully applied our algorithm to biomarker research and drug discovery. In this presentation, we will introduce our recent biomarker discovery results by utilizing quantitative features of Mass++ software.

Evening Seminar 2

Sunday, September 15
18:15-19:45
Room 315

Sponsored by
Agilent Technologies, Inc.

ES-02

2013 HUPO Meeting of the Minds

This unique event provides the opportunity to meet with HUPO keynote speakers and dignitaries during Agilent's cocktail reception.
This unique gathering's format will consist of 15-20 minute intervals for personal discussions with one or two dignitaries at a time, and then rotate to another table for additional discussions with other thought leaders in the proteomics field.

Evening Seminar 3

Tuesday, September 17
19:00-20:30
Exhibition Hall Session Space

Sponsored by Matrix Science Ltd.

ES-03

Mascot Insight: A New Application to Organise, Analyse and Report Mascot Search and Quantitation Results

Dr. Patrick Emery
Matrix Science Ltd. Senior Bioinformatician

As proteomics experiments become larger scale and more complex, one of the main challenges facing researchers and core facilities is the management and data mining of the generated data sets. Mascot Insight is a new platform which can take the results of Mass Spectrometry based sequence database searches, either from a Mascot server or from an MzIdentML export, and allows users to merge, filter, compare and annotate datasets and incorporate additional information from sources such as GO and molecular interaction databases. A variety of reports are supplied which facilitate analysis of discovery searches, quantitation experiments and de novo searches. Users can create additional reports using a simple Java based API.

POS-01-001 Basic Analysis for Elucidation of the Mechanisms of Acute Effects Induced by Excessive Drinking

Yuki Iwahara[1], Kazuma Higashisaka[1], Akiyoshi Kunieda[1], Kota Tanaka[1], Shin-ichi Tsunoda[2,3], Shunji Oshima[4], Yasushi Kitagawa[4], Yasuo Yoshioka[1], Yasuo Tsutsumi[1,2,3]

[1]Laboratory of Toxicology and Safety Science, Graduate School of Pharmaceutical Sciences, Osaka University, Japan, [2]Laboratory of Biopharmaceutical Research, National Institute of Biomedical Innovation, [3]The Center for Advanced Medical Engineering and Informatics, Osaka University, Japan, [4]Asahi Group Holdings, Ltd., Japan

It is known that light to moderate drinking could promote our health mentally and physically. However, excessive drinking has various adverse effects both in acute and in chronic phase. In order to prevent excessive drinking and to promote proper drinking, it is necessary to evaluate and discriminate effects by excessive drinking. Furthermore, it is also important to elucidate the mechanisms how excessive drinking induces such effects. Although the evaluation of chronic effects, including clinical application of biomarkers, has already advanced, those of acute effects have not progressed yet. In this study, to evaluate acute effects of excessive drinking and to elucidate its mechanisms, we investigated comprehensive analysis of proteins in plasma of mice exposed to ethanol using proteomics. Difference of plasma protein levels was analyzed by SDS-PAGE and proteins in fractions showing different staining intensity were identified by LC/TOF/MS. As the result, fibronectin, one of extracellular matrix, was identified only in control group, whereas not identified in excessive drinking group. In addition, ELISA analysis showed that the plasma levels of fibronectin were significantly decreased in excessive drinking group compared with control group. It is reported that fibronectin participates in platelet aggregation and that is suggested that decrease of plasma fibronectin levels could relate to suppress coagulation system. Thus, we are now trying to elucidate the association between coagulation and excessive drinking. It is expected that elucidation of the mechanisms not only of adverse effects by over drink, of positive effects by moderate drink also leads to promote appropriate drinking.

POS-01-002 Enrofloxacin Resistance of Uropathogenic Canine *Escherichia coli* Reveals Mechanism Involved in DNA Repair

Cristian Piras[1], Soggiu Alessio[1], Viviana Greco[2], Piera Anna Martino[1], Andrea Urbani[2,3], Jarlath Nally[4], Luigi Bonizzi[1], Paola Roncada[1,5]

[1]DIVET, Universita' degli studi di Milano, Italy, [2]Fondazione Santa Lucia, IRCCS, Italy, [3]Dipartimento di Medicina Sperimentale e Chirurgia, Universita degli Studi di Roma Tor Vergata, Italy, [4]School of Veterinary Medicine, UCD, Ireland, [5]Istituto Sperimentale Italiano L. Spallanzani, Italy

Urinary tract infections, including cystitis and pyelonephritis, are one of the most common infectious diseases of humans and domestic animals. The pathogen that most frequently is responsible of such diseases is *Escherichia coli*. Commensal *E. coli* in the intestinal tract colonizes the vaginal and peri-uretheral tissues, ascend into the bladder and cause the infection. The pathogenic potential of uropathogenic *E. coli* (UPEC) is thought to be dependent on both host and bacterial factors and, because of its potential zoonotic risk, it represents a major problem for public health(1). One recent concern is the increased resistance to antimicrobials observed in canine urinary tract isolates, in particular for fluoroquinolones. This study was conducted to investigate the proteins involved in mechanisms of antibiotic resistance (enrofloxacin) of *Escherichia coli* isolates from urine of dogs. The experiments were conducted on dog urinary clinical isolates of *E. coli* induced to antibiotic resistance with increasing enrofloxacin concentrations. 2D DIGE of clinical isolated vs antibiotic resistant isolates was performed and differentially expressed proteins were identified through MALDI-MS/MS, as reported in (2). Among the nineteenth differentially expressed proteins, the most important findings, were that mechanism of antibiotic resistance is mediated by the decreased expression of outer membrane proteins and by the increased expression of DNA repair proteins. This data is in agreement with the mechanism of enrofloxacin, a strong inhibitor of DNA gyrase, the major bacterial target of quinolones.

1. Nam, E.H., et al., J Microbiol Biotechnol, 2013. **23**(3): p. 422-9.Harada, K., et al.,
2. Piras, C., et al., Mol Biosyst, 2012. **8**(4): p. 1060-7.

Keywords: UPEC *E. coli*, enrofloxacin antibiotic resistance, canine animal model

POS-01-003 Profiling of Thermostable Proteins in Diabetic Cardiovascular Rat Plasma

Thi Minh Phuong Nguyen, Viet Binh Doan, Kim Dung Nguyen, Thai Thuong Tran, Thi Bich Thao Le, Bich Nhi Nguyen, Dinh Minh Pham, Van Chi Phan

Vietnam Academy of Science & Technology, Vietnam

Cardiovascular disease (CDV) is a major chronic complication and the leading cause of early death among people with type 2 diabetes mellitus (T2DM). People with T2DM often have low HDL cholesterol level, raised triglyceride level, high blood pressure, smoking, obesity and physical inactivity that contribute to their risk for developing CDV. Diabetic cardiovascular disease can damage the structure and function of the heart and it can lead to heart failure and arrhythmias.

Therefore, research on diabetic cardiovascular disease is very important to achieve decreased mortality for patients with T2DM. Nowadays, diabetic cardiovascular disease has been studied on animal models coupled with proteomics techniques. In this study, a rat model of diabetic cardiovascular disease was developed by high fat feeding and low dose injection of streptozotoxin. Plasma of diabetic cardiovascular rats (T2DM_CDV) and controls have been used for thermostable fractionation and proteome profiling. The thermostable proteins have been separated and identified by using two-dimensional electrophoresis combined with nanoLC-MS/MS. Our result was shown that at least five proteins (Fibrinnogen alpha chain, Antithrombin-III precusor, Angiotensinogen 1, Haptoglobin, Haptoglobin alpha 1S) were significantly up-regulated and three proteins (Apolipoprotein A-IV, Apolipoprotein E, Apolipoprotein A-I) were down-regulated in T2DM_CDV samples. In which, Antithrombin-III is the most increased (2.87 folds), Fibrinogen alpha chain is increased 2.02 folds, Angiotensinogen 1 (1.42 folds), Haptoglobin (1.97 folds), Haptoglobin alpha 1S (1.59 folds). Apolipoprotein A-I is decreased 1.37 folds, while Apolipoprotein A-IV and Apolipoprotein E are not appearance in T2DM_CDV rats plasma. Our results suggest that the mentioned above proteins might be related to the generation and progression of diabetic cardiovascular disease.

Keywords: diabetic cardiovascular, rat plasma, thermostable protein

POS-01-004 Integrated Proteomics for the Study of Metastatic Human Tongue Cancer Development in a Heterogeneous Microenvironment

Masayo Wilson Morifuji, Akiko Niibori Nambu, Daiki Kobayashi, Norie Araki

Dept. Tumor Gen. Biol., Grad. Sch. Med. Sci., Kumamoto Univ., Japan

Tongue cancer has been shown to be the most metastatic of oral cancers in patient cases. To understand the behavior of metastatic cells and the mechanism of metastatic cancer cell development in a heterogeneous tumor microenvironment, we studied human tongue cancer cell lines with highly metastatic (HM) and non-metastatic (NM) properties, established from the same patient. We created an orthotopic xenograft mouse model via co-injection of dsRed NM and GFP HM clones, and observed that HM cells grew aggressively in the tumor center and formed metastases, while NM cells translocated to the tumor margin without metastasis. The cell lines were then compared using differential proteomic (iTRAQ) and transcriptomic (DNA chip) analyses, followed by network analysis (KeyMolnet) of the combined mRNA and protein data. With the statistically highest score, we detected the HIF (hypoxia inducible factor) signal pathway as being specifically upregulated in HM cells. Detailed analysis of identified signal network showed that 30 (75%) of 41 proteins displaying higher expression in HM were directly or indirectly related to HIF signal transduction. Immunoblotting and immunocyto-chemistry further revealed up-regulation of the expression and activation of HIF-1α in HM. For biological validation, we prepared HIF-1α knockdown HM (HM-HIF^{KD}) cells and observed the cellular proliferation and motility in 2-/3-dimensional co-culture systems of NM and HM or HM-HIF^{KD} clones. The results showed that growth of NM cells was suppressed with the co-culture of HM cells, while the NM cell growth normalized with HM-HIF^{KD} cells. In time-lapse video analysis, HM cells showed aggressive growth with crowding-induced exclusion of NH cells. HM-HIF^{KD} cells, however, had no effect on NM cell growth. These results demonstrate that HIF-1α related signals are important in the development of highly metastatic tumors in a heterogeneous microenvironment.

Keywords: cancer, metastasis, heterogeneity

POS-01-005 Plasma Proteomic Pattern Analysis for Swine Partial Hepatectomy Model

Kohta Iguchi[1], Masaya Ikegawa[2], Etsuro Hatano[1], Tomohito Sakai[2], Kenya Yamanaka[1], Motohiko Sato[1], Gen Yamamoto[1], Tatsuya Okamoto[1], Yosuke Kasai[1], Satoru Seo[1], Kojiro Taura[1], Kei Tashiro[2], Shinji Uemoto[1]

[1]Department of Surgery, Graduate School of Medicine, Kyoto University, Japan, [2]Department of Genomic Medical Science, Kyoto Prefectural University of Medicine, Japan

Background: Partial hepatectomy is a feasible procedure for the treatment of liver diseases. Monitoring liver function during and after surgery is crucial for the improvement of post-operative course. The pig has recently become increasingly relevant as a model organism for biomedical research. Here we try to elucidate plasma proteomic biomarkers on swine partial hepatectomy model.
Methods: A 70% partial hepatectomy was successfully performed for fifteen micro minipigs (MMPs) which were one to two years of age. Plasma samples were collected at 0, 1, 3, 6, 24, 48, 72, 96, 120, 144, and 168 hours after operation. Pig plasma peptides/proteins were purified with C8 magnetic beads and obtained spectra by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The spectra were analyzed and multivariate statistics and receiver operation characteristics were calculated using ClinProTools 2.2™.
Results and Discussions: Chronological distribution of plasma proteomic pattern was defined with machine learning method. By building a support vector machine classifier, an effect on plasma proteomic pattern of an administration of phosphodiesterase III inhibitor, olprinone, was clearly detected with good cross validation accuracy around perioperative period. Of note, some peaks enabled an annotation of the stages from immediate early to recovery stages after hepatectomy which retained the normal liver function defined by biochemical indices.
Conclusion: ClinProt system is a promising biomarker strategy to evaluate the postoperative liver function through plasma proteomic pattern analysis for swine 70% partial hepatectomy model.
Keywords: pig, hepatectomy, plasma proteomics

POS-01-006 Biomarkers of Diabetes in Plasma of NOD Mice

Juliana Almada Colucci[1], Juliana Perez[1], Maisa Mayumi Sakata[1], Regina Lucia Harumi Watanabe[3], Fernando Cesar Bizerra[2], Dulce Elena Casarini[1]

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Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia and it has become a global epidemic in the past years. The heterogeneity in the determining factors for the development of diabetes and its complications represent a big challenge in the advance of new strategies for prevention and treatment. In the present study we propose the investigation of potential biomarkers of diabetes, as well as biomarkers of early stages of diabetic nephropathy in plasma of non-obese diabetic mice using proteomic analysis. Plasma samples from adult diabetic (n=6) and non diabetic NOD (n=4), as well as Swiss mice (n=5), were collected and processed. Individual protein samples were analyzed by NanoUPLC tandem nanoESI-LC MS^E. A total of 141 proteins were identified in all three groups but 10 proteins were exclusively expressed in the diabetic group. Among those ten, candidates had already been associated to type 1 diabetes prevention, endothelial function impairment, retinopathy, diabetes related oxidative stress and one had been described as early biomarker for the disease. Seven proteins were only present in the Swiss strain and 8 were exclusive of the NOD mouse lineage, independent of the diabetic state. NOD mice are not born diabetic and the incidence on the colony is not 100% so our next step will be to follow NOD from early age to adulthood collecting samples at different time points to better understand the change in the proteomic profile that occurs in the diabetic state.
Keywords: diabetes, NOD mice, biomarkers

POS-01-007 Aortic Stenosis in a Rabbit Model: Proteomic Analysis of Valve Tissue

Laura Mourino-Alvarez, Fernando de la Cuesta, Montserrat Baldan-Martin, Maria G. Barderas

Vascular Physiopathology Department, Hospital Nacional de Paraplejicos, Spain

Degenerative aortic stenosis (AS) is the most common valvular disease in older population, being the main cause of aortic valve (AV) replacement in developed countries. It is characterized by thickening of the leaflets followed by progressive calcification of the AV.
A cholesterol and vitamin D_2-enriched-diet induces the development of AS in rabbit animal models. Therefore, they serve as surrogates for human in obtaining faster information with a lower number of samples. A proteomic approach to study stenotic AV in an AS animal model might provide useful information to understand AS development and it is an invaluable tool to search for potential biomarkers of disease.
Male New Zealand White rabbits were fed *ad libitum* with 1% cholesterol-enriched-diet + 50,000 IU vitamin D_2 for 12 weeks. Additionally, rabbits fed with normal rabbit chow were used as controls (n = 7). The model was monitored through blood analysis and echocardiographic measures to secure an adequate development. After sacrificed, AVs were extracted, lysed and analyzed by 2D-DIGE. Histological analysis confirmed AV degeneration in treated animals.
Fourteen spots with altered expression levels (average ratio≥1.5 or ≤-1.5 and p-value≤0.05) were found altered. Identifications were performed by MALDI-TOF/TOF analysis. Among identified proteins, there is a cardiac tissue-specific subgroup with proteins that have shown evidences of alteration in human AV, which have been further analyzed by immunohistochemistry.
This analysis provides additional information to deepen our knowledge of the pathophysiology of this disease.
Keywords: aortic stenosis, rabbit model, valve tissue

POS-01-008 Proteomic Analysis of Left Ventricular Tissues in Dilated Cardiomyopathy Mouse Models

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Dilated cardiomyopathy (DCM) is an intractable disease, and neither its radical treatment other than cardiac transplantation nor its differential diagnostic procedure has been established. To develop these methods for DCM, it is important to understand its pathogenic mechanism. Because phenotypes of causative gene mutations in the DCM are rather common regardless of the genes, it is essential to analyze proteomic changes associated with its progression. In this study, we performed proteomic analysis of left ventricles (LVs) of 4C30 mouse, a DCM model overexpressing Gal-β-1,3-GalNAc-α-2,3-sialyltransferase 2. 4C30 mouse shows a severe symptom of DCM around 24 weeks. Frozen LV tissues were pulverized, denatured, and digested with trypsin in the presence of sodium deoxycholic acid. After desalting, the digests were analyzed by nanoLC-MS/MS (Triple TOF5600) and differential protein expression was quantitatively evaluated with a 2DICAL software. Among 894 identified proteins, 186 and 145 proteins showed significant increases or decreases in 4C30 mice, respectively, compared with age-matched wild-type mice. Proteins associated with fibrosis, remodeling of extracellular matrix and cytoskeleton, and endoplasmic reticulum stress were increased, while energy metabolic enzymes involved in the glycolysis, TCA cycle, and β-oxidization were remarkably decreased. Since β-oxidization system in particular is a main energy source for the heart, this energy depletion might underlie conditions leading to DCM. In addition, regulatory proteins of intracellular calcium levels were also decreased, suggesting malfunction of the cardiac contraction and relaxation. These proteins in the energy metabolism and calcium regulation may be useful for diagnosis and treatments of DCM. We are currently analyzing another mouse model to find common features for understanding pathogenesis of the DCM.
Keywords: dilated cardiomyopathy, 4C30 mouse

POS-01-009 Plasma Proteomic Pattern Analysis for Murine Experimental Autoimmune Encephalomyelitis (EAE) Model

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Background: Multiple Sclerosis (MS) is the most common demyelinating disease of the CNS where current treatments have limited effectiveness. Experimental autoimmune encephalomyelitis (EAE), has developed with pathology including demyelination and axonal damage and clinical events such as relapsing and remitting episodes of paralysis, all of which are features common to MS. Here we adopt a plasma proteomic pattern analysis using murine EAE model. **Methods**: For active induction of EAE, female SJL/J mice were immunized with myelin basic protein (MBP) derived peptides at 10 wk of age. Mice were observed for clinical signs of EAE and scored on a scale according to the severity of the clinical signs. Murine plasma peptides/proteins were purified with C8 magnetic beads using a robotics (ClinProtrobot system) and obtained spectra by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Autoflex II). The spectra were analyzed and multivariate statistics and receiver operation characteristics were calculated using ClinProTools 2.2™. **Results and Discussions**: EAE have been developed with pathology including demyelination and axonal damage and clinical events such as relapsing and remitting episodes of paralysis, all of which are features common to MS. Plasma proteomic pattern was clearly altered with progression of EAE symptoms by machine learning method. By building a support vector machine classifier, an effect on plasma proteomic pattern of EAE was clearly observed with good cross validation accuracy from 13 post immunization day (pid). Of note, some peaks enabled an annotation of clinical stages such as remission and relapsing of the EAE. **Conclusion:** Plasma proteomic pattern analysis is a promising and a reliable biomarker strategy in applying murine EAE model.

Keywords: multiple sclerosis, Experimental autoimmune encephalomyelitis, proteomic pattern analysis

POS-01-010 Non-Genomic Regulation of Hyperactivation by Progesterone and Estradiol in Hamster Spermatozoa

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Mammalian spermatozoa have to be capacitated in order to be fertilized eggs. During capacitation, they show an acrosome reaction and a hyperactivation. Although the acrosome reaction is regulated by a non-genomic regulation of steroids (i.e. progesterone and estrogen), it is not well known whether the hyperactivation is regulated by steroids.

In the present study, progesterone could enhance sperm hyperactivation through a non-genomic regulation. Most effective concentration of progesterone for enhancement of hyperactivation was 20ng/ml. Moreover, PLC-IP_3 signals, PLC-DAG-PKC signals and PKA signals were associated with a non-genomic regulation of progesterone. Enhancement of hyperactivation by progesterone was suppressed by 17β-estradiol in a dose dependent manner. Threshold of 17β-estradiol was 1/1000 to 1/100 of concentrations of progesterone. Moreover, progesterone-enhanced hyperactivation was suppressed by 17β-estradiol by a non-genomic regulation which was associated with inhibition of tyrosine phosphorylations.

Next, suppression of progesterone-enhanced hyperactivation by 17β-estradiol was disturbed by diethylstilbestrol. Diethylstilbestrol significantly but weakly suppressed progesterone-enhanced hyperactivation. When spermatozoa were simultaneously exposed to non-effective concentrations of 17β-estradiol and diethylstilbestrol, diethylstilbestrol accelerated a suppressive effect of 17β-estradiol, and strongly suppressed progesterone-enhanced hyperactivation together with 17β-estradiol.

From results, it is suggested that sperm hyperactivation is competitively regulated by changing concentrations of progesterone and 17β-estradiol associated with female estrous cycle. Moreover, it is also suggested that diethylstilbestrol disturbs the non-genomic regulation of sperm hyperactivation by progesterone and 17β-estradiol playing as accelerator of 17β-estradiol.

Keywords: non-genomic regulation

POS-01-011 A Peptide Profile of Amniotic Fluid in a Fetal Lamb Model of Gastroschisis

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Objective. To clarify the pathophysiology of gastroschisis, we elucidated a peptide profile of amniotic fluid in a fetal lamb model of gastroschisis. ***Methods.*** Gastroschisis was surgically created in 3 fetal lambs at 60 days gestation. At term of 145 days, amniotic fluid from the 3 fetal lambs with gastroschisis and 4 healthy fetal lambs was extracted, from which peptides were purified by magnetic beads-based weak cation exchange. The extracted peptides were detected by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS). **Results.** 77 peptide peaks were detected in the aminiotic fluid samples by MALDI-TOF/MS. 12 out of the 77 peptide peaks were significantly different between the gastroschisis group and the healthy group ($P<0.05$). Peptides with m/z 2632.83, 2922.12, 2937.53, 5447.09, 5486.58, 6867.34 8851.34, 9157.44 and 9173.59 showed higher ion intensity in the gastroschisis group than in the healthy group. Peptides with m/z 2047.98, 2232.5, and 4134.43 showed lower ion intensity in the gastroschisis group than in the healthy group.

Conclusion. A peptide profile of amniotic fluid in fetal lambs with gastroschisis was different from that in healthy ones. The distinct peptides, which may be involved in the pathophysiology of the gastroschisis, should be identified.

Keywords: amniotic fluid, gastroschisis, peptidomics

POS-01-012 A Method for Simultaneous Quantitation of Underivatized Metabolites in the Rat Lens by LC-q-TOF-MS

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Cataract is the leading cause of vision impairment in the world. Lens metabolites play an important role in the processes leading to protein aggregate formation. The lens does not have vascular system, and the antioxidant protection is provided by the metabolite diffusion into the lens from outer tissues. Therefore, the study of changes in the metabolite concentrations in a cataractous lens compared to a normal lens may elucidate the possible mechanisms of cataract formation.

This report provides the data on the lens quantitative metabolomic analysis of two rat strains - senescence-accelerated OXYS rats and Wistar rats. The method of quantitation without a preliminary derivatization step is based on the HPLC separation of the lens protein-free extracts with ESI-q-TOF mass spectrometric detection. The compounds in LC-MS experiment were identified by the combination of several parameters: comparison of accurate m/z values with theoretical, MS/MS data, and the comparison of the retention time of analytes with standards. Then the areas of peaks on extracted ion chromatograms of the target metabolites had been compared with the calibration curves to obtain quantitative data. This allowed us to detect and quantify more than 40 metabolites in each sample. The measured values were in range from thousands to tens nmole/g. The most abundant amino acid is taurine (~5000 nmoles/g), the most abundant antioxidant is GSH (~1500 nmoles/g); the least abundant are AMP (~9 nmoles/g) and asparagine (~8 nmoles/g).

A comprehensive quantitative metabolic profile of the rat lens has been acquired for the first time. The obtained data can be used for the analysis of changes in the lens chemical composition occurring with age and with the cataract development.

We appreciate: Projects 11-04-00143, 11-0300296, 12-04-31244; Grants 11.G34.31.0045 and 8094; Grant NSh-2429.2012.3.

Keywords: cataract, aging, quantitation

POS-01-013 Phosphoproteome Dynamics of WHV/myc Transgenic Mouse Model During HCC Carcinogenesis

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As the rapid improvement of mass spectrometry instruments and phosphopeptide enrichment technologies, phosphoproteomics has gained the capability to identify and quantify large amounts of phosphosites with high accuracy and efficiency, and has been widely used in diseases research and drug development. In this work, we combined the Titania enrichment, TMT labeling, and SCX fractionation to elucidate the dynamic phosphoproteome regulation of HCC (Hepatocellular carcinoma) pathogenesis through comparison of WHV/*myc* transgenic mouse and normal C57BL/6 mouse at 6 different stages (10day, 2mon, 3mon, 5mon, 7mon, 11mon). A total of 4161 unique phosphopeptides and 1900 phosphoproteins were quantified across 6 stages, with protein FDR set at 0.01. These phoshopeptides were clustered into different groups according to change trends at 6 stages by HCA and C-means clustering. Combining the biological function analysis revealed that, in WHV/*myc* transgenic mouse, many phosphoproteins participating in DNA metabolism, RNA metabolism, nucleotide metabolism, mitosis and cell mobility were up-regulated significantly from 5mon to 7mon, and some recovered at 11mon. Many other phosphoproteins involved in carbohydrate metabolism, endocytosis, mTOR signaling pathway, ErbB signaling pathway and apoptosis were down-regulated from 5mon to 7mon. However, these phosphoproteins showed little change in normal C57BL/6 mouse. Also, many key metabolism enzymes and kinases were regulated dramatically during hepatocarcinogenesis, which may be potential HCC biomarkers and therapeutic targets.

Keywords: HCC carcinogenesis, WHV/myc transgenic mouse, phosphoproteome dynamics

POS-01-014 Protein Networks and Cellular Pathways Altered in Severe Cases of West Nile Virus Infection

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Background: The recent West Nile virus (WNV) outbreaks in developed countries have been associated with significantly higher neuropathology incidence and mortality rate than previously documented. The changing epidemiology and the lack of effective human antiviral therapy or vaccines make understanding the pathogenesis of severe disease a priority. Thus, to gain insight into the pathophysiological processes in severe WNV infection, a kinetic analysis of protein expression profiles in the brain of WNV-infected mice was conducted using samples prior to and after the onset of clinical symptoms. *Methodology/Principal findings:* Using quantitative proteomic approaches, a set of 148 proteins with modified abundance was identified. The bioinformatics analysis (Ingenuity Pathway Analysis) of each protein dataset originating from the different time-point comparisons revealed that four major functions were altered during the course of WNV-infection in mouse brain tissue: i) modification of cytoskeleton maintenance associated with virus circulation; ii) deregulation of the protein ubiquitination pathway; iii) modulation of the inflammatory response; and iv) alteration of neurological development and neuronal cell death. *Conclusion:* This study provides novel insights into the *in vivo* kinetic host reactions against WNV infection and the pathophysiologic processes involved, according to clinical symptoms. This work offers useful clues for anti-viral research and further evaluation of early biomarkers for the diagnosis and prevention of severe neurological disease caused by WNV.

Keywords: West Nile Virus, pathways deregulated, protein networks

POS-01-015 Nutritional Proteomics for the Study of the Suppression of High Fat Diet-Induced Pre-Diabetes by Omega-3 Polyunsaturated Fatty Acids

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Omega-3 polyunsaturated fatty acids (PUFAs) have been reported to render health beneficial effects based on numerous nutritional and epidemiology studies. We are interested in the molecular mechanisms underlying the nutritional health effects of omega-3 PUFAs. In this study, we investigated the molecular mechanisms underlying the suppressive effect of type-2 diabetes by omega-3 PUFAs by mass spectrometry-based proteomics.

We have raised C57BL/6 mice under different dietary conditions in the high fat (55%) diet background to modify the *in vivo* concentrations of omega-3 and omega-6 PUFAs. Omega-3 PUFAs diet exhibited significant suppression of pre-diabetic parameters compared to omega-6 diet as revealed by glucose tolerance test. By shotgun proteomics analyses of the livers of these mice we identified several classes of proteins that were up-regulated or down-regulated. These protein changes are induced by diets and appear to underlie the metabolic disturbance caused by the high-fat diet. Furthermore, addition of omega-3 PUFAs to the same high fat diet background suppressed or modulated some of these protein changes. These protein changes are likely to be in the causal chain of molecular signaling processes in which omega-3 PUFAs function as an ameliorating modulator of pre-diabetic conditions.

Thus our study demonstrates that application of proteomics would be a powerful protocol to understand the molecular mechanisms underlying the nutritional effects of omega-3 and omega-6 PUFAs in health and disease.

Keywords: omega-3 polyunsaturated fatty acids, nutritional proteomics, diabetes

POS-01-017 Analysis of the Secretome of Cultured Cells with Antibody Microarray

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Antibody microarray is a growing proteomics methodology endowed with high potential in terms of specificity, sensitivity and simplicity. The method virtually enables the analysis of various sample types like body fluids as well as cells and tissue specimens. The proteomic analysis of cell conditioned media however is restricted to the sandwich-based rather than the direct antibody array. This is feasible due to the fact that direct antibody microarray entails a protein concentration high enough (≥1 mg/ml) for successful labeling as a prerequisite step for analysis, which is not the case with the sandwich arrays that require no protein labeling. Still, the sandwich antibody microarray allows for a very limited multiplexing, where the superiority of direct array platform prevails. In the present work, a protocol is described for the first time that allow the analysis of secretome from cell culture media via direct antibody microarrays, hence allowing for further multiplexing and throughput analysis of such samples. The presented protocol showed high quality and reproducibility as it used to analyze 16 pancreatic cancer cell lines culture media as well as different cell types under treatment/stimulation. With the growing interest of cell secretome as a promising source for diagnostic and biomarker discovery the present method provides a broader coverage sample types for high multiplexing proteome analysis using antibody microarrays

Keywords: antibody microarray, secretome, cell culture

POS-01-018 Development of a MSIA UPLC-MS/MS Assay for the Analysis of Interleukins

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Interleukins, a family of proteins involved in intercellular signaling are present at pg/mL levels in blood. The mass spectrometric measurement of secreted interleukins has been challenged by the low levels at which these markers are found in a highly complex matrix. A sensitive, robust, fast and easy to use solution can be achieved through use of mass spectrometric immunoassay (MSIA) tips for the targeted enrichment of proteins of interest at a clinically relevant level. We present a targeted MSIA workflow for the detection of multiple interleukins of interest in the study of sepsis. Sepsis results from severe local or systemic infections such as appendicitis, pneumonia, bacteremia, diverticulitis, pancreatitis, and necrotizing fasciitis. Fatality from sepsis is typically 25-50%. Early diagnosis of sepsis is critical in patient survival. Gram- negative bacteria account for 50% of all cases of sepsis, and a major component, lipopolysaccharide (LPS), contributes greatly to septic shock. LPS activates secretion of proteins involved in intercellular signaling such as the interleukin family of proteins. Interleukins of interest were extracted with high-throughput from human serum. Following affinity co-enrichment and co-elution, samples were reduced, alkylated and trypsin digested prior to analysis by LC-MS.

Keywords: MSIA, cell signaling, sepsis

POS-01-019 Establishment of Peptide Immunoaffinity Enrichment-Coupled Mass Spectrometric Assays for Oral Cancer Biomarker Validation in Body Fluids

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Mass spectrometry-based multiple reaction monitoring or selected reaction monitoring assay (MRM/SRM assays) using isotopically coded peptides as standards to trace the presence and abundance of target peptides provides a high throughput and multiplexed protein expression profiling of clinical samples without antibody. Using stable isotope standards and capture by anti-peptide antibodies (SISCAPA), the sensitivity of SISCAPA-MRM assay could further be improved hundred-to-thousand times than the traditional MRM assays. Oral squamous cell carcinoma is one of the common cancers in the world, and is ranked the 6th leading cause of death from cancer for the whole population in Taiwan since 2004. Using genomic and proteomic approaches, many genes/proteins dys-regulated in oral cancer have been discovered. However, there are still no markers approved by the Food and Drug Administration to support early detection and prognostic prediction of oral cancer. To find useful markers for aiding oral cancer management, we surveyed potential marker candidates from hundreds of published literatures and our in-house oral cancer marker datasets discovered from tissues and cancer cell lines by proteomics and genomics. Fifty protein candidates preliminarily verified in small cohort of oral cancer specimens were selected for monoclonal antibody production and SISCAPA-MRM assay development. To improve the performance of SISCAPA assay, methods for immunoaffinity enrichment of target peptides from saliva/plasma are optimized and discussed.

Keywords: SISCAPA, MRM, oral cancer

POS-01-020 Structural Comparability of the Biosimilar and Innovator Version of a Recombinant Monoclonal Antibody

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Biosimilars will likely see considerable growth in the near future. The consensus from regulatory bodies is that a biosimilar must be highly comparable to the reference therapeutic with any differences justified. Herceptin® (Roche) is a therapeutic recombinant monoclonal antibody approved in 1998 for the treatment of breast cancer. It has been effective in treating the 20-30% of metastatic breast cancer cases - where overexpression of HER2 is the cause - by binding to the extracellular domain of the receptor. A biosimilar to Herceptin® has been developed; we have demonstrated a structural comparison of the former to several lots of the latter. The data takes into account aggregation, charge heterogeneity, disulfide bonding structure, glycosylation and other post-translational modifications (PTMs) and higher-order structure of the therapeutic antibodies using size-exclusion chromatography, SDS-PAGE, ion-exchange chromatography, glycan analysis, peptide mapping with LC-MS, subunit mass spectrometry analysis, circular dichroism and intrinsic tryptophan fluorescence for analytical comparison. The structural integrity of an antibody is essential to its efficacy and safety as a therapeutic. Modifications can affect the overall structure, antigen binding and effector functions, which all contribute to the effectiveness of the therapeutic. The data show that the biosimilar is highly comparable to the Herceptin® lots with the exception of a C-terminal lysine present on the former that is absent on the lots of the latter. This modification is known to not affect the action of the therapeutic. Other differences were minor and are acceptable given the guidelines of the regulatory agencies.

Keywords: biosimilars, analytical protein chemistry

POS-01-021 Raising Monoclonal Antibody Against Cell Membrane Proteins Based on Flow Cytometry Screening and Protein Array Identification

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High-quality antibodies against membrane proteins and their associated partners have been challenging to generate using conventional means. In this report, we immunized mouse with whole intact cancer cell and screened the hybridomas by the intact cell in ELISA assay after cell fusion. The positive hybridomas were further analyzed by flow cytometry to confirm if the antibody recognize cell membrane-related protein. The corresponding antigen of hybridomas was identified using protein array and verified by western-blot. As results, we obtained eight positive hybridomas in cell-based ELISA screening. Among them, two have higher affinity. Several proteins showed reactive to these two monoclonal antibodies by NAPPA protein array analysis, the potential antigen was further verified by western-blot. This strategy of generating monoclonal antibodies against membrane-related proteins may provide useful tools for exploring of the physiological process on cell surface.

Key words: Membrane-related protein, monoclonal antibody, flow cytometry, protein array

Keywords: membrane-related protein, monoclonal antibody, protein array

POS-01-022 Classification of Cancer by Multiple Discriminant Analysis for Relationship between Cancer and Expression of Human Cellular Phosphoprotein

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This is the first study to investigate the potential for classification of cancer using anti-phosphoprotein monoclonal antibodies (PPmAbs) and multiple discriminant analysis (1). Although numerous cancer proteome studies have been performed in many laboratories across the world, these studies have been focused on the expression of relatively abundant and specifically cancer-related proteins. We have generated over 150 hybridoma clones producing monoclonal antibodies (mAbs) against a human phosphoprotein mixture derived from a human leukemia cell line. The expression profiles of 22 cell lines from 9 different types of cancer using PPmAbs were examined. Multiple discriminant analysis is a mathematical method that may be used for the profiling of phosphoprotein expression in cancer cells and may improve the classification power, thereby increasing the final discrimination rate (2). The relationship between cancer cells and the expression of human phosphoprotein in the cells was used to construct a diagnostic system for cancers. Multiple discriminant analysis was able to successfully classify the cell lines into the correct cancer group by using the diagnostic system for cancers. These results show that multiple discriminant analysis based on phosphoprotein expression in cells or tissues may be a potentially valuable method for assisting in the classification of several cancers. REFERENCES (1) Motofuji Y., et al., Potential of classification of cancer by multiple discriminant analysis for relationship between cancer and expression of human cellular phosphoprotein. Biomed Res 33:139-43 (2012). (2) Duda R., et al., Pattern classification, Second edition. New York, NY, USA: John Wiley and Sons (2001).

Keywords: monoclonal antibody-based phosphoproteomics, multiple discriminant analysis, cancer diagnosis

POS-01-023 High-Throughput Production of Antibodies Within the Swedish Human Protein Atlas Project

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The Swedish Human Protein Atlas (HPA, www.proteinatlas.org) project was set up to systematically characterize the human proteome using polyclonal antibodies. After more than nine years of production, 75% of the human protein-coding genes have been studied. To achieve this, lots of effort has been put into development of high-throughput protocols for cloning, antigen production and affinity-purification of antibodies.

The phase where the great majority of the "low hanging fruits" have been successfully produced has now been reached. Therefore, the focus has gradually shifted towards antigens that are difficult to produce and non-functional antibodies. These antigens and antibodies need to be studied one-by-one. To face this

challenge, different alternative protocols for successful cloning, antigen production as well as successful purification of the antibodies have been developed. Optimizations of the standard protocols are, and will be, essential for a successful production of the more challenging clones, antigens and antibodies to be able to cover the remaining protein-coding genes.

Keywords: cloning, protein production, antibody

POS-01-024 Subcellular Localization of the Full Human Proteome Calls for a Selective Choice of Cells

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Knowing the subcellular location of a protein is key to understand the complex machinery of the living cell. One major challenge in systematic efforts to characterize the full human proteome is to find suitable samples, expressing each and every target protein.

The Subcellular Protein Atlas aims to systematically localize the entire human proteome on a subcellular level using an antibody-based approach, combining immunostaining and high-resolution confocal imaging, as part of the Human Protein Atlas Project. A new approach has been adapted, using RNA-sequencing as a proxy for protein expression levels to select suitable cell lines individually for each and every protein to be studied. By combining a panel of 15 human cell lines of different tissue origin, a high coverage (78%) of the human genome can be obtained, resulting in a significantly higher success rate of protein localization.

In order to reach the goal of localizing the full human proteome, cell sources need to be found for the remaining 22 % of the genes not expressed in these cell lines. A bioinformatics analysis of the non-expressed genes showed that genes involved in neurological or hematopoetical functions as well as membrane proteins were highly overrepresented. In order to cover this gap we have optimized our high-throughput protocols to also enable characterization of cells in suspension, stem cells and differentiated mature neurons.

Here, we present results showing that a combination of high-throughput methods, and a diverse array of cells and cell lines that allow characterization of rarely expressed proteins, is crucial in order to successfully analyze the full human proteome on a subcellular level.

References:
1. Wiking et al, J Proteome Res, 2013 Jan 4

Keywords: Antibody, Human Protein Atlas, Subcellular localization

POS-01-025 Mgl-1 Regulated by Antagonistic Functions of STIP1 and DUB Enzyme USP-t

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The *lgl* gene encodes a cortical cytoskeleton protein, Lgl, which is involved in maintaining cell polarity and epithelial integrity. In our previous study, we have observed the ubiquitin-mediated degradation of Mgl-1, a mammalian homolog of *Drosophila* tumor suppressor protein Lgl. The protein degradation of Mgl-1 is prevented by a scaffolding protein RanBPM and enhances Mgl-1-mediated cell proliferation and migration. Using matrix assisted laser desorption/ionization -time of flight-mass spectrometry (MALDI-TOF-MS) analysis and co-immuno -precipitation assay, both HspB and STIP1 were identified as Mgl-1 interactors. Additionally, we have identified the deubiquitinating enzyme ubiquitin-specific protease-t (USP-t) as a novel regulator of Mgl-1 that counteracts on its protein degradation. The interaction between Mgl-1 and USP-t was confirmed by co-immunoprecipitation and GST pull-down assays. USP-t deubiquitinates and stabilizes Mgl-1 protein in a dose-dependent manner. In RanBPM knockdown cells, USP-t-mediated Mgl-1 protein stabilization was inhibited, indicating that RanBPM is essential for USP-t to deubiquitinate Mgl-1. Furthermore, we showed that the stabilization and function of Mgl-1 on cell migration were not regulated by USP-t in the absence of RanBPM. In addition, soft agar assay showed that the tumor mass was significantly increased in USP-t-depleted cells. Taken together, our study indicates that RanBPM acts as a modulator protein and recruits deubiquitinating enzyme USP-t to prevent Mgl-1 protein degradation.

Keywords: deubiquitinating enzyme

POS-01-026 Analysis of Differential Expression in a Four-Stage Human Cell Line Model for Malignant Transformation

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We have combined a quantitative transcriptomics strategy using deep RNA sequencing with an antibody-based protein profiling approach to study a human fibroblast cell line model representing four different stages on the route to malignancy. The cell line model was created in order to mimic the process of malignant transformation and goes from primary contact-inhibited cells, through immortalized and transformed cells to finally metastatic cells. RNA sequencing revealed that approximately 6% (n=1357) of the human protein coding genes is differentially expressed in at least one of the steps from normal to metastatic cells, with a majority of the genes being down-regulated. The group of up-regulated genes is highly enriched for genes involved in proliferative activity while the down-regulated genes are overrepresented by secreted proteins or proteins exposed on the cell surface, supporting the principle of dedifferentiation during malignant transformation. We demonstrate how a transcriptomics approach with following protein analysis can be used to define the changes that accompany the mechanisms related to immortalization, transformation and invasion/metastasis separately. The RNA sequencing data is currently analyzed on transcript level to also look for differential expression of splice variants, which will be followed by protein analysis using antibodies targeting different protein isoforms.

Danielsson, F., et al., *Majority of differentially expressed genes are down-regulated during malignant transformation in a four-stage model.* Proceedings of the National Academy of Sciences of the United States of America, 2013. **110**(17): p. 6853-8.

Keywords: cancer, antibody based proteomics, transcriptomics

POS-01-027 Detection and Clinical Validation of Circulating Autoantibodies as a Useful Sero-Diagnostic Markers for Non-Small Cell Lung Cancer

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Cancer tissues are comprised of various components including tumor cells and the surrounding tumor stroma, which consists of extracellular matrix and inflammatory cells. Since the tumor stroma plays important roles in tumor development, investigation of the tumor stroma in addition to tumor cells is important to identify useful tumor-associated markers.

To discover novel useful sero-diagnostic markers, a comparative study of tumor-associated autoantibodies (AAbs) in sera from lung adenocarcinoma (AD) patients was performed by two-dimensional immunoblotting with AD cell lines and each autologous AD tissue. Autoantigens identified from tissue and cell line samples comprised 58 spots (45 antigens) and 53 spots (41 antigens), respectively. Thirty-six proteins including Keratoepithelin (BIGH3), Link protein 1 (HAPLN1), Annexin A2 and Macrophage capping protein were detected only in tissues, 32 proteins were detected only in cell lines, and 9 proteins were observed in both. The expressions of BIGH3 and HAPLN1 were confirmed in the tumor stroma of tumor tissues, but not in AD cells by immunostaining and immunoblotting. Finally, the usefulness of autoantigens were validated using large number of sera from lung AD patients.

We suggest that investigation combining the two different abovementioned methods might be important to identify tumor-associated AAbs which play important roles in tumor development in tumor cells and tumor stromal components, because different AAbs were obtained based on the methodology used. Furthermore, these four autoantigen can be detected even in early lung ADs, it is suggested that these are useful as candidate novel sero-diagnostic markers for lung AD.

Keywords: lung cancer, tumor-associated autoantibody, cancer stroma

POS-01-028 Detection of Tumor Associated Antigens in Culture Supernatant Using Autoantibodies in Sera from Patients with Bladder Cancer

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The expression pattern of tumor secreted proteins are greatly different under various conditions. Due to their essential roles in process of tumorigenesis, the analysis of tumor secreted proteins has been suggested that the promising strategy for identifying cancer biomarkers. Subsequently, elicited humoral immune responses generate autoantibodies (AAbs) against these secreted proteins in the tumor microenviroment. It is reported that harnessing of humoral immune response to identify novel tumor biomarkers is an attractive strategy, because the immune system perform biological amplification.

In this study, we performed proteomic analysis to identify secreted antigens that are recognized by AAbs in sera of BC patients. Two-dimensional gel electrophoresis was performed to separate proteins that secreted from BC cell lines. Then, separated proteins were transferred onto PVDF membranes, and were reacted with mixed sera from patients with high-grade or low-grade BC, respectively. By comparing the immunoreacted patterns, 25 and 32 spots were specifically detected with sera from patients with high-grade and low-grade BCs, respectively. Autoantigens that were recognized by AAbs specifically in sera of high-grade or low-grade were identified by mass spectrometry.

In further study, we will confirm whether the levels of AAbs detected in this study are associated with tumor progression or prognosis of BC using by dot blot analysis with recombinant proteins, which are immunoreactive with AAbs.

Keywords: autoantibody

POS-01-029 Development of High-Throughput Screening System Using Autoantibody Library for Discovery of Scirrhous Gastric Cancer Biomarker

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Scirrhous gastric cancer is unique among gastric carcinomas. It is characterized by poorly differentiated carcinoma cells or signet-ring cells which diffusely infiltrate the gastric wall with provoking reactive fibrosis. The prognosis of patients with scirrhous gastric cancer is extremely poor and the 5-year survival rate is significantly lower than patients with other gastric cancer. In this study, we report on high-throughput screening system using a single-chain Fv (scFv) autoantibody phage library for finding novel diagnostic biomarker of scirrhous gastric cancer. The scFv-autoantibody phage library was constructed from peripheral blood lymphocytes from gastric cancer patients using polymerase chain reaction. We panned the constructed library with scirrhous gastric cancer, non-scirrhous gastric cancer, and disease-free control serum to make a sublibrary of antibodies that bind proteins differentially expressed, structurally changed, and post-translationally modified. This sublibrary antibody was printed on microarray and was incubated with labeled serum from multiple sets of scirrhous gastric cancer patients and gastric cancer patients or controls. Some antigens were differentially expressed in scirrhous gastric cancer as compared with the other groups. The results indicate that autoantibody phage library will be a powerful tool for discovery of diagnostic biomarker proteins.

Keywords: autoantibody library, scirrhous gastric cancer, biomarker

POS-01-030 Diagnostic Significance of MUC5B and TTF-1 Expression in Resected Non-Small Cell Lung Cancer

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To develop the diagnostic markers for lung adenocarcinoma (AD), we generated monoclonal antibodies using AMeX-fixed AD tissues as an antigen by employing the random immunization method. From obtained monoclonal antibodies, we selected the antibody which recognized MUC5B. To investigate the relationship between the expression of MUC5B and clinicopathological parameters, the expression of MUC5B in tumor cells was immunohistochemically studied in 167 consecutive cases of non-small cell lung cancer (NSCLC). The MUC5B expression was significantly higher in ADs than in squamous cell carcinomas (SCC) (P<0.00001). Clinicopathologically in ADs, MUC5B expression was significantly associated with poorer differentiation (P=0.0324) and poorer prognosis (P=0.0148). Multivariable analysis confirmed that MUC5B expression was significantly associated with survival (P=0.0136, Hazard Ratio:2.69, 95%Confidence Interval:1.23-5.90). To attempt improving the diagnostic accuracy of AD, we immunohistochemically studied in combination with the expression of TTF-1 known as lung AD marker and MUC5B in 167 cases of NSCLC. As a result, MUC5B yielded a sensitivity of 66.4% and a specificity of 90.6%, and TTF-1 yielded a sensitivity of 81.3% and a specificity of 96.9%. Combining data from MUC5B and TTF-1 improved the diagnostic accuracy when compared to TTF-1 alone, yielding a sensitivity of 97.0% and a specificity of 90.6%. Moreover, patients which are MUC5B (+) and TTF-1 (-) predict poor 5-year survival. The present study suggests that the markers combined MUC5B with TTF-1 should be a useful for discriminating ADs from SCCs and for a poorer prognostic indicator for lung AD patients. Immunostaining of TTF-1 and MUC5B are useful for definite diagnosis of lung ADs.

Keywords: lung cancer, biomarker discovery, Ramdom immunization method

POS-01-031 Analysis of Autoantibodies Related to Tumor Progression in Sera from Patients with pT1G3 Bladder Cancer

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Approximately 75% of the patients with bladder cancer (BC) are classified as a non-muscle invasive bladder cancer (NMIBC). NMIBC have a high recurrence rate after initial surgical resection and may progress to muscle invasive bladder cancer (MIBC) which are considered to be the poor prognosis. Easy selection of the patient with NMIBC who should receive more aggressive treatment is clinically important. In this study, autoantibodies (AAbs) against tumor associated antigens (TAAs) in the patients sera with pT1G3 BC were detected by proteomic analysis.

The equivalent amount of proteins, From BC cell lines were mixed and separated by two-dimensional gel electrophoresis, and transferred to the PVDF membrane. Then, the membranes were reacted with pooled sera from patients with each three of pT1G3 BC who progressed to MIBC or maintained in NMIBC as a primary antibody. Autoantigens that detected by circulating AAbs were identified by mass spectrometry.

As a result, 35-protein spots, 34 TAAs and protein spots, 24 TAAs were only detected in pooled sera from pT1G3 BC patients who progressed to MIBC and maintained in MIBC, respectively.

Further study may be need to be classified the utility of AAbs detected in the study as a sero-diagnostic marker which enable to predict the progression of NMIBC to MIBC.

Keywords: autoantibody

POS-01-032 Towards Automated Enrichment and Quantitation of Insulin Using Immuno-MALDI

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Synthetic insulins are commonly employed for the treatment of insulin-dependent diabetes. However, a usual and severe side effect of insulin therapy or treatment of diabetic patients with type 1 or type 2 diabetes is hypoglycemia. An overdose of insulin produces an hypoglycemic effect and can result in unconsciousness, seizure and even death. In order to detect, treat and prevent hypoglycemia, the need of an easy and fast quantitative method for insulin is needed.

Identifying and detecting proteins is a difficult task due to the low concentration of insulin and the complexity of plasma proteome. However, immuno-Matrix Assisted Laser Desorption/Ionization (iMALDI) has shown to be a rapid method minimizing the need of tedious sample preparations prior to Mass Spectrometric detection. iMALDI involves the use of antibodies to capture specific peptides and thus is well suited to quantitate low abundant proteins such as insulin. The antibodies are bound to magnetic beads, making them easy to handle and spot onto MALDI targets. Due to the minimized transfer between tubes and plates during enrichment and washing sample losses are kept to a minimum. As the beads are deposited directly onto the MALDI target prior to elution, there are no losses occurring during the final elution step. These advantages allow us to detect targets in lower concentrations than standard immunoprecipitation methods. Magnetic beads, and the easy procedures present in iMALDI, allow for automation of the iMALDI procedure, resulting in high sample throughput required for use in a clinical setting.

Keywords: immuno-matrix assisted laser desorption/ionization, insulin, automation

POS-01-033 Development of an Automated Immuno-MALDI Assay for the Clinical Measurement of Plasma Renin Activity

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The renin angiotensin aldosterone system (RAAS) is crucial for the regulation of blood pressure. Dysfunctions of the RAAS can lead to severe diseases related to hypertension. A well-established biomarker for the diagnosis of primary aldosteronism, a form of secondary hypertension, is plasma renin activity (PRA). It is commonly determined by radioimmunoassay (RIA), which has the disadvantages of using radioisotopes and the possibility of cross-reactivity.

To overcome these issues we have developed a mass spectrometric PRA assay based on immuno-MALDI (iMALDI) with the final goal of implementing it in the clinic. Plasma samples are split into two aliquots. The first aliquot is incubated at 37°C for Angiotensin I (Ang I) generation, the second one is placed on ice as a blank. The aliquots are then incubated for 1 hour with stable isotope-labeled Ang I analogues and anti-Ang I antibodies bound to magnetic beads. The beads are washed and spotted directly onto a MALDI target with the peptides being eluted from the beads by addition of HCCA matrix. Ang I quantitation of both aliquots allows for PRA determination.

64 clinical samples were prepared manually and analyzed using our iMALDI method. Results were compared with RIA (R^2 = 0.9412) and LC-MS/MS (R^2 = 0.9471) results determined at St. Paul's Hospital in Vancouver, Canada, exhibiting strong correlation to clinical measurement.

In order to achieve the requirements for clinical assays (robustness, high throughput and accuracy) we have optimized and automated the sample preparation on an Agilent Bravo liquid handling platform for analysis on a Bruker Microflex MALDI instrument.

Keywords: iMALDI, plasma renin activity, automation

POS-01-034 Antibody-Based Proteomics Using an Automated Tissue Microarray Quantification System

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Antibody-based proteomics is a concept that emerged in association with the progression of genome-wide antibody production projects. The Human Antibody Initiative aimed to raise at least one monospecific antibody to all >20,000 proteins encoded by the human genome. It is anticipated that production of a large antibody library will greatly accelerate the discovery and validation of biomarkers. A tissue microarray (TMA) is a glass slide on which hundreds of small formalin-fixed paraffin-embedded (FFPE) tissue cores are arrayed. TMA is especially useful for characterization of a particular protein in large clinical cohorts, but has been rarely used for exploration of biomarkers. Here we report the development of a new rapid TMA quantification system. High-resolution TMA fluorescence images are obtained using a flatbed line scanner. Tumor cell areas are then automatically discriminated from stromal components, and their images are digitalized in a series of algorithms. Using this system we performed high-throughput immunohistochemical screening of 1012 antibodies across 139 ovarian cancers and identified a novel biomarker associated with poor patient prognosis and chemothrepy resistance. This system can screen antibodies on the basis of their reactivity with a large number of patient samples with reasonable comprehensiveness and throughput. We discuss the potential application of the technology to large-scale clinical studies.

References

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Keywords: tissue microarray, antibody-based proteomics, immunohistochemistry

POS-01-036 A Modified ELISA Strategy Using Graphene Oxide Sheets and Gold Nanoparticles Functionalised with Different Antibody Types

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Enzyme-linked immunosorbent assay (ELISA) is an effective and powerful method for protein detection and has being the most commonly used analytical strategy for detecting and measuring trace biomarkers or other proteins. Despite its popularity, ELISA still has some shortcomings, such as the high cost and large amounts of antibodies, a poor LOD and so on.
In this study, Gold nanoparticles (GNPs) and graphene oxide (GO) sheets were introduced, and a novel ELISA strategy called 3G-ELISA was developed by using antibody-functionalised GO sheets and GNPs. This modification significantly enhanced the sensitivity and greatly reduced the cost of this assay. The applicability of the method was demonstrated by detecting HSP70 in a human serum sample. The result suggests that the 3G-ELISA method is feasible to detect an antigen in a complex mixture, and the limit of detection (LOD) is up to 64-fold and the cost is as low as one-tenth of the conventional ELISA method. We believe that this strategy could be exploited to widen its applicability for the analysis of trace biomarkers in complex mixtures.
Keywords: graphene oxide, gold nanoparticles, 3G-ELISA

POS-01-035 Study of Usefulness of Established Monoclonal Antibodies as a Sero Diagnostic Marker for Lung Cancer

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An early diagnosis of lung cancer is usually difficult, because there are few useful sero diagnostic markers. This is a factor leading to the poor prognosis of patients with lung cancer. The aim of this study was to identify sero diagnostic markers in lung cancer patients and healthy controls with our established monoclonal antibodies (MoAb) employing the random immunization method. We focused on one MoAb which recognizes Cytoskeleton Associated Protein 4 (CKAP4). For the results of immunoblot analysis, CKAP4 was detected at 63 kDa in tumor tissues at various levels in the cases, but not in their non tumor peripheral lung tissues. In immunohistochemistry, CKAP4 was localized in the cytoplasm of tumor cells and their tumor stromal fibroblasts, but no obvious staining was detected in their non tumor peripheral lung tissues. The positive rate of CKAP4 expression in squamous cell carcinoma (SCC) was higher than in adenocarcinoma (AD). CKAP4 levels were significantly higher in sera from lung cancer patients than healthy controls. Furthermore, CKAP4 levels in sera from SCC patients were significantly higher than in AD patients.
Keywords: CKAP4, lung cancer

POS-01-037 Towards the Absolute Quantification of Therapeutic Proteins by Immunoaffinity Purification Mass Spectrometry

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LC-MS has emerged as a promising technique for therapeutic proteins quantification, particularly in complex biological matrixes such as serum, plasma, tissues, etc. A key limitation to this technique is its limited sensitivity in comparison to ligand binding assay. In this study, immunoaffinity purification by magnetic beads as a sample pretreatment step was developed to isolate target protein from serum, followed by on-bead digestion with trypsin to release surrogate peptides. These surrogate peptides are subsequently measured by LC-MS/MS as quantitative surrogates for the therapeutic protein. Combination of affinity purification and LC-MS/MS (refer as immuno-LC/MS) results in selective purification and matrix simplification, thus enabling improvements in assay sensitivity to be achieved. The progress of the asay dvelopment was evaluated. USing the optimized conditions, this method enables absolute quantification of therapeutic proteins in non-human primate serum at low ng/mL level. The validated method is then applied to obtain the PK profile of therapeutic protein in animal serum.
Keywords: Immunoaffinity-LC-MS, therapeutic protein, magnetic bead

POS-01-038 XIM Cross Species Immunoassays - Analyzing Proteins Across the Barrier

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Biologists typically strive to transfer results from animal models to human beings. Genetic differences limit the use of antibodies as generic tools in immunoassays across species borders. Such a constraint does not exist for mass spectrometry-based approaches. However, sensitivity of pure chromatography and mass spectrometry-based systems is restricted to a lower μg/ml concentration range in plasma.

Here we address cross-species assays by using a special set of TXP-antibodies binding to short c-terminal peptide epitopes. Such antibodies enable the enrichment of classes of peptides, sharing the same terminal epitope. Using an *in silico* selection process, it is possible to identify peptide sequence motifs which are common for targeted peptides/proteins in rodents, dogs and primates. We have used sets of such short c-terminal peptide epitopes to generate antibodies targeting potential pre-clinical kidney, liver and vascular toxicity biomarkers. These antibodies are used to enrich peptides from tryptically digested plasma and urine of five species (mouse, rat, dog, monkey, and human). The enriched peptides were identified by data-dependent LC-MSMS to demonstrate the cross-species applicability of TXP-antibodies in preclinical toxicology studies.

Keywords: cross-species immunoassays, immunoaffinity MS, SISCAPA

POS-01-039 DigiWest: High Content Western Blotting

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The profiling of central signal transduction pathways requires the detection of changes in protein expression and differences in protein modification or activation. Mass spectrometry based analysis systems showed their power by allowing unbiased discovery approaches, whereas antibody-based approaches such as reverse phase protein microarrays allow a more targeted analysis of high numbers of limiting sample material. With the large efforts that have been put into the generation of binding molecules for array-based proteomics large numbers of antibodies are available and screening approaches that take advantage of these valuable resources are of interest.

Here, we describe an approach that uses the principles of protein blotting (protein separation by SDS gel electrophoresis; protein immobilisation on a solid support) and combine it with a multiplexed bead array as a readout system. Thereby, the throughput and sensitivity of bead-arrays is combined with the well-established Western-blot. Taking advantage of our approach, we performed a matched-pair analysis of ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) directly on the protein level. Carcinoma cells (DCIS, IDC) were collected by laser capture micro-dissection from one tissue specimen containing both types of cancer cells and both were analysed using our system. 160 different antibodies could be employed to detect expression of known tumour markers and to analyse the activation state of different signalling cascades. The analysis shows that the transition to invasiveness is accompanied with defined changes in intracellular signal transduction cascades.

Keywords: signal transduction, tumor characterisation, antibody-based proteomics

POS-01-040 Identification of Altered Cell Signaling Pathways in B-Lymphocytic Chronic Leukemia (B-CLL) by Functional Proteomics Approaches

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In post-genome era having sequence the human genome, one of the most important pursuits is to understand the function of gene-expressed proteins. The overwhelming size and complexity of human proteome requieres very high-throuhgput techniques for rapid analysis. Despite the significant advancements in molecular biology and genetic tools, this demand has not been satisfied and only a small fraction of the proteome has been understood at the biochemical level. Systems biology adn Proteomics strive to create detailed predictive models for molecular pathways based upon quantiative behavior of proteins. Understanding these dynamics networks provides clues into the consequence of aberrant interactions and why they lead to B-chronic lymphocytic leukemia. Historically, methods capable of collecting quantitative data on biochemical interactios could only be used for one or for a few proteins at the time. Protein microarrays allow hundreds to thousands of proteins to be analyzed simultaneously, providing an attractive option for high-throuhgput studies such as protein interactions, differential protein profiles,...A novel bead suspension array system, based on color coded beads, which are compatible with a flow-cytometry, allows measuring many proteins simultaneously because this novel approach offers the advantage that hundreds of different proteins or antibodies can be codified in specific color combination; in addition, it is combining with size exclusion chromatography and subcellular fractionation, by this way, it is possible to determine protein complexes and/or specific protein identification. We will present differential protein profiles (i.e. Bcl-2, SOS, Lyn,..., among others) obtained from normal B cells and aberrant B-cells from Chronic Lumphocytic leukemia(with different cytogenetic alterations and immunophenotype).

Keywords: chronic lymphocytic leukemia, protein arrays, antibody based proteomics

POS-01-041 Signatures of Human Proteins in Plasma Discriminate Syndromes of Childhood Malaria

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Human malaria is a life-threatening disease causing an estimated 600,000 deaths in 2010 and although the mortality rates have decreased during the last decade, deaths in Africa due to childhood malaria are still elevated. A week after being exposed to malaria, complications may develop abruptly and turn out to be fatal, so an early prognosis of disease development is needed to find a suitable therapy. Using the resource of antibodies from the Human Protein Atlas and antibody suspension bead arrays, we profiled for human proteins in plasma of children from Nigeria that suffered from different malaria syndromes. Out of more than 1,000 human proteins studied with arrays built on targeted and untargeted selection criteria, 41 profiles differed significantly between malaria infected and control individuals from the community. Another 13 were found to discriminate uncomplicated malaria, severe malaria anemia and cerebral malaria. Markers of oxidative stress were identified in plasma of anemic individuals while markers of endothelial activation, platelet adhesion and muscular damage were identified in children with cerebral malaria. These findings suggest the presence of deep lesions into their micro-vasculature and surrounding tissues concurrent. This increased knowledge of the pathogenesis of malaria serves as a basis for development of novel diagnostic strategies that may predict whether a child will develop the severe forms of the disease.

Keywords: suspension bead arrays, plasma profiling, multivariate analysis

POS-01-042 A Kinase Activity-Estimating Method Using LC-MS/MS

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Protein phosphorylation is regulated by protein kinases and recognized to be the major general mechanism by which almost all intracellular events are controlled. Alterations in activation levels of protein kinases often cause human diseases, including cancer. Although several kinase inhibitors are used for cancer therapy, the resistance is a serious clinical problem. The resistance is caused by not only mutations of target kinases but also constitutive activation of downstream or alternative kinases of target kinases; thus, comprehensive analysis of activation levels of various kinases may enables prediction of the sensitivity of kinase inhibitor drugs in cancer patients. Since kinases are generally activated by phosphorylation at specific amino acids, antibodies to the activating phosphorylation are used to estimate the activation level of kinases. Western blotting with active kinase-antibodies is the major strategy to estimate activation levels of kinases but time-consuming to analyze for various kinases; thus we are trying to develop a LC-MS/MS technique to comprehensively estimate the activation level of various kinases. This kinase activity-estimating (Kin-Act) LC-MS/MS is a combined strategy of affinity-purification using active-kinase antibody and a LC-MS/MS-based quantitation technique, such as SILAC. Since Kin-Act LC-MS/MS requires active kinase-antibodies able to be used for affinity purification, we identified 24 active kinase-antibodies for affinity purification. Since amino acid sequences around activating phosphorylation sites of similar kinases are very homologous, at least 40 kinases can be affinity-purified by the 24 active kinase-antibodies. Kin-Act LC-MS/MS using a combination of multiple active kinase-antibodies makes possible comprehensive analysis of activation levels of various kinases.
Keywords: kinase activity, anti-active kinase antibody, affinity purification

POS-01-043 Benefits of Fluorescent Detection for Quantitative Western Blotting

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Western blotting is a well-established and widely used technique to confirm the identity and presence of proteins from a variety of sources. Improved detection methods and imaging equipment makes Western blotting a tool for quantitative protein analysis. Fluorescent detection systems are typically characterized by their high sensitivity and broad linear dynamic range, and are as such well adapted to quantitative Western blotting. Moreover, fluorescence detection enables simultaneous detection of more than one protein on the same blot (multiplexing), even when the proteins have overlapping size, by utilizing secondary antibodies conjugated to fluorophores that are spectrally differentiated from each other. This makes fluorescent Western blotting a useful tool for accurate monitoring of changes in protein abundance and for detection of posttranslational modifications. Here we demonstrate the benefits of Amersham™ ECL Plex™ fluorescent detection system in quantitative Western blotting applications and how to reach precise quantitative analysis. Protein levels are quantified in order to monitor variation in protein expression as a response to various treatments in some typical Western blotting applications for cell signaling studies.
Keywords: Western blotting, fluorescence, quantitative

POS-01-044 Can We Detect Usually Undetectable Cytokines in Human Body Fluids?

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Background: Cytokines, chemokines and growth factors are potential bio-makers but they are very low in abundance in various matrices e.g. body fluids, tissues, cell culture supernatants, etc. Cytokines are currently being analyzed as a semi-targeted proteome approach more frequently these days than in the past using bead-based, multiplexing, immuno-detection systems. Most of the detected cytokines are low to mid pg/ml concentrations and approximately 10-20% cytokines are not being detected at all. Reagents used to detect cytokines using multiplexing systems are expensive and most of the reported work has been done as instructed by the manufacturers without further optimizing assay conditions due to the high cost of the reagents. Therefore, often it is ignored the potential of detecting those un-detectable cytokines. I have investigated whether it is possible to detect undetectable cytokines or increase the sensitivity of those detected at very low levels by optimizing sample preparation methods. *Methods:* Three sample preparation methods have been tested for analysis of various cytokines: (i) human plasma analyzed either un-diluted or diluted, analyzed 48 cytokines; (ii) human plasma analyzed after removal of the top 14 high abundance proteins (HAP) and compared with neat plasma, analyzed 48 cytokines; and (iii) concentrated human urine was equalized with ProteoMiner (combinatorial peptide ligand library method) and compared with un-equalized urine, analyzed 27 cytokines. Results: (i) concentrations of 52% cytokines were increased and 21% were decreased in diluted plasma compared to undiluted plasma, and 17% cytokines showed no differences in concentrations; (ii) more cytokines were detected in neat plasma compared to HAP removed plasma which was counterproductive; (iii) updated results on the detection of cytokines before and after ProteoMiner treatment of human urinary proteins will be presented.
Keywords: high abundance protein depletion, proteominer protein equalization, detection of cytokines

POS-01-045 Intact Mass Analysis of Monoclonal Antibody (MAb) Charge Variants Separated Using Linear pH Gradient

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Julia Baek, Andreas Huhmer, Remco Swart, Srinivasa Rao,
Yury Agroskin, Chris Pohl

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Recombinant monoclonal antibodies (MAbs) can be highly heterogeneous due to modifications such as sialylation, deamidation and C-terminal lysine truncation. Salt gradient cation exchange chromatography has been used successfully in characterizing MAb charge variants. However, additional effort is often required to tailor the salt gradient method for an individual MAb, such as changing the buffer and salt concentration. In the fast-pace drug development environment, a platform method is desired to accommodate the majority of the MAb analyses. In this study, we present a linear pH gradient separation method. The integration of a high pressure biocompatible HPLC system, a series of small particle high resolution cation-exchange columns, and a linear pH gradient provides an effective platform for MAb. Using this method, MAbs containing lysine variants are successfully separated on a SCX column. These variant are collected, desalted, and analyzed by a benchtop quadrupole Orbitrap mass spectrometer, Q Exactive. The full MS spectra of the MAbs show a complete charge envelope distribution. MS measurements are able to completely characterize those charge variants and localize the charge modification. The MS measurements also confirm the separation by charge using the pH gradient.
Keywords: Linear pH gradient, monoclonal antibody, intact mass

POS-01-046 Secretome-Based Identification of Novel Serum Biomarkers for Ovarian Clear Cell Adenocarcinoma

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The conventional ovarian cancer biomarker, CA125, frequently exhibits normal levels in patients with ovarian clear cell adenocarcinoma (CCA), a highly malignant type of ovarian cancer. Therefore, new diagnostic biomarker that would allow more reliable detection of CCA is needed. Secretome, the diverse set of proteins secreted or released from cells, including proteins shed from their surface, is a source for identifying circulating biomarkers in the blood. Here, we report discovery of new diagnostic biomarkers for detection of CCA by secretome analysis using ovarian cancer cell lines. Of over 2000 proteins identified, four potential biomarker candidates were sieved out by our bioinformatics analysis. Western blot and real time RT-PCR analyses showed that protein and mRNA levels of these biomarker candidates were elevated in tissue sample from patients with CCA. Furthermore for clinical validation, serum levels of these proteins were measured by MRM and ELISA assays. Serum levels of these proteins were significantly changed in patients with CCA, even those with normal CA125 levels. In terms of the receiver operating characteristic curve analyses, these biomarkers have higher diagnostic power than CA125 for detection of CCA. These findings might be of special relevance in the diagnosis of CCA.

Keywords: secretome, ovarian cancer, biomarker

POS-01-047 Search and Identification of Peptide Biomarkers of Colorectal Cancer in Sera

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Colorectal cancer (CRC) is a common and deadly disease in the world. The average lifetime risk to develop nonhereditary, sporadic CRC is approximately 5%. Each year more than one million people are diagnosed with CRC and about half of them die from this malignancy.Stage of the disease, is an important prognostic factor, with five year survival rates of more than 90% for localized CRC (stage I) and only about 10% for CRC that metastasized to distant organs (stage IV). The aim of the present work was a search and identification of peptide markers of CRC in sera using modern mass spectrometry techniques.

Blood sera obtained from 50 patients with CRC and 50 healthy donors (control) were used for isolation and identification of peptides.Serum samples of each analyzed groups were fractionated using magnetic beads with weak cation exchange surfaces, obtained eluates were heated at 98°C for 15 min, desalted using STAGE tips and analyzed by nanoLC-MS/MS using ABSciexTripleTOF 5600+ with or without (in case of label-free quantitative mass spectrometry analyses) preliminary fractionation by off-line strong anion exchange chromatography. Label-free quantitative mass spectrometry analyses of serum samples were made by SWATH (sequential windows acquisition of all theoretical ion-fragments spectra) approach.

Suggested workflow for serum peptides isolation and identification allows us to identify more than 6000 unique peptides originated from the almost 1000 unique proteins with high confidence. SWATH analyses of serum samples obtained from CRC patients and healthy donors among 2761 selected peptides revealed 163 peptides which content in CRC samples were more than 3 times higher than in control samples, and 57 peptides which content in control samples were more than 3 times higher than in CRC samples. We believe that obtained results provide solid background for a search of new potential biomarkers for colorectal cancer.

POS-01-048 Nuclear N-myc Downstream-Regulated Gene 1 Protein (NDRG1) as a Prognostic Biomarker in Renal Cell Carcinoma

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In renal cell carcinoma (RCC), prognostic biomarkers have been required for the risk stratification therapy. We aimed to identify prognostic biomarkers in RCC. The protein expression profiles of primary tumor tissues from 9 RCC patients were created by two-dimensional different gel electrophoresis (2D-DIGE). Among 3,771 protein species, 73 ones exhibited statistically ($p < 0.01$) and significantly (> 3-fold) different expression level in tumor tissues compared with non-tumor tissues. Protein identification was achieved using mass spectrometry. We focused on a multi-functional tumor suppressor protein, namely N-myc downstream-regulated gene 1 protein (NDRG1). NDRG1 is regulated by hypoxia-inducible factor 1-alpha, an important transcriptional factor, and reported in many malignancies but not in RCC. The gene silencing assay by siRNA *in vitro* revealed that the decrease of NDRG1 significantly ($p < 0.01$) promoted proliferation and invasion of the RCC cells in which NDRG1 localized in the nuclei. We validated immunohistochemical analysis on the new 82 RCC cases. The nuclear NDRG1 expression level was significantly ($p < 0.01$) associated with favorable prognosis. Multivariate analysis demonstrated that nuclear NDRG1 expression level was an independent prognostic factor ($p < 0.01$). Our findings suggested that nuclear NDRG1 had tumor suppressive effects and was associated with favorable prognosis in RCC. Proteomics with 2D-DIGE is a useful approach to the biomarker discovery. Nuclear NDRG1 may provide additional prognostic information for the risk stratification therapy in RCC.

Keywords: 2D-DIGE, renal cell carcinoma, NDRG1

POS-01-049 Discovery of a Urinary Biomarker for Renal Cell Carcinoma Based on a Proteomic Analysis of Cyst Fluid

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Renal cell carcinoma (RCC) is usually detected by imaging performed for reasons unrelated to RCC. AS a result, the incidence of RCC and the rate of mortality due to RCC are both increasing, indicating that a new screening procedure for urine or serum is necessary. Although various samples, including tissue, cells, serum, and urine, from patients with RCC have been analyzed, biomarkers with diagnostic value have not yet been identified. We used a proteomics approach to analyze cyst fluid in cases of cyst-associated RCC to identify accessible and abundant proteins that are overexpressed and/or secreted by RCC cells. Proteins in the cyst fluid were separated by reverse-phase highperformance liquid chromatography and agarose twodimensional gel electrophoresis and were identified by tandem mass spectrometry. We conducted a National Center for Biotechnology Information search and a MEDLINE search to predict the function of these identified proteins and to select a tumor-marker candidate protein. Our search resulted in the identification and selection of the differentially regulated protein known as 14-3-3 protein beta/alpha, which was overexpressed in cyst fluid from cyst-associated RCC but has not been previously associated with RCC. We then measured its incidence through Western blotting of various normal and RCC samples (serum, urine, tissue, and cyst fluid). The expression levels of 14-3-3 protein beta/ alpha were higher in urine samples from patients with RCC than in samples from healthy volunteers. Receiver operating characteristic (ROC) curve analyses were performed to assess this potential biomarker; these data (area under the ROC curve value was 0.8813) indicate a high degree of accuracy for this screening method. 14-3-3 Protein beta/ alpha may be a diagnostically useful biomarker for early diagnosis of RCC.

POS-01-050 Revealing Annexin A4 as Potential Biomarker: New Order Towards Liver Cancer Therapy

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Hepatocellular carcinoma (HCC) is the third-leading cause of cancer-related mortality worldwide. A potential biomarker for hepatocellular carcinoma is the protein annexin A4, an intracellular Ca2+ sensor, mainly found in epithelial cells. In respect to cancer, ANXA4-overexpression and Ca2+ elevation is an important risk factor of carcinogenesis that results in liver cancer. Despite this correlation, the role of ANXA4 in the progression of HCC in remains obscured. In this study we aimed to identify ANXA4 as a potential biomarker involved in HCC progression. In order to clarify how the aberrant expression of this protein and its post translational modification, participates in the development of liver carcinoma from fibrosis. We analyzed human clinical samples of HCC (n=50) and fibrotic liver (n=65) and control. By using comparative proteomics approaches, we observed over expression of ANXA4 in HCC with respected control. It's over expression and localization in the HCC patients is further validated by western blot and immunohistochemistry. Furthermore, in-silico analysis revealed phosphorylation and S- nitrosyltaion sites and functional association network by STRING reveal the interaction partner of ANXA4. Over expression of ANXA4 may regulate the genes that are known to be related to cancer and may induce cell proliferation. It might be possible that ANXA4 triggers a signaling cascade, leading to increased epithelial cell proliferation, ultimately promoting carcinogenesis. This observation suggests that ANXA4 along with PTMs sites may decipher critical mechanism of progression of fibrosis to cirrhosis to carcinoma and therefore provide a new insight for liver cancer therapy.

Keywords: hepatocellular carcionoma, annexin A4, phosphorylation

POS-01-051 Discovery and Subsequent Validation of Biomarkers for Colorectal Cancer by Large-Scale Proteomic Analysis and Tissue Microarray Analysis

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Recent advances in quantitative proteomic technology have enabled the validation of biomarkers on a large scale. In this study, we performed quantitative proteomic analysis of membrane fractions from colorectal cancer tissue for biomarker discovery and extensively validated biomarker candidate proteins. A total of 5287 of 5566 identified proteins were annotated by GO cellular components analysis and 3087 (58.4%) were predicted to be membrane proteins. Also, 1567 proteins were predicted to have a transmembrane domain by TMHMM algorithm. A total of 159 membrane proteins and 55 extracellular proteins were differentially expressed between polyps and cancer without metastasis, while the expression of 32 membrane proteins and 17 extracellular proteins was altered between cancer with and without metastasis. Among these biomarker candidates, 105 proteins were further quantitated by selected (or multiple) reaction monitoring (SRM/MRM) using synthetic stable isotope-labeled peptides as internal control and 78 were verified. Some of the results were also confirmed by Western blotting and immunostaining. Furthermore, analysis of multi-cancer tissue microarray consisting of 1150 cores from 14 cancer tissues showed that two of the candidate proteins were highly expressed not only in colorectal cancer but also in several cancer tissues. These results suggested that our method for biomarker discovery and subsequent validation using SRM/MRM will contribute to identify useful biomarkers for various cancers. We are currently evaluating the possibility of quantitation of the colorectal biomarker candidate proteins in blood samples and some of them have been detected in sera.

Keywords: membrane proteomics, colorectal cancer

POS-01-052 A Study of Protein and Glycoprotein Biomarker Discovery in Nipple Discharges from Breast Cancer by Nano-LC-ESI-Mass Spectrometry

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Breast cancer is one of the leading causes of death among women around the world. Early detection is beneficial in the fight against the mortality of this multifaceted disease. Currently, there are no clinical biomarkers available for early detection of breast cancer. Most proteomic studies of cancer tissue and serum from breast cancer published so far did not lead to the clinical marker yet. Nipple discharge (ND) has been suggested to be a sauce of secreted proteomes in the early microenvironment modification in pathologic condition of cells in ductal-bibular epithelium. We have conducted a study with such a minute amount of ND that newly developed two dimensional nano-LC/ESI-MS/MS proteomics technology. A nano-LC UltiMate 3000 (Thermo Scientific DIONEX) with a two-dimensional LC system (1st reversed phase (RP) under basic condition followed by fractionation and 2nd RP under acidic condition were utilized). The LC setup was coupled online to HCTultra mass spectrometer (Bruker Daltonics) using a nanoelectrospray ionization source. The resulting mass spectra were analyzed using Mascot (Matrix Science) and Scafford (Proteome Software) search engines. As a result of a study using ND samples, we found unique protein expression patterns, and protein and glycoprotein markers between breast cancer patients and non breast cancer patients. The pattern and markers may provide the new way of distinguishing breast cancer patients. We will discuss that an advanced proteomic analysis of ND warrants the search of a new breast cancer marker for early detection of disease.

Keywords: breast cancer, nipple discharge, biomarkers

POS-01-053 Development of Novel Membrane Protein Marker for Lung Cancer Diagnosis and Therapy

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Lung cancer is the one of common cancers and the fetal disease causing high death rate. Lung cancer is histologically divided into four types: adenocarcinoma, large-cell carcinoma, small-cell carcinoma, and squamous-cell carcinoma. High death rate of lung cancer is because of late diagnosis and absence of effective treatment. Thus, it is important to develop an efficient method for diagnosis and treatment. Targeted therapy is a rising method for cancer therapy and has a potential clinical benefit. Especially, cancer specific membrane proteins are useful as biomarker. In this study, we found candidate proteins for biomarker of lung cancer and confirmed that they can target four types of human lung cancers.

We analyzed proteins expressed in normal human lung tissue and in four types of human lung cancer cell lines. We got the list of 1,340 proteins by MS/MS. Among them we chose four promising proteins which can be used as biomarker of lung cancer. For further validation, we prepared antibodies against candidate proteins and carried out various experiments to verify the specific expression of candidates in the membrane of lung cancer cell. Also, we performed antibody treatment in lung cancer cells and in the xenograft model to analyze whether antibody can regulate proliferation of lung cancer cells.

According to results of western blot and immunocytochemistry and immunohistochemistry, we concluded that candidate proteins are expressed in human lung cancer cell specifically. In addition, the result of flow cytometry showed candidate proteins are located in membrane of human lung cancer cell lines. Furthermore, we found that antibodies against candidate proteins can kill lung cancer cells and block the growth of lung cancer tumor in xenograft model. Besides, *in vivo* and ex vivo imaging represented antibodies can target lung cancer tumor. These results suggest four candidates can play a significant role in targeting lung cancer for diagnosis and treatment.

Keywords: lung cancer, membrane protein, biomarker

POS-01-054 Proteomics Identification of Novel Prognostic Biomarkers Associated with Relapse of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is one of the most deadly cancers in the world. The prognosis after surgical resection remains poor due to the high relapse rate. Patients with early relapse often develop into stage 4 metastatic cancer, which results in poor survival. There is an urgent need to identify prognostic biomarkers which could guide treatment options and possibly lead to development of novel therapeutics. Using iTRAQ labeling combined with 2D-LC-MS/MS, we compared the proteome of the tumour and the adjacent non-tumour liver tissues of two groups of HCC patients: (i) patients who experienced early relapse (ER) within two years of resection and (ii) patients who were relapse-free within two years of resection (NR). For each patient, the expression levels of proteins in the tumour (T) tissues were normalized against the adjacent non-tumour (N) tissues. Subsequently, the T/N ratio was compared between the ER and the NR groups to identify differentially regulated proteins between the two groups. 42 differentially regulated proteins were identified, and this panel of proteins was able to successfully segregate the patient samples according to relapse status during clustering analysis. Verification of iTRAQ results would be performed using SWATH-MS and western blot, and expression of selected candidate proteins would be evaluated on a larger cohort of HCC patients by tissue microarray analysis.

Keywords: Hepatocellular carcinoma, Relapse, iTRAQ

POS-01-055 Low Expression of Chromatin Remodeling Gene *ARID1A* Induces Gastric Cancer Cell Proliferation and Migration

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AT-rich interactive domain-containing protein 1A (*ARID1A*) encodes BAF250a protein, a subunit of human SWI/SNF-related chromatin remodeling complexes. The tumor suppressor function of *ARID1A* remains elucidative. In current study, we found that knockdown of *ARID1A* in gastric cancer (GC) cell lines promoted cell proliferation and colony formation *in vitro* and modulated cell-cycle related genes, while restoration of *ARID1A* expression suppressed cell proliferation. Silencing of *ARID1A* stimulated cellular growth and glucose consumption. *ARID1A* silencing upregulated the phosphorylation of AKT, and the expression of PI3K and PDK1, suggesting PI3K-AKT pathway might be a downstream target of *ARID1A*. Luciferase reporter assays indicated that SWI/SNF complex bound to the promoters of PI3K and PDK1. Endogenous *ARID1A* silencing enforced cell migration and invasion whereas ectopic expression of wild type *ARID1A* inhibited migration. E-cadherin expression was down-regulated by *ARID1A* knockdown and was raised by overexpression of *ARID1A*. Elevated E-cadherin significantly inhibited cell migration and invasion *in vitro*, while knockdown *CDH1* promoted migration and invasion. The expression of BAF250a and E-cadherin showed strong correlation in GC tissue samples. BAF250a could bind to *CDH1* gene promoter and enhance its transcription. GC tissues had lower *ARID1A* expression than paraneoplastic tissues ($p < 0.0001$). GC patients with low expression of BAF250a and E-cadherin have worse prognosis, as revealed by Kaplan-Meier survival curve analyses. Low *ARID1A* expression was significantly associated with local lymph node metastasis ($p = 0.032$) and tumor infiltration ($p = 0.0056$). Taken together, *ARID1A* suppresses gastric tumor growth, migration and invasion by modulation of PI3K-AKT and E-cadherin pathways.

Keywords: AT-rich interactive domain-containing protein 1A (ARID1A), gastric cancer, SWI/SNF complex

POS-01-056 Identification of Plasma Membrane Proteins of Gastric Cancer Cells Reveals SYNJ2BP and C19ORF52 as the Pro-Proliferative Factors

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Gastric cancer (GC) is one of the most frequent cancers worldwide and still remains a significant cancer burden nowadays in China. In this study, we used an optimized avidin/streptavidin affinity method to isolate and enrich cell surface proteins from one normal gastric cell line GES-1 and three GC cell lines, HGC-27, MGC803 and SGC7901. Membrane proteins were separated by SDS-PAGE and analyzed by repeated label-free LC-MS with LTQ Orbitrap. We identified 3,178 proteins at 1% false positive rate. Half of them (1,680; 53%) were membrane-related proteins, including 920 plasma membrane proteins, 373 membrane-integral proteins and 26 proteins exposed to extracellular space like extracellular matrix protein or secreted proteins. Interestingly, 33 and 43 proteins were found to be up or down-regulated more than three fold in GC cells, respectively, as revealed by normalized spectra count method. Four down-regulated and 8 up-regulated proteins were chosen for further evaluated by Western blot and quantitative RT-PCR in in additional GC cell lines. These genes were subjected to RNA interference assay in 4 cell lines. Five genes were found to be associated with cellular proliferation when silenced by small interfering RNAs. Further long term knockdown assay by short hairpin RNAs revealed that SYNJ2BP and C19ORF52 were potential pro-proliferative genes. Taken together, our current GC cell plasma membrane proteomic analyses may provide new insight into the carcinogenesis mechanism of gastric cancer and reveal potential novel targets or biomarkers for gastric cancer treatment and diagnosis.

Keywords: gastric cancer, proteomics, plasma membrane

POS-01-057 Quantitative Phosphoproteome Analysis of Cultured Stomach Cancer Cell Lines Aimed at Development of Biomarkers for Prediction of Drug Efficacy

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More than 30 of approved molecular target drugs are in use for cancer treatment, and numerous drugs are currently undergoing clinical trials. Genetic tests based on detection of gene mutation and amplification are frequently used for selection of patients sensitive to the drugs. However, these diagnostic tests have limitation in selection of the effective patients, because resistance to drugs is often occurs due to bypassing pathways, even the target itself remains unaltered and continues to be inhibited by drug. In order to predict drug efficacy more precisely, signaling pathways responsible for drug resistance need to be investigated. Protein phosphorylation plays a major role for the signaling pathways, thus a large scale phosphoproteome analysis is useful for studying the pathways comprehensively. The aim of this study is to find useful biomarkers for prediction of efficacy of two molecular target drugs currently undergoing clinical trials for stomach cancer, Lapatinib (an EGFR/HER2 dual inhibitor) and AZD4547 (an FGFR inhibitor). We, first, evaluated protein expression level and phosphorylation status of receptor tyrosine kinases by Western blot analysis and determined drug sensitivity (IC50) in a panel of 20 stomach cancer cell lines isolated from both primary and metastatic sites. Each selected cell lines were SILAC-labeled, and mock- and drug-treated cells were mixed. After preparation of whole cell lysates, sample was reduced and alkylated, prior to tryptic digestion. Phosphopeptides were concentrated by Fe-IMAC, peptides were identified using LC-MS/MS. Phosphopeptides which have a phospho-site probability above 75% determined by MaxQuant software were defined as truly phosphorylated peptides. Our data would provide a useful information for establishment of forceful diagnostic method for drug efficacy.

Keywords: phosphoproteome, biomarker, drug efficacy prediction

POS-01-058 Optimizing Techniques in Proteomics-Based Biomarker Discovery and Validation in Head and Neck Carcinoma

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Introduction: Discovery of tumor biomarkers using proteomic methodologies is coming of age, but large databases are a major challenge. While optimizing a biomarker discovery project in head and neck cancer, we combined traditional and cutting edge techniques to establish reproducible workflow patterns.
Methods: Tissue was micro-dissected from paraffin-embedded tumors from twelve head and neck squamous cell carcinoma (SCCA) cases, and normal (control) tissues. Protein extractions using commercial and in-house reagents liberated peptides after trypsin digestion, and mass spectrometric analysis was performed.
Results: Data was entered as spectral quantitative counts derived from the Mascot/Scaffold/X-!Tandem program. We calculated P-values on the 162 proteins generated, and the 'normal' and 'tumor' columns were sorted by ascending values. This brought 'zero' values to the top of the 'normal' column, enabling potential biomarkers to be expressed only on the 'tumor' side. Using Ingenuity Pathway Analysis (IPA) software, we narrowed our search down to around 10 candidates. Four of these with high P-values, and good IPA interactions were selected for validation. Antibodies to candidate markers were first tested on tissue microarray (TMA) slides containing material from normal oropharyngeal and SCCA (head and neck) tissues.To date, two of the candidates, HNRPK (nuclear), Profilin (cytoplasmic) have emerged as promising biomarkers, while validation on two more are nearing completion. The TMA results will provide a valuable 'training set' to strengthen validation of data on the original experimental cases.
Conclusions: Discovery times for biomarkers can be optimized by combining mass spectrometry with traditional statistics, bioinformatics, and TMA immunohistochemistry.
Keywords: proteomics, mass spectrometry, carcinoma

POS-01-059 Biomarker Proteins in Head and Neck Squamous Cell Carcinoma. A Brief Review

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Introduction: As part of a proteomics-based biomarker discovery project, we performed a retrospective meta-analysis of proteins expressed in head and neck squamous cell carcinoma (SCCA).
Methods: A PUBMED search of papers from 2009 - 2013 formed the study material. Expressed proteins in SCCA from various head and neck sites were carefully tabulated and analyzed, in an effort to identify unique biomarkers
Results: 49 publications were available for review. The sources of SCCA studied ranged from fresh to archived human tissues and cell lines. The tumor location involved mostly oropharyngeal tissues, and rarely the upper esophagus. The type of study varied from traditional proteomic methods like 2D gel electrophoresis, and immunohistochemistry to mass spectrometry. A total of 168 proteins had been reported or studied in association with SCCA, but it was unknown whether these were unique, or deferentially expressed (compared to normal tissues). By grouping proteins that were commonly expressed across studies, at least 10 potential biomarker proteins that merited further investigations were isolated.These were either consistently upregulated (CD147, HSP70, E-Cadherin, MMP2, Proteasome activators), downregulated (Annexin), or indeterminate (p63, Profilin, Stratifin, VEGF). Increased expression of heat shock proteins was a unique feature in this group, as was observed in a pilot project on head and neck SCCA done in our laboratory earlier. Its significance as a biomarker is unknown.
Conclusions:.Although this meta-analysis provided an interesting insight into the field, more meaningful data from larger studies (using comparable proteomic techniques) may provide a better understanding of the protein profile in SCCA
Keywords: proteomics, meta-analysis, squamous cell carcinoma

POS-01-060 Proteome Analyses of Mammal Tumor Cells in Response to Treatment with Fibroblast Growth Factor 2

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FGF2 transiently blocks mice tumor cells on G1→S transition of the cell cycle and then blocks irreversibly in G2→M, leading to the appearance of cells with senescent phenotype. Several evidence points to an important role of nucleus and chromatin in eukaryotic cycle control and cellular senescence. Recent results of our group pointed out that nuclear proteins are preferentially altered after treatment with FGF2. We are conducting a comparative and quantitative MS analysis of mouse nuclear cell extracts before and after stimulation with FGF2. MS analyses after 0, 3, 5, 10 and 48h treatment are being conducted using *Spike-in* SILAC and *label free* quantification. We are specially interested in the analyses of proteins differentially expressed mainly related to gene expression, management of cellular phenotype in cell replication, mediation in the remodeling of chromatin and DNA damage and repair. These are indispensable cellular processes that appeared to be potentially involved in cycle blockage and senescence induction, based on our previous results, including cyclin-dependent kinase inhibitor 1A (p21), which is a key protein in the arrest of the cell cycle in G1. After onthology analyses and data processing using DAVID we are determining optimal treatment times to characterize the most important biochemical changes induced by the presence of FGF2 in the cell. While proteins related to biosynthesis, cell death and transcription regulation will be validated using gene knockout; RNA binding, spliceosome, chromatin and mitochondrial proteins are going to be scrutinized when superexpressed. Using inmunofluorescence and tandem affinity purification assays we are constructing new paradigms of FGF2 models. The validation and implications of these changes will be presented in order to explain induction and maintenance of defense mechanisms triggered by FGF2.
Keywords: FGF2, senescence, cell nucleus

POS-01-061 Characterization of the Breast Cancer Marker Candidate LAG3 Protein in Human Plasma by Direct SPRi-MALDI-MS Analysis from Antibody Arrays

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Proteomics plays an important role in biomarker discovery for clinical applications. In this study, we coupled Surface Plasmon Resonance imaging (SPRi) with MALDI-TOF mass spectrometry to permit the multiplexed quantification of binding by SPRi and the molecular characterization of interacting partners by subsequent MS analysis. This adds a dimension of specificity as MS permits the differentiation of molecules that are difficult to tell apart by use of antibodies, such as truncation variants or protein isoforms. The LAG3 protein was spiked in human plasma at ~1 μg/mL in a proof of concept study to detect, identify and characterize it as potential breast cancer marker. LAG3 was bound to a-LAG3 antibodies that were covalently attached to the chip surface. SPRi binding kinetics were obtained in real time, followed by tryptic digestion, matrix deposition and MALDI MS and MS/MS spectra acquisition. LAG3 was identified through Mascot interrogation. The density of bound antibody on the surface (~7fmol/mm²) was compatible with both, quantitative determination of binding parameters and the identification thru bottom-up MS/MS analysis for the SPRi-MALDI workflow. MALDI image analysis of the chips confirmed the co-localization of LAG3 peptides with the array spots whereas serum albumin - used to block the reactive chip surface after a-LAG3 deposition - was only detected between the array spots. This indicated that the matrix coating and trypsin application processes employed (piezoelectric nebulisation) did not significantly cause any delocalisation of the array content. The rapid, multiplexed and automated on-chip MALDI-MS analysis shows robustness at the femtomole level and opens numerous applications in the proteomic field such as ligand screening and lead optimization.
Keywords: breast cancer marker

POS-01-062 Proteomic Approach for Prognostic Biomarker in Myxoid Liposarcoma

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[Objective] Myxoid liposarcoma (MLS) is the second most common subtype of liposarcoma, accounting for 10% of all soft tissue sarcomas. The clinical course of MLS spans a wide spectrum from a curable disorder to a highly malignant disease that leads to metastasis and death. Thus, the molecular background of MLS has been studied to predict the behavior of individual tumors and to optimize therapeutic strategies. The adaptation criteria for adjuvant therapy often depend on the rate of round cell component in the primary tumor tissue. However, the negative predictive values of the round cell component for good prognosis remained 60-80%, and a novel molecular diagnostic modality has been required for better clinical outcome. In this study, we aimed to develop novel prognostic biomarker in MLS. [Materials and methods] This study included 26 MLS cases, and their primary tumor tissue. The tissues were grouped by those with and without distance metastasis. We employed two-dimensional difference gel electrophoresis (2D-DIGE) to create protein expression profiles. Proteins were extracted from the frozen tumor tissues, and labeled with ultra high sensitive fluorescent dye. The labeled protein samples were separated by a large expression profiles were obtained as a gel image. The structure of interesting proteins was examined by mass spectrometry and database search. The results of proteomic study were validated by specific antibodies by western blotting or formalin-paraffin embedded tissue samples. [Results] We observed up to 3,500 protein species by 2D-DIGE. The comparative proteomic study revealed the presence of protein species which were statistically and significantly associated with the status of distant metastasis. Those proteins were subjected to mass spectrometry and database search for protein identification. [Conclusions] Comparative proteomic study in MLS, identified the candidates for prognostic biomarker, whose clinical utilities are worth further validating.
Keywords: myxoid liposarcoma, biomarker, metastasis

POS-01-063 Secretome Analysis of Three-Dimensional *In Vitro* Model Cholangiocarcinoma

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Cholangiocarcinomas (CCA) is a malignant neoplasm of biliary tract epithelium with an incidence and mortality progressively increase over the past decades. Although the occurrences of this cancer are variable amongst countries, Thailand has the highest incidence rate in the world. As this aggressive and poorly understood malignancy remains largely incurable, developing more effective biomarkers and therapeutic modalities could significantly extend survival rate for these patients. Nowadays, study of cancer cell lines secretome as a means to identify diagnostic and prognostic markers has been widely performed. However, conventional method is to collect conditioned media obtained from monolayer culture that may not be wholly representative of dynamic features in which tumors exist *in vivo*. At present, three-dimensional (3D) culture has been extensively used since it provides more realistic microenvironment in natural physiology than routine method. Therefore, scaffold-based 3D culture of human intrahepatic cholangiocarcinoma isolated from Thai patient (HuCCA-1) was established and differentially secreted proteins between 3D and monolayer cultures were identified. In total, 25 distinct proteins which belong to categories of metabolic enzymes, signal transduction, stress response, cytoskeleton, and protein synthesis and degradation upregulated in 3D culture. These altered proteins might indicate similarity between this model and *in vivo* secretion from solid tumor and would provide more valuable data about bile duct cancer pathogenesis that could improve future diagnosis and therapeutic strategies.
Keywords: three-dimensional culture, cholangiocarcinoma, secretome

POS-01-064 Investigation into the Mechanisms of Prostate Cancer Androgen Independence Using Label-Free Data-Independent Quantitative LC-IM-DIA-MS and Pathway Analysis

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Patients diagnosed with localised prostate cancer (PCa) are often given androgen deprivation treatment (ADT), which fails in up to 35% of cases. However, over time many show disease remission with the development of androgen insensitive tumours progressing to uncontrolled metastatic disease. LNCaP cells are a prostate cancer cell line showing androgen dependent growth. Sustained maintenance of LNCaP cells in steroid depleted medium resulted in the development of LNCaP-Abl and Hof sublines, both growing independently of androgens. The progression from androgen-dependent to androgen-independent growth provides an excellent model of the development of androgen insensitive PCa following ADT.
LNCaP, Abl and Hof cells were prepared in triplicate using unfractionated and SDS-PAGE fractionated protein lysates. Quantitative label-free LC-MS was performed using a nanoAcquity LC system and ion-mobility enabled Synapt G2 mass spectrometer operating in data independent ion mobility analysis mode (IM-DIA). Quantitative, bioinformatic and statistical analysis of the data was undertaken with TransOmics and PLGS software, supporting relative quantification and molar amount estimation. Pathway analysis was conducted with Panther Classification System.
1111 proteins were identified with mean sequence coverage of 15.0%. The average numbers of peptides/protein was 6 with about 10% of the analytes separated from interferes using IM. Unsupervised PCA demonstrated good clustering of replicates and separation of the cell types. Comparing LNCaP and Abl cells, 428 proteins were observed in at least two biological replicates of ABL and LNCaP of which 188 were significantly regulated. Combined trend and pathway analysis showed changes in the gonadotropin releasing hormone receptor pathway and glycolysis. Pathway analysis revealed changes in the androgen-independent cells and provide rational targets for intervention in androgen-insensitive metastatic PCa.
Keywords: cancer proteomics, label-free LC-MS, ion mobility

POS-01-065 Bladder Cancer Proteome: A Multiplexing Approach Using Online 2D RP-RP Chromatography Coupled with Data Independent Ion Mobility

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Bladder cancer arises from malignancy of cells located within the epithelial lining with over 380,000 diagnosed cases worldwide. Therefore using a qualitative and quantitative proteomic approach, we aim to provide data for potential biomarkers based on three different human cancer cell lines. Protein extracts were tryptically digested and separated using 1D or 2D on-line nanoscale chromatography. Data were acquired in HDMS^E (data independent acquisition) where the collision energy is increased following ion mobility separation to produce fragments that exhibit the same drift time as their precursors. Post processing software was used to correlate precursor and fragment ions before database searching. Data initially collected from the 1D chromatography experiments yielded an average of 3200 protein identifications in two out of three replicate injections. For 2D based experiments, identifications returned on average were 5690 (5 fractions) and 7000 (10 fractions) proteins. In all cases, the resulting data were searched against a curated human database, constructed from the results of in-house genomic and transcriptomic studies. Based upon the database of bladder cancer variation, a total of 1622 mutated sites were identified in all three cell lines with minimal overlap. 171 mutated sites shared with the two bladder cancer cell lines; most proteins only contained the single unique mutated peptide. Of these mutated proteins, 35 are considered as the putative protein biomarkers of bladder cancer as previously reported. Therefore, our proteomic evidence has established a solid database, qualitative or semi-quantitative, to explore the functional molecules related to the type of tumour cells.
Keywords: biomarker discovery, bladder cancer, ion mobility

POS-01-066 Proteomic Analysis Displays Overexpression of Lactoylglutathione (GLO1) is Associated with Tumor Progression in Murine Fibrosarcoma

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Lactoylglutathione (GLO1), ubiquitously expressed methylglyoxal detoxification enzyme, is implicated in the progression of human malignant diseases. The role of Glo1 in development or progression of murine fibrosarcoma is still unclear. We performed proteomic analysis for identification of intracellular proteins in the regressive murine fibrosarcoma cell line QR-32 and the inflammatory cell-promoting progressive tumor cell clone QRsP-11 by two-dimensional gel electrophoresis and mass spectrometry. Seven up-regulated protein spots in QRsp-11 compared to QR-32 were identified as lactoylglutathione, annexin A1, adenylate kinase isoenzyme 1, transcription factor BTF3, myosin light polypeptide 6, low molecular weight phosphotyrosine protein phosphatase and nucleoside diphosphate kinase B. Western blot demonstrated that HSPB1, a methylglyoxal-adducted protein, was over-expressed accompanied by GLO1 in QRsp-11 compared to QR-32. Moreover, the level of GLO1 was increased in nuclear extract of QRsp-11 compared to QR-32. Our data suggest that high expression of GLO1 is associated with tumor progression in murine fibrosarcoma.

Keywords: GLO1, cancer progression, proteomics

POS-01-068 Up-Regulation of Type I Collagen during Tumorigenesis of Colorectal Cancer Revealed by Quantitative Proteomic Analysis

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Colorectal cancer (CRC) is one of the most prevalent cancers in the world. Discovery of noninvasive biomarker candidates promising for diagnosis and prognosis is of great importance for the management of CRC. In this study, we performed proteomic profiling of serum from patients with different stages of CRC using a 2D-LC-MS/MS based approach combined with the APEX quantitative method. A total of 917 proteins were identified and 93 were found differentially expressed in four groups (normal, stage I, II and III), which mainly relate to cell adhesion, immune responses, the coagulation process and metabolism. Importantly, we found collagen I was dynamically changed from stage I to IV with a maximum in stage II detected in serum by MS analysis and validated in tumor tissues of the same batch of CRC patients by real-time PCR and western blotting. And it is worthy to note that the serum level of collagen I degradation telopeptide (CTx) was correlated with UICC staging and poor 3 years disease-free survival of CRC patients by ELISA analysis. These results suggested that (1) serum proteomics may reflect the biological changes in colorectal tumor tissues, (2) collagen I may be an early event in tumorgenesis of CRC and CTx may provide additional information for prognosis of CRC.

Keywords: colorectal cancer tumorigenesis, collagen I, CTx

POS-01-067 Proteomics Approach to Identify a Glycosylphosphoatidylinositol Specific Phospholipase D (GPLD-1) as a Lung Cancer Biomarker and Its Regulation in Lung Cancer

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Lung cancer causes the highest mortality than any other cancers. Therefore early detection for lung cancer is essential. In order to discover potential biomarkers for lung cancer, we applied label free LC-ESI MS/MS analysis to compare SQLC patients' sera and healthy control sera after albumin and IgG depletion. In this proteomic study, we identified GPLD1 as a serum biomarker and validated the GPLD1 protein level in healthy (n=56) and SQLC sera (n=56) and human lung cancer patients' tissues and their adjacent normal tissues (n=22) by western blot analysis. The results showed that GPLD1 level of 40 SQLC patients sera and human lung cancer tissues (n=15) were lower than healthy sera and adjacent normal lung tissues. Furthermore, we performed sandwich ELISA on total 72 serum samples of HEC (16), SQLC (16), ADC (16), and SCLC (16). In these results, GPLD1 level was significantly lower in all lung cancer samples than healthy control. Further, in vitro experiments showed that GPLD1 reduced in Chang cells (normal liver cell line) by the interaction with lung cancer cells, THP-1 monocytes or IL-6, IL-1β treat. Additionally, in lung cancer mouse model, we also found GPLD1 level was lower in serum, lung cancer tissues, and in liver of cancer induced mouse compared to normal control mouse. Furthermore, IL-6 i.p. injections to mouse abrogate GPLD1 expression in serum and GPLD1 transcription in liver. Our data suggest that GPLD1 might be a potential candidate for lung cancer serum biomarker and further studies for GPLD1function in cancer progression might be needed.

Keywords: GPLD1, serum biomarker, lung cancer

POS-01-069 Treatment Efficacy of a Novel Polybisphosphonate on Soft Tissue Tumor Lesions

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Abstract Background: ODX is a novel bi-functional and macromolecular polybisphosphonate developed for treatment of bone metastasis in prostate and breast cancer. High treatment efficacy has previously been demonstrated *in vitro* and *in vivo*. The present study investigates whether ODX has efficacy also on soft tissue tumor lesions using Expression Proteomics.

Methods: Twelve female nude mice were injected with approximately 1-1.5 million MDA 231 cells subcutaneously around the mammary pad region. Tumors developed and were allowed to grow until ≤1cm before initiating treatment with ODX, administered i.v. 2.5mg/kg, once/week for 5 weeks. Serial measurements of tumor growth during the treatment period were conducted. The animals were sacrificed after 5 doses of ODX and tumor tissues were resected in treated and non-treated animals and histo-pathological evaluation and proteomic analysis were performed.

Results: The non-treated mice group developed multiple large size tumors with pronounced ulcerations, while the treated mice showed significantly smaller and less developed tumors without ulcerations. The general conditions of the treated mice were much better than the non-treated group. Multivariate analysis of a panel of proteins with significant expression levels unambiguously differs between treatment and no treatment groups, using 2-DE+MALDI-TOF. These results were further confirmed using non-gel based Nano-LC coupled with Synapt G2.

Conclusion: This study using nude mice with breast cancer implants indicate that ODX has very significant treatment efficacy on soft tissue lesions exclusive of its efficacy on bone metastasis. Proteomic analysis results provide a general view of the changes in protein expression profiles after ODX treatment. The data will enable screening for markers to monitor therapy response and to identify proteins associated with the efficacy of ODX therapy.

Keywords: breast-prostate cancer, polybisphosphonate, expression proteomics

POS-01-070 Analysis of Proteome Expression of Peripheral Blood Mononuclear Cells in Pancreatic Cancer Patients for Noval Biomarker Discovery

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Pancreatic cancer is one of the malignanttumors with poor prognosis .Early detection and resection of tumors can apparently lengthen the life time of patients. Blood is one of the promising samples for early detection of tumors and peripheral blood mononuclear cells (PBMCs) comprise thecirculating mononuclear cells, including monocytes, T-cells, Bcells, and natural killer (NK) cells, and have emerged in recentyears as surrogate markers of several diseases including inflammatory(e.g. preeclampsia, rheumatoid arthritis, and chronicpancreatitis) and malignant (chronic lymphocytic leukemia andrenal cell carcinoma) diseases .We used 20 cases of PBMCs from normal people,20 from benign tumor patients and 20 pancreatic cancer patients for analysis.3235proteins were identified, with 45 proteins were differentially expressed apparently in pancreatic tumor samples using strict statisticalanalysis. These proteins are highly enriched in transcription pathway .Some up-regulated proteins shows consistency with our tissue and serum samples with a high potential to be a biomarker candidates. Further quantitation of these proteins and functional analysisare on the way. **Keywords:** PBMCs/pancreatic carcinoma/ iTRAQ **References** Michael J. Baine, SubhankarChakraborty,eta1.PLoS ONE,2011,e170142. Natalie C. Twine, Jennifer A. Stover, Bonnie Marshall, *eta1*. Cancer Res 2003;63:6069-6075

Keywords: PBMCs/pancreatic carcinoma/ iTRAQ

POS-01-071 Quantitation and Evaluation of Candidate Biomarkers of Pancreatic Cancer in Plasma Using Multiple Reaction of Monitoring (MRM) Method

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Pancreatic cancer is one of the most highly aggressive and lethalof all solid malignancies .Systematically discovering and validating new biomarkers for early diagnosis of pancreatic cancer is under urgent demand. Targeted mass spectrometry (MS) through multiplereaction monitoring (MRM) has emerged as an alternative to affinity-based measurementsof defined protein sets with faster and cost-efficient assay development, high sensitivity and high reproducibility. We previously analyzeddifferential expressed proteome of serum from healthy people, people bearing benign pancreatic diseases and pancreatic cancer people using ITRAQ labeling coupled with LC MS/MS method. Through restrict data analysis, we find that 29 proteins were overexpressed in pancreatic cancer patients' serum. Based on these, we further quantify and validate these proteins in huge sample herd, evaluate their sensitivity and specificity, analyze them and chose a combination of new biomarkers with development prospect. **Keywords:** pancreatic carcinoma/ iTRAQ/serum **References** 1. Faca, V.M., Song, K.S., Wang, H., Zhang, Q. *etal*. PLoS Med.2008, 5, e123. 2. Pannala R, Leirness JB, Bamlet WR, *eta1*. Gastroenterology, 2008, 134(4): 981-987.

Keywords: pancreatic carcinoma/ iTRAQ/serum

POS-01-072 Differential Glycan Profiling of MY.1E12-positive MUC1 to Discover Serological Glyco-Biomarker for Cholangiocarcinoma Diagnosis

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Glycoproteomics allows comprehensive identification of glyco-biomarker candidates, which are glycoproteins with disease-specific glycosylation. Our aim in this study is the development of a glycan-related cholangiocarcinoma (CC) marker. Previously, we reported that *Wisteria floribunda* agglutinin (WFA) was the best probe lectin to discriminate between the normal bile duct epithelia and CC lesion in CC tissue sections by the lectin microarray and histochemical analysis. Furthermore, we identified sialyl-MUC1, which was recognized with a specific monoclonal antibody MY.1E12 as a WFA-positive glycoprotein in surgical tissue and bile specimens. An increase of WFA-positive sialyl-MUC1 in CC patients was confirmed by WFA-coated MY.1E12 sandwich ELISA (Matsuda A., *et al*., *Hepatology, 2010*). In this study, we validated the assay system for serological diagnosis using comparative analysis. We performed glycan profiling of serum sialyl-MUC1 using lectin microarray. Although some lectins showed a significant difference between CC and others (healthy and benign disease controls), we concluded that WFA was the best probe and WFA positive sialyl-MUC1 was feasible marker compared with other markers between CC and benign bile duct diseases. Currently, we are conducting further validation using serum specimens obtained from several clinical sites.

This work was supported by a grant from New Energy and Industrial Technology Development Organization (NEDO) in Japan.

Keywords: biomarker, cholangiocacinoma, MUC1

POS-01-073 Novel Diagnostic Marker Candidates of Hepatocellular Carcinoma Revealed by Quantitative Proteomics

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Hepatocellular carcinoma (HCC) is the seventh most common cancer worldwide and shows a high mortality and an increasing incidence. The success of therapy for HCC patients strongly depends on the diagnosis time. To increase sensitivity and selectivity during immunohistochemical diagnosis, the use of multiple biomarker panels is necessary due to heterogeneity of the tumor. In this context, novel biomarker candidates are needed in order to improve existing marker panels or to setup even new ones. To find novel diagnostic HCC biomarker candidates, malignant (n = 7) and non-tumorous tissue samples (n = 7) were analyzed by means of quantitative proteomics. Herein, we applied complementary techniques in order to improve proteome coverage, namely a gel-based 2D-DIGE as well as label-free MS-based approach. Using text mining tools the significantly regulated proteins were analyzed regarding their known association to HCC, liver diseases or other tumors. Based on these results, selected candidate proteins were chosen for an initial verification using Western blots and immunohistochemistry (n = 28). In a further validation set (n = 288), altered expression levels were further confirmed for several candidate proteins. In particular, major vault protein (MVP) and 14-3-3 sigma protein also known as stratifin (SFN) showed significant over-expressions in HCC tumor tissues. Hence, we further tested whether a combination of one of these marker candidates with an established biomarker panel can improve the diagnostic performance. In this respect, marker panels containing HSP70, glypican 3 and glutamine synthetase in combination with MVP or SFN were tested. Here, an increased diagnostic performance was found for the marker panel containing SFN in comparison to the panel without it, which makes 14-3-3 sigma protein a promising diagnostic marker candidate for immunohistochemical detection of HCC.

Keywords: quantitative proteomics, HCC biomarker, immunohistochemistry

POS-01-074 Ratio of Glycochenodeoxycholate-3-Sulfate to Its Precursor as a Novel Biomarker of Small Hepatocellular Carcinoma

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To discover novel serum marker(s) for the diagnosis of hepatocellular carcinoma (HCC), especially those small ones, bile fluids from HCC and benign hepatobiliary cases were analyzed using an ultra performance liquid chromatography-mass spectrometry. Acquired data were subjected to multi-variant analysis with respect to HCC, and the differential metabolites found were reevaluated with sera from 416 hepatic disease cases, including 91 small HCCs (solitary nodule with diameter less than 2cm). A sulfated bile acid - glycochenodeoxycholate-3-sulfate (GCDCS) was screened out with elevated concentrations in the bile fluids and in the sera of HCC patients. Of note, the ratio of GCDCS to its precursor, glycochenodeoxycholic acid (GCDCA), was significantly higher in HCC patients than that in the normal, hepatitis B and hepatitis B-related cirrhosis cohorts. GCDCS/GCDCA had a sensitivity of 75% and a specificity of 81% at the optimal cutoff point with an area under the receiver operating curve (AUC) of 0.83 *vs* 0.74 for AFP (p=0.001). Furthermore, combinational use of GCDCS/GCDCA and AFP improved the diagnostic sensitivity for small HCC from 58% (AFP alone) to 87% (p<0.001) with an AUC of 0.92 (GCDCS/GCDCA plus AFP) *vs* 0.72 (AFP alone, p=0.001). Additionally, the ratio was also higher in tumor tissues than that in the adjacent cirrhotic tissues. Conclusion: GCDCS/GCDCA was a novel serum indicator for HCC diagnosis of both the general population and individuals at high risk.

Keywords: hepatocellular carcinoma, bile acids, metabonomics

POS-01-075 Proteomic and Cytokine Plasma Biomarkers for Predicting Progression from Colorectal Adenoma to Carcinoma in Human Patients

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In the present study, we screened proteomic and cytokine biomarkers between patients with adenomatous polyps and colorectal cancer (CRC) in order to improve our understanding of the molecular mechanisms behind tumorigenesis and tumor progression in CRC. To this end, we performed comparative proteomic analysis of plasma proteins using a combination of 2-DE and mass spectrometry as well as profiled differentially regulated cytokines and chemokines by multiplex bead analysis. Proteomic analysis identified 11 up-regulated and 13 down-regulated plasma proteins showing significantly different regulation patterns with diagnostic potential for predicting progression from adenoma to carcinoma. Some of these proteins have not previously been implicated in CRC, including up-regulated leucine-rich α-2-glycoprotein, hemoglobin subunit β, Ig α-2 chain C region, and complement factor B as well as down-regulated afamin, zinc-α-2-glycoprotein, vitronectin, and α1-antichymotrypsin. In addition, plasma levels of three cytokines/chemokines, including interleukin-8, interferon gamma-induced protein 10, and tumor necrosis factor α, were remarkably elevated in patients with CRC compared to those with adenomatous polyps. Although further clinical validation is required, these proteins and cytokines can be established as novel biomarkers for CRC and/or its progression from colon adenoma.

Keywords: 2-DE, carcinoma, adenoma

POS-01-076 Inhibition of Transketolase by Oxythiamine Altered Dynamics of Protein Signals in Pancreatic Cancer Cells

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Oxythiamine (OT), an analogue of anti-metabolite, can suppress the nonoxidative synthesis of ribose and induce cell apoptosis by causing a G1 phase arrest *in vitro* and *in vivo*. However, the molecular mechanism remains unclear yet. In the present study, a quantitative proteomic analysis using the modified SILAC method (mSILAC) was performed to determine the effect of metabolic inhibition on dynamic changes of protein expression in MIA PaCa-2 cancer cells treated with OT at various doses (0 μM, 5 μM, 50 μM and 500 μM) and time points (0 h, 12 h and 48 h). A total of 52 differential proteins in MIA PaCa-2 cells treated with OT were identified, including 14 phosphorylated proteins. Based on the dynamic expression pattern, these proteins were categorized in three clusters, straight down-regulation (cluster 1, 37% of total proteins), upright "V" shape expression pattern (cluster 2, 47.8% total), and downright "V" shape pattern (cluster 3, 15.2% total). Among them, Annexin A1 expression was significantly down-regulated by OT treatment in time-dependent manner, while no change of this protein was observed in OT dose-dependent fashion. Pathway analysis suggested that inhibition of transketolase resulted in changes of multiple cellular signaling pathways associated with cell apoptosis. The temporal expression patterns of proteins revealed that OT altered dynamics of protein expression in time-dependent fashion by suppressing phosphor kinase expression, resulting in cancer cell apoptosis. Results from this study suggest that interference of single metabolic enzyme activity altered multiple cellular signaling pathways.

Keywords: quantitative proteomics; pancreas cancer; 15N stable isotope; phosphorylation; turnover rate; metabolic inhibitor; metabolic therapy; transketolase; oxythiamine

POS-01-077 In-Depth Proteomic Characterization of the Secretome of Colorectal Cancer Metastatic Cells Identifies Key Proteins in Liver Metastasis

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Colorectal cancer is the second cause of cancer-associated mortality in developed countries. Tumor metastasis depends on different factors, such as growth factors and receptors, proteases, chemokines and extracellular matrix proteins. Many of these factors are secreted by the metastatic cells. To identify and characterize proteins associated with colon cancer metastasis, we have compared the conditioned serum-free medium of highly metastatic KM12SM colorectal cancer cells with the parental, poorly metastatic, KM12C cells using quantitative SILAC analyses. In total, 1337 proteins were simultaneously identified in SILAC forward and reverse experiments. For quantification, 1098 proteins were selected in both experiments, with 155 proteins showing >1.5 fold-change. Three secreted proteins GDF15, S100A8/A9 and SERPINI1 showed capacity to discriminate cancer serum samples from healthy controls using ELISA assays. *In silico* analyses of deregulated proteins in the secretome of metastatic cells showed a major abundance of proteins involved in cell adhesion, migration and invasion. A panel of six proteins was selected for functional analysis. Knocking-down expression of NEO1, SERPINI1 and PODXL showed a significant effect on cellular adhesion. Silencing or blocking experiments with SOSTDC1, CTSS, EFNA3, CD137L/TNFSF9, ZG16B and MDK caused a significant decrease in migration and invasion of highly metastatic cells. In addition, SOSTDC1, EFNA3 and CD137L/TNFSF9 were involved in liver colonization capacity of KM12SM cells. Finally, the panel of six proteins involved in invasion showed association to poor prognosis and overall survival after dataset analysis of gene alterations. In summary, by using quantitative proteomics we have defined a collection of proteins that are relevant to understand the mechanisms underlying adhesion, migration, invasion and metastasis in colorectal cancer.

Keywords: secretome, metastasis, colon cancer

POS-01-078 N-Linked Glycoprotein Profiling of Prostate Cancer Tissue by ^{18}O Labeling and High Resolution Mass Spectrometry

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Prostate cancer (PCa) represents the second leading cause of cancer-related death in men. Prostate specific antigen (PSA) is currently used for PCa screening. However, because of its low specificity, new diagnostic biomarkers are required. Glycosylation is known to play a central role in cancer transformation and progression. Indeed, available biomarkers are mostly glycoproteins. On these basis, the aim of this study was to quantify N-linked glycoproteins expressed in paired normal and tumor tissue from twelve PCa patients who underwent radical prostatectomy. Glycopeptides from normal and cancer tissue were enriched using solid phase extraction of N-linked glycopeptides through a hydrazide support and then released by PNGase F. Then, glycopeptides were quantified by ^{18}O labeling and high resolution mass spectrometry. This strategy allowed the extensive profiling of normal and cancer tissue and the identification of specific PCa glycoprotein profiles. More than 300 glycoproteins were quantified in a single run. As expected, PSA was found among differentially expressed proteins, and thus acted as positive control. The analysis of the molecular function, cellular localization and cancer association of these proteins, showed that there was a global increase of proteins involved in cancer-related functions in PCa tissue. As many of the identified candidates are detectable in serum using highly sensitive, targeted methods (ELISA, SRM), these data constitute a promising starting point for the development of a new specific biomarker signature in serum for PCa early diagnosis.

Keywords: prostate cancer, glycoproteins

POS-01-080 Down-Regulation of the Tumor Suppressor Gene PML is a Novel Prognostic Biomarker in Gastrointestinal Stromal Tumor: Integrated Proteomic and Transcriptomic Approach

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Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the gastrointestinal tract. Adjuvant therapy with imatinib prolongs disease-free survival after the complete resection, however nearly all patients suffer from some adverse effects. In addition, half of the operative GIST patients are cured by surgery alone, and only limited patients may have benefit from imatinib treatments. Therefore, prognostic markers are urgently needed to optimize the adjuvant therapy. GIST of the small intestine (SI-GIST) exhibit worse clinical behaviors than that of the stomach (S-GIST). Because further understanding of the molecular backgrounds of malignant potentials of SI-GIST may provide us a clue to develop prognostic modalities, we examined the proteomic and transcriptomic differences between S-GIST and SI-GIST. We generated the protein expression profiles of surgically resected 4 S-GISTs and 4 SI-GISTs using label-free proteomics. For the transcriptomic analysis, DNA microarray datasets of 23 S-GISTs and 9 SI-GISTs in Gene Expression Omnibus were examined. Among the 2555 genes analyzed, we showed that promyelocytic leukemia (PML) was significantly down-regulated in SI-GIST at both protein and mRNA level. PML acts as a tumor suppressor by controlling cellular growth and apoptosis. The correlation between loss of PML and tumor progression was reported in various malignancies, but not in GIST until our study. Immunohistochemical study in multi-institutional 254 cases showed that down-regulation of PML was significantly frequent in SI-GIST, and was significantly correlated with shorter disease-free survival in the entire GIST and in S-GIST stratified by the tumor location. Multivariate analysis revealed that PML expression was an independent prognostic factor. Our study demonstrated that PML is a novel prognostic marker, and is worth investigating for the mechanistic significance and the potential of being the therapeutic applications in GIST.

Keywords: gastrointestinal stromal tumor, promyelocytic leukemia, label-free proteomics

POS-01-079 Nerve Growth Factor and Its Precursor as Cancer Biomarkers and Targets: Using Proteomics from Discovery to Validation

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Nerve growth factor (NGF) and its precursor (proNGF) are well know for their role in the development of the nervous system where they participate in neuron survival and differentiation. We have discovered that NGF can stimulate breast cancer cell growth and using proteomics as well as other molecular biology approaches we have shown that it is overexpressed in breast tumours. Moreover, ProNGF is also secreted by breast cancer cells and has the effect of enhancing tumour cell invasion. In animal models, targeting NGF and proNGF or their receptors TrkA, $p75^{NTR}$ or sortilin resulted in an inhibition of tumour growth and metastasis and therefore NGF and proNGF are potential clinical biomarkers and targets in breast cancer. Bench to bedside translation of biomarkers involves validation studies with large cohorts of cancer samples as well as the development of tools for clinical applications. An integrative approach involving immunohistochemistry, ELISA, and multi reaction monitoring (MRM) mass spectrometry has been developed to analyse tumours and blood samples and to explore correlation between the levels of proNGF/NGF and their receptors with clinicopathological parameters including tumour subtype, estrogen and progesterone receptors, HER2, node invasion and patient survival. These studies have highlighted a number of features, such as a higher level of proNGF in the blood of breast cancer patients as compared to normal controls, suggesting proNGF as a possible biomarker for diagnosis, and a relationship with node invasion indicating that it may also be a prognosis biomarker for the risk of developing metastasis. Interestingly, overexpression of NGF/ProNGF was also found in lung, thyroid, and prostate tumours, expanding their clinical value to other cancers.

Keywords: cancer, breast, lung, thyroid, prostate, biomarkers and therapeutic Targets

POS-01-081 Screening for Early Intestinal Metaplasia Biomarkers in Gastric Cardia Adenocarcinoma (GCA) by Navigated LCM and Proteomics

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Objective: To identify GCA associated proteins and early intestinal metaplasia (IM), protein biomarkers. **Methods:** We performed navigated laser capture microdissection (LCM) to enrich the malignant (group A), IM (group B) and nonmalignant (group C) gastric cardia epithelial cells from surgical specimens of human GCA. The proteins extracted from these cells were separated by 2-DE. Protein spots were identified by MS and database searching. **Results:** (1) The 2-DE patterns with high resolution and reproducibility of human GCA were obtained. The mean detected number of protein spots was: 867 $\pm$ 51 in A, 836 $\pm$ 50 in B, and 905 $\pm$ 74 in C. The percent of matched spots between them was: 77.6% between A and C, 86.7% between A and B, and 79.5% between B and C. (2) Seventy two proteins of GCA including their cellular localization and physiological function were successfully identified. (3) Twenty three proteins were consistently differential regulated in IM. They were classified into cell proliferation and differentiation (ANXA2, ANXA4), apoptosis (Prx-2, GSTP, VDAC, BCL2L11), metabolism (ADH1C, AKR1C3, CA II, GATM, Sulfotransferase 1A1, ZFYVE1, GPR175), protease related (PCNC1), cystoskeleton (Keratin 8), chaperones (Hsp27, PDIA3), RNA binding and transcription (hnRNPH3, ZNF511, ENO1, ATPA), unknown (ERp29, Galectin-3). Expressions of Hsp27 and Prx-2 were further confirmed by immunohistochemical and western blot analysis. **Conclusion:** We identified 72 proteins of GCA, which may be helpful to construct the database and elucidate the molecular mechanisms of the carcinogenesis of GCA. Twenty three proteins regulated in IM may have a potential role in early detection targets of GCA.

Keywords: gastric cardia adenocarcinoma, intestinal metaplasia, biomarker

POS-01-082 The Proteomic Study to Identify Proteins Specific to Invasion of Myxofibrosarcoma

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Backgrounds: Myxofibrosarcoma (MFS) is a common type of soft tissue sarcoma in the elderly. High invasive activity of MFS results in frequent local recurrence after surgery. As MFS is refractory to chemotherapy and radiotherapy, the results of surgery directly related to tumor prognosis. However, the surgery plan for deciding surgical margin considering the tumor invasion depends on empirical criteria. Identification of proteins related to invasions may improve the clinical outcome of MFS.
Methods: We created the proteomic profiles of 11 biopsy samples from MFS patients using two-dimensional difference gel electrophoresis (2D-DIGE). 6 cases had invasive tumors, and 5 had non-invasive ones on MRI imaging.
Results: The protein expression profiles by 2D-DIGE consisted of 3,453 protein spots, including 59 spots whose intensity exhibited >2-fold difference (P<0.01) between the two sample groups. Mass spectrometry showed the 59 spots corresponded to 47 distinct gene products. The expression level of 36 of the 47 proteins was increased in MFS with more invasive phenotypes. These 43 proteins play a key role in cell adhesion, migration, and formation of invadopodia in tumor cells. The expression of these proteins was validated immunohistochemistry and optimized along the tumor invasive area.
Conclusion: We identified novel proteins specific to invasion by proteomic approach in MFS. Elucidation of these proteins will lead to further understanding of invasive features of MFS. The expression of these proteins was especially along the tumor invasive area. These findings will help to decide appropriate surgical margin which leads to novel clinical applications.
Keywords: myxofibrosarcomsa, invasion, 2D-DIGE

POS-01-083 Serum Metabolomics as a Screening Method for Pancreatic Cancer

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Background: To improve the prognosis of pancreatic cancer patients, more sensitive serum screening methods with high accuracy are needed. We applied serum metabolomics as a diagnostic tool for pancreatic cancer.
Methods: Sera from pancreatic cancer patients (PC), healthy volunteers (HV), and chronic pancreatitis patients (CP) were collected at multiple institutions. The PC and HV were randomly allocated to the training or the validation set. All of the CP were included in the validation set. Metabolites in sera of the subjects were analyzed by gas chromatography mass spectrometry and a data processing system using an in-house library. The diagnostic model constructed via multiple logistic regression analysis in the training set was evaluated on the basis of its sensitivity and specificity in validation set study.
Results: In the training set study, which included 43 PC and 42 HV, the model possessed high sensitivity (86.0%) and specificity (88.1%) for PC. The utility of the model was confirmed in the validation set study, which included 42 PC, 41 HV, and 23 CP; i.e., it displayed highest sensitivity (71.4%) for cancer patients; and furthermore, it had higher sensitivity in early PC (77.8%) and lower false positive rate in CP (17.4%) than conventional tumor markers.
Conclusions: Serum metabolomics could accurately detect pancreatic cancer patients earlier than tumor markers. It is a promising screening method for pancreatic cancer.
Keywords: biomarker, metabolomics, GC/MS

POS-01-084 Cancer Proteomics for Biomarker Development Toward Personalized Medicine

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Cancer is a clinically diverse disease, and biomarkers for further characterization of cancer cells are required to optimize the therapeutic strategy for individual patients. Tissue proteome is a rich source for biomarker research as it is a direct functional translation of genome. With this notion, we conduct tissue biomarker study for personalized medicine using surgical specimens and biopsy samples. Proteomics for biomarker development requires an interdisciplinary collaboration, because proteomics has been mainly driven by basic researchers, and in contrast, identifying the clinical problems and addressing the need for biomarker are performed by clinicians. Thus, we established tight collaboration between researchers and clinicians, and tackle urgent subjects in the hospital, such as prognostic and predictive biomarkers. High quality clinical information is the main pillar of biomarker study, and the effective use of materials deposited in the biobank system requires the knowledge and experience about medicine. We found that the bench-to-bed side collaboration is indispensable in this sense too. As no proteomic technology allows uncover entire aspects of proteome, we use multiple proteomics tools based on different principles, such as two-dimensional gel electrophoresis, LC-MS/MS and antibody libraries for protein expression profiling. We found that these established methods are productive in tissue biomarker study. Biomarkers are the molecules whose expression level is highly associated with important clinical parameters such as metastasis and resistance to treatments, and the biomarker study may lead to novel cancer biology and therapeutic targets. We discuss these issues introducing recent research outcomes from our laboratory.
Keywords: cancer, biomarker, personalized medicine

POS-01-085 N-Linked Glycoprotein Profiling for Colorectal Cancer Biomarker Discovery Using High Resolution Quadrupole-Orbitrap Mass Spectrometry and ^{18}O Isotopic Labeling

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Colorectal cancer (CRC) is a common cause of death, and the discovery of high-specificity and sensitivity biomarkers could become an essential diagnostic tool for the early detection of CRC. Post-translational modifications are known to play an important role in cancer progression. Particularly, glycosylation has been recognized as one of the most prominent cancer-associated biochemical alterations. Starting from these assumptions, we profiled expressed N-linked glycoproteins in CRC tissues using a quantitative proteomic technique based on ^{18}O stable isotope labeling in order to identify differentially expressed proteins in CRC compared to unaffected colorectal tissue. Twenty consecutive patients underwent surgery for CRC, and tissues were dissected from excised tumors and adjacent normal mucosa. First, N-linked glycopeptides were isolated from tissue samples by Solid Phase Extraction of N-linked Glycopeptides and analyzed with High Resolution Quadrupole-Orbitrap Mass Spectrometry. Afterwards, identified glycopeptides were selected for quantification in N-glyco-enriched tissue with labeling ^{18}O samples. Finally, the identified and quantified N-linked glycoproteins, differentially expressed between normal and cancer tissues, were associated with patients clinical features. Data showed global alteration in the N-linked glycoprotein profile of CRC tissues compared with normal tissues, especially for proteins involved in biological processes of tumorigenesis. Of these, some are detectable in serum by mass spectrometry with current technology. Our findings suggest that N-linked glycoproteins that are differentially expressed at specific cancer stages might act as potential biomarkers of CRC. Furthermore, a subset of these proteins is of potential interest for stage-specific diagnosis and prognosis of patients with CRC using targeted proteomic quantification in serum.
Keywords: colorectal cancer, glycoproteins

POS-01-086 Analysis of Secretome and Quantitative Tissue Proteome Identified Fibulin-4 and Melanotransferrin as Serological Markers for Colorectal Cancer

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Early clinical diagnosis of colorectal cancer (CRC) through serological markers would be convenient and decrease the mortality rate. In order to discover serological CRC markers, we analyzed cell line secretome to gather proteins of higher potential to be secreted from tissues into circulation. A total of 898 human cell secreted proteins were identified from 2 CRC cell lines, of which 62.2% were predicted to be released or shed from cells. The identified proteins were compared to tissue proteomes to find candidate proteins whose expressions were elevated in tumor tissues compared to normal tissues as revealed by data mining of: (i) quantitative information on protein expression levels generated by using cICAT-mTRAQ and reported previously, or (ii) immunohistochemical images piled in Human Protein Atlas public database. By applying various stringent criteria, 11 candidate proteins were selected. Among these, we validated an increase of fibulin-4 (FBLN4), RPE-spondin (RPESP) and melanotransferrin (TRFM) at the plasma level of CRC patients through Western blotting (all p values <0.01) using 114 plasma samples from 20 healthy controls, 71 CRC patients, 11 colorectal adenoma patients, and 12 patients with other inflammatory diseases. By using commercial ELISA on 71 plasma samples from 10 healthy controls and 61 CRC patients, we further demonstrated that FBLN4 and TRFM are complementary to CEA in diagnosis of CRC. We suggest FBLN4 and TRFM as potential serological markers for CRC and expect our strategy helps to identify highly cancer-specific and body fluid-accessible biomarkers.

Keywords: secretome, serological marker, colorectal cancer

POS-01-087 Cancer-Specific Genome Changes Observed at Proteome Level in Colorectal Cancer

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Cancer genome includes thousands of somatic mutations, some of them being drivers of cancer development. It is not quite clear how many of them may be observed at proteome level. To this end, we made a database of aminoacid changes predicted from exome sequencing from 276 cancers and controls (published be The Cancer Genome Atlas Network, *Nature*, 2012). Amino acid polymorphisms were searched in LC-MS/MS data by Mascot engine software using specifically developed algorithm. Cancer cell line data available from repositories were used and own mass-spectra were recorded with LTQ OrbiTrap from 4 samples of colorectal carcinoma with corresponding controls. Some translated single amino acid polymorphisms (SAPs) were detected with high score and 1% peptide FDR, e.g. in the heat shock cognate 71 kDa chaperone, adenomatous polyposis coli protein, serine/threonine-protein kinase B-raf and some other cancer-related proteins. Peptides with amino acid polymorphism may, in perspective, be used for targeted mass-spectrometry proteomics as possible highly specific biomarkers.

Keywords: colorectal cancer, proteome, single amino acid polymorphism

POS-01-088 Differentially Glycosylated Circulating Protein Biomarker Discovery for Barretts Esophagus and Esophageal Adenocarcinoma

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Esophageal adenocarcinoma (EAC) arises from precursor metaplastic condition Barrett's Esophagus (BE). BE patients are frequently screened using endoscopy-biopsy for early neoplastic changes. However, being an asymptomatic condition, it is very difficult to identify BE patients and to recruit them for screening. Also endoscopy is not suitable for large scale population screening due to high cost and patient non-compliance. Taken together, majority of EAC cases are diagnosed very late during pathogenesis and showed high mortality.

To facilitate early diagnosis, we focused on alterations in circulatory protein glycosylation, using a panel of 20 lectins to isolate different glycan structures on serum glycoproteins. Serum samples from control (n=9), BE (n=10) and EAC (n=10) patient groups were analyzed by lectin magnetic bead array-coupled mass spectrometry [1]. Customized database "GlycoSelect" was developed which incorporates outlier detection and sparse Partial Least Squares regression Discriminant Analysis[2]. We identified a ranked list of candidate glycobiomarkers that distinguish a) EAC from BE and b) BE from control group. In general, glycoproteins bound several lectins, reflecting heterogeneity and multiplicity of glycosylation. Specific glycan structure changes were observed as loss and gain of binding to a single lectin while maintaining binding to other lectins. Future work will validate the candidate protein-lectin pairs using a customized lectin-affinity array-coupled with quantitative mass spectrometry using independent cohort of 100+ patients. The specificity and sensitivity of panels of glycobiomarkers will be determined to develop a serum screening test for BE/EAC.

[1] Choi et al., Electrophoresis 32, 3564-3575 (2011)
[2] Lê Cao et al., BMC Bioinformatics 12, 253-268 (2011)

Keywords: esophageal adenocarcinoma (EAC), barrett's esophagus (BE), biomarker

POS-01-089 Proteomic Analysis of Human Malignant Pleural Mesothelioma Cells Compared to Normal Mesotherial Cells

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Malignant pleural mesothelioma (MPM) is the malignancy arising from pleural mesothelial cells and asbestos-related disease. Its prognosis is very poor because of the difficulty of early detection. The incidence may increase in the next two decades. We performed proteome analysis with the aim of investigating protein expression in MPM cell lines and pleural mesothelial cell line. The proteins were separated by 2-DE, then the protein spots that showed differential expression between in MPM cell lines and MeT-5A were identified by LC-MS/MS. We identified seven proteins showing higher expression in MPM cell lines than MeT-5A. Then we examined the expression of 78 kDa glucose regulated protein (GRP78) and heat shock 70 kDa protein (HSP70) in MPM cells and MeT-5A cells by Western blotting. It showed up-regulation of GRP78 and HSP70 in MPM cell lines compared to MeT-5A and normal pleural tissues. In addition, we examined the expression of DDX39 in MPM cells and MeT-5A cells by Western blotting, because we interested in the relation between malignant pleural mesothelioma cells and these proteins which have been reported to be associated with metastasis and invasion. The expression of DDX39 was higher in MPM cell lines compared to MeT-5A These results suggest that GRP78, HSP70 and DDX39 may play important roles in malignant pleural mesothelioma, and could lead to early detection and new therapy.

Keywords: malignant mesothelioma, HSP70, GRP78

POS-01-091 Search for Biomarker Proteins Related to Cisplatin-Susceptibility in Malignant Mesothelioma

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Introduction

Although cisplatin (CDDP) is a highly effective anticancer drug for malignant mesothelioma (MM), its response rate is only 20%. For this reason, it is important to elucidate the molecular mechanism of CDDP-resistance and to distinguish responders from non-responders. In order to identify proteins related to CDDP-susceptibility, we quantitatively compared protein expression between MM cells that display high and low susceptibility to the drug.

Materials and Methods

CDDP-cytotoxicity test: MM cells were cultured with different concentrations of CDDP for 24 h. Cytotoxicity was evaluated by the WST-8 assay.

Two-dimensional differential in-gel electrophoresis (2D-DIGE): Cell lysates from MM cells, H28 and H2052, were labeled with Cy3 or Cy5 and then separated by IEF-PAGE and SDS-PAGE. Differentially expressed proteins were identified by mass spectrometry (MS).

AnnexinA4 (ANXA4) expression analysis in clinical samples: A FFPE tissue microarray slide was stained using anti-human ANXA4 antibody.

Results and Discussion

Among the five MM cells tested in this study, CDDP-susceptibility was the highest in H2052 and the lowest in H28. Differentially expressed proteins between H28 and H2052 were subsequently analyzed by 2D-DIGE and identified by MS. Our results identified ANXA4 as showing higher protein expression in H28. Moreover, ANXA4 expression was higher in clinical MM tissues than normal tissues. Thus we evaluated the function of ANXA4 in CDDP-susceptibility by a genetic engineering procedure. Cytotoxicity assay showed CDDP-susceptibility was significantly increased by ANXA4-siRNA transfection and decreased by ANXA4-cDNA transfection. These data suggested ANXA4 expression was related to CDDP-resistance in MM cells. We are currently analyzing the relationship between ANXA4 expression and CDDP-response in MM patients.

Keywords: Malignant mesothelioma, Cisplatin-resistance, Biomarker protein

POS-01-092 Autoantibody Biomarkers for the Detection of Early Stage Ovarian Cancer

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Ovarian cancer is the leading cause of death from gynaecologic malignancies. The majority (80%) of ovarian cancer cases are identified at advanced stages where prognosis is poor. Detection at an early clinical stage dramatically improves the 5-year survival rate from only 35% in late stages to 90%. However, screening for early ovarian cancer is currently not possible. Autoantibodies raised against tumour associated antigens (TAAs) during early stages of cancer development have potential as biomarkers for ovarian cancer diagnosis. Our lab developed an immunoproteomic strategy that enabled the identification of autoantibodies that are differentially raised in ovarian cancer patients compared to healthy/benign controls. Here, captured autoantigens eluted from paired cancer and control immunoaffinity columns were differentially labelled using isotope coded protein label (ICPL) technology. Pooled samples (n =12) were analyzed using liquid chromatography coupled to an LTQ XL Orbitrap mass spectrometer. Relative quantification was performed to identify 141 autoantigens, and therefore their corresponding autoantibodies, to be differentially present in the sera of ovarian cancer patients by ≥1.5 fold ratio compared to the controls. Upon bioinformatic prioritization, 50 autoantibody candidates were selected for verification using protein microarray (n=98). Of these, eleven autoantibody candidates were found to be significantly different in the cancer patients compared to controls. A panel of the top four candidate biomarkers showed combined ROC curves with 100% sensitivity and 98.3% specificity using 60 controls and 18 early stage patient sera. We will further validate those candidates, in combination with CA125 as a diagnostic indicator for early ovarian disease using enzyme-linked immunosorbent assay (ELISA) and lager patient cohorts.

Keywords: auto antibody, ovarian cancer, quantitative proteomics

POS-01-093 Glioblastoma Cell Secretome Analysis and Relevance with Tumor Associated Pathways

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Glioblastoma multiforme (GBM) is the most aggressive malignant tumor of the central nervous system. We studied secretome of three GBM cell lines (HNGC-2, U87MG and LN-229) as an effort to identify proteins, which may be targeted in the plasma from GBM patients as markers for disease surveillance. By direct analysis of the conditioned media proteins of these cells without any pre-fractionation, we identified 148 non-redundant list of proteins, of which more than 40% belonged to extracellular/membrane class and many were cancer related. Out of these cell lines, HNGC-2 is a tumorigenic cancer stem cell line derived from a primary tumor and exhibits typical features of GBM when developed into mouse xenografts. So we further expanded secretome protein analysis by SDS-PAGE pre-fractionation followed by LC-MS/MS analysis and identified 996 proteins. Majority of them have transcript evidence from HNGC2 transcriptome and mapped to important pathways commonly affected in tumor cells such as Phosphatidyl inositol 3 kinase (PI3K/AKT) and Integrin linked kinase (ILK) signalling pathway. Comparison with the published cancer cell secretome datasets showed an overlap of 348 proteins and several proteins with signal/transmembrane domains or extracellular localization, with potential of secretion from cells. On integration, we arrived at a non-redundant list of 597 proteins; 233 of them already detected in normal cerebrospinal fluid or plasma, thus offering a strong resource for investigation in the plasma of GBM patients. The analysis included an observation that suggested fragmentation of astrocyte specific protein, GFAP, and release of its fragments into the condition medium, which may be the basis of detection of GFAP in the plasma of GBM patients (Jung et al 2007).

Keywords: glioblastoma, secretome, mass spectrometry

POS-01-094 In-Depth Proteomic Characterization of Pancreatic Ductal Adenocarcinoma Subtypes

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with a median overall survival of six months. One reason for the short median survival is that PDACs have a high propensity for locoregional invasion and additionally feature the early development of distant metastases. Furthermore, four out of five patients present with a locally advanced or even metastatic disease precluding curative surgery. So far, curative resection presents the single most important factor for the determination of the outcome in patients with pancreatic adenocarcinoma.

Recently, three PDAC subtypes (classical, quasi-mesenchymal, and exocrine-like) have been proposed based on transcriptomic profiling of primary tumors. However, no protein biomarkers for the subclassification of PDAC are known. Using a set of twelve primary human patient-matched cell lines grown under serum free stem cell conditions, we applied LC-MALDI-MS and LC-ESI-MS to uncover novel subtype specific biomarkers relevant for the development of diagnostics and/or therapeutics. The identification of differentially expressed proteins was achieved by:

(i) *in vitro* biotinylation of cultured cell followed by mass spectrometric analysis of purified biotin-tagged proteins for the cell surface proteome

(ii) GeLC-MS-based analysis of the total proteome

(iii) analysis of proteins secreted into the chemically defined, serum-free cell culture medium.

The biotinylation-based approach for the determination of the accessible cell surface proteome resulted in the identification of more than 2500 proteins. The GeLC-MS-based analyses of the total proteome lead to the identification of more than 5000 proteins, while the secretome consisted of more than 2000 proteins. This first global analysis of the proteome of twelve primary, patient-matched PDAC cell lines and their xenografted tumors resulted in the identification of subtype specific biomarkers which are currently validated by antibody-based and antibody-free techniques.

Keywords: pancreatic ductal adenocarcinoma, biomarker discovery, cancer

POS-01-095 Proteomics of High Grade Gliomas: New Molecular Insights

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Gliomas are primary tumors of the central nervous system among which with Gr III anaplastic astrocytoma and Glioblastoma (GBM, WHO Gr IV) are highly malignant, GBM being the most common and aggressive form. We have been engaged in studying differentially expressed membrane proteins from the clinical specimens of these tumors. We have integrated our results from GBM tissue specimens and GBM cell lines with the transcriptomic analysis of TCGA and other datasets for GBMs. This integrated view of potentially tumor associated proteins reveals important regulatory proteins, pathways and networks that can be taken further for clinical investigations. Changes in Ca^{+2} homeostasis and Calcium signalling appeared to be a major event in GBM, being reported for the first time. Transcriptomics data integration yielded not only better global view of the pathways involved but also revealed important regulatory linkages between miRNAs, target mRNAs and proteins. Another important group of regulatory proteins observed in these high grade tumors (Grade III) is a large number of hnRNPs. The presence of these multifarious regulatory molecules with the membrane fraction of these tumors and their differential status in the tumor context opens up new possibilities of their additional or yet unknown functions. Interestingly, many of the differential gene/protein expressions observed in tumor tissues were also included in the secretome of GBM cell lines or some were detectable in blood plasma enhancing their potential of clinical applications.

Keywords: glioblastoma, membrane proteins, calcium signaling

POS-01-096 Towards the Mechanism of EGFR Inhibitor Resistance in Non-Small Lung Cancer Cells

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While tyrosine kinase inhibitors (TKIs) against Epidermal Growth Factor Receptor (EGFR) have positive therapeutic effects in a subset of Non-Small Cell Lung Cancer (NSCLC) patients, their clinical efficacies are limited due to the emergence of TKI resistance. To study the mechanism of TKI resistance, two NSCLC Erlotinib-resistant cell lines were established. To identify differences in protein expression between parental and drug-resistant cells after EGF and/or erlotinib treatment, the high multiplexing capabilities of Tandem Mass Tag (TMT) reagents and mass spectrometry (MS) analysis were utilized. Parental and H358 erlotinib-resistant NSCLC cell lines were treated with 10 μM erlotinib in the presence or absence of EGF. Proteins from the eight conditions were trypsinized then labeled with TMT6plex reagents plus two $^{13}C/^{15}N$ isotope variants of the TMT6-127 and TMT6-129 reagents. The peptides were then combined and analyzed using nano-LC coupled to a Orbitrap Elite or a novel quadrupole dual cell linear ion trap Orbitrap hybrid mass spectrometer by FT MS^2 or MS^3. Proteome Discoverer 1.4 was used to identify/quantify proteins. Pathway analysis/protein profiling was performed by Protein Center 3.9. Using HCD MS^2 or MS^3 fragmentation methods, more than 1500 protein groups were identified on the Orbitrap Elite with nearly 99% being quantifiable. The novel Orbitrap hybrid noticeably increased the number of identified proteins with a comparable quantitation level. Protein expression ratios between the multiple conditions showed a noticeable decompression using the multinotch MS^3 method with many more proteins exhibiting a statistically significant ratio change. The H358 cell line showed a marked increase in expression of DNA translation/transcription factors and replication licensing proteins, apoptosis inhibitors, and some Ras associated signal transduction elements.

Keywords: drug resistance

POS-01-097 Proteomic Analysis of Tumor-Derived Exosomes Derived from Cultured Human Lung Cancer Cell as Tumor Biomarkers

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Introduction : Lung cancer is the leading cause of cancer deaths worldwide. Detection and measurement of drug-target or biomarker proteins of lung cancer patients are important processes for cancer therapy. Only a limited number of biomarker proteins have been identified from proteomic analysis. In this study, we performed a proteomic analysis of exosomes derived from cultured lung cancer cell lines to detect membrane proteins as cancer biomarkers and evaluated their usefulness for tumor diagnosis.
Material and Method : **Exosome preparation**: The culture medium derived from human lung cancer cell lineswere sequentially centrifuged to eliminate cells and debris, followed by filtration through a nitrocellulose membrane. Then, exosomes were precipitated by ultracentrifugation. **Proteomic analysis**: Exosome samples were subjected to reduction, alkylation with iodoacetamide, and tryptic digestion. Extracted tryptic peptides from each sample were concentrated by centrifugal lyophilization and analyzed by LC-MS/MS fitted with a nanoflow RP-HPLC. RP-HPLC of peptide mixtures was performed on an C18 column developed using a linear 60-min gradient from 0% to 100% B (acetonitrile).
Results and Conclusion: The isolated vesicles were classified as exosomes based on a structural analysis using transmission electron microscopy. The proteome analysis identified more than 50 proteins that were expressed in exosomes derived from three lung cancer cell lines, but not in normal cells. The amount of identified proteins expressing in plasma exosomes can be quantified using different antibodies against exosome markers and tumor expressing proteins. The results of this study will provide a new diagnostic method for detecting exosomes secreted from cancer into the blood, which will be particularly useful for developing tests for minimally invasive personalized cancer therapy.

Keywords: exosome, lung cancer, diagnosis

POS-01-098 The Role of 14-3-3 Protein in Cholangiocarcinoma

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Cholangiocarcinoma is a tumor that derived from the biliary tract epithelium cell (cholangiocyte). This cancer has poor prognosis, is difficult to diagnose and is associated with high mortality. Recent data show that incidence of cholangiocarcinoma is increasing in several areas worldwide. Indeed, it is noteworthy that the incidence of intrahepatic cholangiocarcinoma in Thailand is highest in the world. From previous study we have shown that 14-3-3 protein is significantly upregulated in anoikis induced cholangiocarcinoma cells. However, the role of 14-3-3 protein in cholangiocarcinoma pathogenesis is still largely unknown. Therefore, the aim of this study is to identify binding partners of 14-3-3 protein in cholangiocarcinoma cells using co-immunoprecipitation technique coupled with mass spectrometry analysis. By identifying the binding partner of this protein, we might discover certain function of this protein in cholangiocarcinoma. We were able to identify multiple binding partner proteins by mass spectrometry and further confirmed by immunoblot assay. Interestingly, we have identified a 58 kDa protein as the major binding partner of 14-3-3 in cholangiocarcinoma cells. This protein appears to play an important role in cancer pathogenesis and maybe regulated by 14-3-3 protein. These data might shed some light on the functions of 14-3-3 protein in cholangiocarcinoma pathogenesis.

Keywords: 14-3-3 protein, cholangiocarcinoma, co-immunoprecipitation

POS-01-099 Search for New Urothelial Cancer Biomarkers

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[Purpose] Recently, many studies have focused on biomarker exploration for various diseases. Biomarkers, have the advantage of being less of a burden to the patient, since only a sample of serum or urine is required. Urothelial cancer is one of many cancers that affect the urinary tract. Currently the most effective biomarker for urothelial cancer, NMP22 (nuclear matrix protein) has the problem of presenting a very high rate of false positives. In this study, we set out to search for a new diagnostic biomarker to complement NMP22, using urine from urothelial cancer patients in order to attempt early detection of urothelial cancer. [Methods] We performed SDS-PAGE on urine protein of urothelial cancer patients and transferred the protein to PVDF membrane. The transferred proteins were analyzed using SNA lectins and the protein bands that reacted strongly were digested using trypsin. Glycoproteins were identified using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) and we analyzed the structure of the N and O-linked oligosaccharides. [Results] Protein bands detected at 75kDa and 50kDa reacted strongly to SNA lectin in the urine of patients with urothelial cancer. A stronger reactivity was correlated with an increase in malignancy. Furthermore the 75kDa and 50kDa band were identified as protein X and Y by analysis of LC/MS/MS. The analysis of the sugar chain structure of protein X and Y determined that they were N-linked glycans attached to the sialic acids. O-linked sugar chains were not detected. [Conclusions] Glycoprotein X and Y reacted strongly to SNA lectin, showing the possibility of a new diagnostic marker to complement the urinary NMP22 in urothelial cancer. We are currently considering the creation of an antibody which specifically reacts with glycoprotein X and Y.

Keywords: biomerker, urothelial cancer, lectin

POS-01-100 Oligosaccharide Structure Analysis of Acute Phase Protein in the Serum of the Prostate Cancer Patient

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[Purpose]
Increase of prostate specific antigen (PSA) in the serum has been recognized as the most important prostate cancer marker. However, PSA is often present in patients with non cancerous diseases such as prostatitis, showing a gray zone of PSA between 4-10ng/ml. Therefore, in this study, we set out to discover a method that would complement the carrier protein in the serum to detect cancer-specific proteins.
[Methods]
We were separated high-abundance proteins of serum using Agilent Multiple Affinity Removal System. We performed SDS-PAGE on high abundance proteins and low abundance proteins and transferred them to a PVDF membrane. Glycoproteins were analyzed using various lectins. Glycoproteins were analyzed with a lectins binding assay using the BIAcore 2000 system (Surface Plasmon Resonance). Finally we performed oligosaccharides structure analysis of N or O-linked IgG by Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI TOF MS).
[Results]
The most abundant Oligosaccharides structure in the serum was $(Hex)_2(HexNAc)_2(NeuAc)_2$ + $(Man)_3$ $(GlcNAc)_2$. In particular, the oligosaccharide was increased in high abundance proteins of the serum of patients with prostate cancer.
$(Hex)_1$ $(HeNAc)_2$ $(Deoxyhexose)_1$ + $(Man)_3$ $(GlcNAc)_2$ was reduced but $(HeNAc)_2$ $(Deoxyhexose)_1$ + $(Man)_3$ $(GlcNAc)_2$ increased in the heavy chain of IgG in patients with prostate cancer.
[Conclusions]
It is possible to distinguish between healthy individuals with prostate cancer or prostatitis by principal component analysis (PCA) from the result of lectins binding assay of IgG.

Keywords: prostate cancer, oligosaccharide, lectin

POS-01-101 Glycoproteomic Approaches Enable to Find Glycoproteins with Aberrant Glycans as Novel Biomarkers for Epithelial Ovarian Cancer

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Epithelial ovarian cancer (EOC) is often asymptomatic, and thus more than two-thirds of EOC cases are diagnosed in the advanced stages and their five-year survival rate is less than 30%. By contrast, chemotherapy is very effective for EOC patients with stage I or II when the tumor mass is limited. Therefore, the diagnosis at an early stage would improve the prognosis of EOC. CA125, an EOC biomarker being used universally, has both false-positive and false-negative problems. To address the search for an EOC biomarker more reliable than CA125, we employed a combination of glycoproteomic techniques. Lectin microarray analysis of conditioned media from cancer cell lines showed high AAL signals in general. To select tumor biomarker candidates exhaustively, peritoneal washing (PW) from EOC patients was subjected to the affinity chromatography using AAL columns and followed by IGOT-LC/MS analysis. As a result, more than 200 glycoproteins were found from the EOC samples. A validation study was performed using PW from EOC patients. Several glycoproteins were analyzed with both the lectins to recognize distinctive glycans and the antibodies to recognize the protein portion. In the sandwich ELISA of the ascites fluids, one candidate showed a higher signal in the EOC patients compared to that in the benign disease patients. The result suggests glycoproteins with aberrant glycans are capable of EOC marker, although the protein quantities were not specifically elevated in EOC patients. This work was supported by a grant from New Energy and Industrial Technology Development Organization (NEDO) in Japan.
Keywords: glycoproteomics, ovarian cancer, biomarker

POS-01-102 Substrates and Regulation Mechanisms for the SIRT5

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Lysine succinylation is a novel post-translational modification. Diverse metabolic enzymes located in mitochondria can be succinylated. It has been well-known that SIRT5 is a lysine deacetylase on the basis of sequence similarity. But recent progress has shown that SIRT5 is a NAD-dependent protein lysine demalonylase and desuccinylase. SIRT5 can remove the succinyl moieties from target lysines, which may regulate the biological activity of the target proteins.Dysfunction of acetylation process is often associated with several diseases, especially cancer. It is unknown whether SIRT5 is implicated in cancer pathogenesis. To establish the relationship between SIRT5 expression and tumorigenesis, we examined its expression level in a number of human cancer cell lines, including cervical cancer, gastric cancer, breast cancer, colorectal cancer and hepatocellular carcinoma. Human cancer cell lines express varying levels of SIRT5 protein, ranging from clearly detectable to almost undetectable. In human hepatocellular carcinoma cell line MHCC97H and HCCLM3, both of which exhibit a rather lower SIRT5 expression level, SIRT5 overexpression in the two cell lines can inhibit cell proliferation and promote cell apoptosis. Therefore, our findings indicate possible important link between SIRT5 and tumorgenesis.Furthermore, overexpression of Sirt5 and/or IDH2 (isocitrate dehydrogenase 2) in cultured cells show that SIRT5 directly interacts with IDH2,a key enzyme in the TCA cycle. It has been shown that IDH2 can be succinylated responding to different physiological conditions. SIRT5 desuccinylates IDH2,resulting in change of IDH2 activity. In our study, we found SIRT5 has a direct interaction with IDH2,which indicates that SIRT5 may be involved in regulating cell metabolism and energy production by regulating this kind of reversible modification, and then has an important function in tumorgenesis.

Keywords: SIRT5, succinylation, IDH2

POS-01-103 Comparative and Targeted Proteomic Analyses of Urinary Exosomes/Microparticles from Bladder Cancer and Hernia Patients for Biomarker Discovery and Verification

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Bladder transitional cell carcinoma is a major epidemiological problem whose incidence continues to rise each year. Urinary exosomes/microparticles are an attractive material for non-invasive bladder cancer biomarker discovery. In this study, we applied isotopic dimethylation labeling coupled with LC-MS/MS to discover bladder cancer biomarkers in urinary microparticles isolated from hernia (control) and bladder cancer patients. This approach identified 2964 proteins based on more than two distinct peptides, of which 2058 had not previously been reported as constituents of human urine exosomes/microparticles. By performing comparative proteomics between hernia and bladder cancer using pooled samples and individual samples, we identified 107 differentially expressed proteins as noninvasive candidate biomarkers. Differences in the concentrations of 29 differential proteins (41 signature peptides) were precisely quantified by LC-MRM/MS in 48 urine samples of bladder cancer, hernia, and urinary tract infection/hematuria. Concentrations of 24 proteins changed significantly ($p<0.05$) between bladder cancer (n=28) and hernia (n=12), with area-under-the-curve values ranging from 0.702 to 0.896. Finally, we quantified tumor-associated calcium-signal transducer 2 (TACSTD2) in raw urine specimens (n=221) using a commercial ELISA and confirmed its potential value for diagnosis of bladder cancer. At a cutoff-value of 2.47 ng/mL, TACSTD2 was able to differentiate all bladder cancer subgroups from hernia ($p<0.001$, AUC=0.80, n=221) with a sensitivity, specificity, the positive predictive value, and negative predictive value of 73.6%, 76.5%, 84.4% and 62.6%, respectively. Our study reveals a strong association of TACSTD2 with bladder cancer and highlights the potential of human urinary microparticles in the non-invasive diagnosis of bladder cancer.

Keywords: Biomarker, bladder, multiplexed quantitation

POS-01-104 Differential Analysis of LG and HG pTa Bladder Tumors in Comparison to Controls for the Detection of Potential Biomarkers

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Introduction: Bladder cancer is the 9th most common cancer worldwide. The disease is associated with high recurrence and mortality rates. Common diagnostic tools such as cystoscopy and cytology are cost intensive and lack acceptance by patients. Alternative methods are rare and until now there are no known tumor markers in blood or urine specific enough to detect bladder cancer which leads to ongoing research in the field of biomarker detection. The aim of this work was to detect differences in protein expression between papillary low grade (LG) and high grade (HG) bladder tumors against normal urothelia within the context of tumor progression.

Methods: Laser microdissection was used to isolate tumor cells LG (n=7), HG (n=5) and additional normal urothelium (n=8) from 10 μm thin serial section of HE stained papillary bladder tumor tissue. Cells were lysed, labelled (saturation-labeling) and subsequently separated using Difference In Gel Electrophoresis (DIGE). Protein differences were analysed using the Decyder Software. Promising candidates were validated by western blot analysis.

Results: A total of 56 differentially expressed proteins matching the predefined criteria t-test ≤ 0.05 and an average ratio of ≤ -1.5 or ≥ 15 were identified via MS-analysis. Out of those 41 were up-regulated and 15 down-regulated in HG tumors. Proteins were further characterised with the help of the Ingenuity Pathway Analysis software which revealed that 80% were derived from the cytoplasm. Interesting proteins such as Peroxiredoxin 6 and Transgelin 2 will be further validated on a lager collection of tissue samples as well as urine.

Keywords: differential analysis (DIGE), bladder cancer, biomarker search

POS-01-105 Targeted Proteomics for the Verification of Prostate Cancer Biomarkers in Clinically Stratified Expressed Prostatic Secretions and Urines

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The ability to predict which patients have aggressive prostate cancer, warranting a radical course of treatment, from patients with slow-growing tumours that would be better suited for active surveillance, is a major challenge in personalized care for prostate cancer. Further, as the incidence of prostate cancer rises, more patients are being over-treated, partly, to due a lack of reliable prognostic biomarkers. A successful biomarker panel would, in a timely manner, clearly demarcate these patient groups; this is a considerable challenge using biopsies, which often miss malignant lesions and are associated with risks and discomfort. In a recent publication, we described a workflow that utilizes LC-MS for the identification of potential soluble protein markers in expressed prostatic secretions (EPS). As a result, we introduced several putative prostate cancer prognosticators, demonstrating their differential expression between patient groups with extracapsular and organ-confined tumours, as well as recurrent vs. non-recurrent individuals. These analyses resulted in 100 biomarker candidates that warrant verification in large cohorts of clinical samples. For the quantitative assessment of this list, we have optimized a targeted mass spectrometry platform using stable isotope dilution-selected reaction monitoring mass spectrometry. This tool can be used to quantify specific proteins directly in complex biological samples, offering a rapid and economical pre-validation step of high numbers of biomarker candidates. Using this approach, we are in the process to verify our candidates in urinary EPS from patients with organ-confined (n=41) versus extracapsular tumors (n=14), as well as controls (n=26). Furthermore, we have obtained individual urine samples from patients with differing biopsy and post-prostatectomy pathological grades (n=300). With this, we anticipate to identify a robust panel of prognostic protein biomarkers, directly quantifiable in urinary EPS and urine.

Keywords: SRM-MS, prostate cancer, biomarker

POS-01-106 Serum Protein Biomarkers Discovery for Early Detection/Monitor Treatment Response of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor in Non-Small Cell Lung Cancer

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Epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) have been developed and used clinically in the treatment of advanced non-small cell lung cancer (NSCLC) with EGFR mutation. It provides better life quality and prolongs patients' survival comparing to chemotherapy. Although EGFR mutation predicts responsiveness to TKI treatment efficiently, unfortunately 30% of patients with activating EGFR mutation represent no effect after EGFR TKI treatment. Moreover, around 10% of patients without EGFR mutation have response to EGFR TKI treatment. Therefore, it is worthy to search other biomarkers to assist EGFR mutation test for prediction and early detection of TKI treatment. Herein we established a potential dataset to generate a protein signature for this purpose by quantitative proteomic approaches from 20 pared sera (before and after 7 days TKI treatments), including 11 partial-response (PR) patients with activating EGFR mutation and 9 progress-disease (PD) patients with wild type of EGFR. The top 14 high-abundance sera proteins were removed and iTRAQ-based quantitative proteomic analysis was performed followed by Proteome Discoverer software analysis. We identified 536 reproducible proteins (524 quantified) from two-independent analyses. To search the potential predicator of TKI treatment, we compared the proteins identified from PD and PR groups before treatment and found that there were 36 differential expressed proteins with mean±1.5 SD fold change between these two groups. We also found that the levels of 39 and 31 proteins were changed in PD and PR groups after 7 days EGFR TKI treatment, respectively. Currently, two interested candidates were verified by ELISA assay.

Keywords: non-small cell lung cancer, quantitative proteomic, epidermal growth factor receptor tyrosine kinase inhibitor

POS-01-107 Positive Regulation of Brain-Specific Serine Protease 4 by Thyroid Hormone Receptors Leads to Enhanced Tumor Motility in Human Hepatoma Cells

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The thyroid hormone, 3, 3', 5-triiodo-L-thyronine (T_3), has been shown to modulate cell growth, development, differentiation and metabolism via interactions with thyroid hormone receptors (TRs), but the secretory proteins that are regulated to exert these effects remain to be characterized. In a previous study, brain-specific serine protease 4 (BSSP4) was identified as a T_3-regulated secretory protein within the secretome via stable isotope labeling with amino acids in cell culture (SILAC)-based quantitative proteomic approaches. BSSP4, a member of the human serine protease family, participates in extracellular matrix remodeling. However, the physiological role and underlying mechanism of T_3-mediated regulation of BSSP4 in hepatocellular carcinogenesis are yet to be established. Data from the present study revealed upregulation of BSSP4 at both the mRNA and protein levels after T_3 stimulation in a time- and dose-dependent manner in hepatoma cell lines. Additionally, the regulatory region of the BSSP4 promoter stimulated by T_3 was identified at positions -609/-594, using reporter and chromatin immunoprecipitation assays. BSSP4 overexpression led to enhanced tumor cell migration and invasion, both *in vitro* and *in vivo*. Subsequent experiments further indicated that BSSP4-induced migration occurs through the ERK 1/2-C/EBPβ-VEGF cascade, similar to that observed in HepG2-TRα1 and J7-TRα1 cells. Western blot and quantitative reverse transcription polymerase chain reaction analysis confirmed BSSP4 overexpression in clinical hepatocellular carcinoma (HCC) patients, compared with normal subjects. *Conclusion*: Our findings collectively support a potential role of T_3 in cancer cell progression through regulation of the BSSP4 protease. BSSP4 may thus be effectively utilized as a novel marker and anti-cancer therapeutic target in HCC.
Keywords: thyroid hormone receptor, secreted protein, SILAC

POS-01-108 Label-Free Quantitative Personalized Tissue Membrane Proteomics and Targeted Membrane Glycoprotein Profiling for Gastric Cancer Biomarker

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The glycoproteins and/or membrane proteins hold promises for discovery of cancer biomarker because most FDA-approved cancer biomarkers are secreted glycoproteins. Unfortunately, some of the current cancer biomarkers, such as CEA, CA 19-9, and CA 72-4 have low sensitivity and specificity for gastric cancer (GC) detection. Recent studies suggest that analysis of tumor tissues can directly lead to the investigation of the origin of cancer and generate potential markers with higher specificity and sensitivity. In this study, we applied an informatics-assisted label-free quantification method for personalized membrane proteomics analysis of paired of cancerous and adjacent normal tissue from patients with gastric cancer. In this study, individual membrane proteins were first purified from paired tumor and adjacent normal tissues of 24 GC patients with different stages. The purified membrane proteins were mixed with internal standard protein, subjected to gel-assisted digestion. Finally, extracted peptides were analyzed in triplicate by LC-MS/MS. The analysis quantified 1752 proteins across different patients; 1058 were classified as membrane proteins or membrane-associated proteins including 525 plasma membrane proteins annotated by Gene Ontology, Ingenuity Pathway Analysis Knowledge Base, and TMHMM prediction. There are 497 significantly up-regulated expressed proteins in the four different stages of GC patients including the well-documented GC biomarkers: EGFR, ENO1 and PPIA. These previous biomarker candidates show up-regulation in <40% patients, suggesting their low sensitivity in for clinical utility. Therefore, we select 8 biomarker candidates which satisfy criteria including structural characterization as glycosylated membrane proteins and ability to secret or shed in serum; five of these proteins had been reported to associate with GC. Those candidates will be validated extensively in well-defined retrospective and prospective clinical samples.
Keywords: membrane protemoics, gastric cancer, biomarker

POS-01-109 Proteomic Analysis of DLBCL Tumor Tissue Reveals Quantitative Differences in Regulators of Cytoskeletal Architecture between R-CHOP Responding and Non-Responding Patients

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Diffuse large B-cell lymphoma (DLBCL) is an aggressive lymphoma of B-cells. The lymphoma grows quickly, and can spread fast to different parts of the body. Despite molecular sub-classification of DLBCL, the International Prognostic Index (IPI) is still the only prognostic tool used in daily clinical practice to risk stratify patients. However, there are survival differences within the same IPI group. Therefore there is an urgent need to establish markers for disease progression and outcome of therapy.
Among patients diagnosed with DLBCL and treated with curative intent, two subgroups were identified; A) patients with progressive disease or relapse within 1 year after completion of treatment and B) patients with over 5-year progression-free survival. Five patients from each subgroup were selected and proteins were extracted from cryostat sectioned tumor samples. In parallel, five established DLBCL cell lines were metabolically labeled with $^{13}C_6$-arginine and $^{13}C_6$-lysine. Lysates from these labeled cell lines were combined and used as an internal protein standard for relative protein quantification. Proteins were separated, trypsin digested and fractions were analyzed by LC-MS using a LTQ-FT/MS instrument.
The relative protein expression in tumor samples was calculated as the ratio between sample signal and reference signal. The analysis identified more than 3,500 proteins and among the proteins differentially expressed when comparing the two subgroups of DLBCL were several regulators of cytoskeletal architecture. Through their association with the actin cytoskeleton these proteins may interfere with cellular processes including cell morphogenesis, migration, proliferation, survival and also invasion and metastasis.
Keywords: biomarker, cancer, DLBCL

POS-01-110 Proteomic Analysis of Highly Invasive Colorectal Cancer Cells Established by Orthotopic Xenograft Mouse Model

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Early detection of cancer metastasis is crucial to improve the prognosis. Cancer progression and acquisition of metastatic potential are influenced by surrounding tissue environment. Orthotopic implantation of human cancer cells into nude mice provides a valuable *in vivo* model for cancer metastasis research. Here, we established metastatic colorectal cancer cells by surgical orthotopic implantation of HCT-116 human colon cancer cells stably expressing green fluorescent protein (GFP). Matrigel invasion assay demonstrated that these established cells were highly invasive. In this study, we performed differential proteome profiling of metastatic HCT-116 cells for identification of colorectal cancer metastatic biomarker candidates. For this purpose, multiplex peptide stable isotope dimethyl labeling for relative quantification were used for differential proteome profiling. Gene ontology analysis and Ingenuity Pathway Analysis (IPA) were performed to search the biomarker candidate proteins. Metastatic potential of candidate proteins were examined by siRNA knockdown experiment.
Keywords: colorectal cancer, metastasis, biomarker

POS-01-111 Proteomic Analysis of Saliva from Patients with Oral Cancer

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Oral cancer (OSCC) is the sixth most common cancer in the world with a survival rate of only 50%. Early diagnosis using saliva based molecular markers would be a non-invasive solution to improve the outcome. The aim of the present study is first-stage identification of potential protein biomarkers present in saliva using LC-MS/MS based quantitative proteomics for OSCC. The study subjects were Leukoplakia patients (n=5), Lymph node Negative (n=5) and Lymph node Positive (n=5) patients with OSCC and healthy controls (n=5). Equal quantities of proteins in cell-free saliva from each subject in a group were pooled and depleted of abundant molecules - amylase and albumin. Proteins in the saliva pools were trypsinized and peptides were differentially labeled with iTRAQ reagents, fractionated by Strong Cation Exchange (SCX) chromatography and subjected to LC-MS/MS analysis using Orbitrap Velos high resolution mass spectrometry. Protein identification was carried out by searching the data using Sequest through Proteome Discoverer workflow (Thermo Fisher Scientific). We identified a total of 890 proteins among which 300 were found with altered levels when combined from various paired comparisons. Thirty of these proteins were found to be common in saliva of all the three patient groups and were identified with multiple, unique peptides, quantity ratio of at least 2 fold. Seventeen of these 30 molecules were either extracellular or associated with plasma membrane indicating their potential for secretion and 22 are known to be associated with OSCC according to published literature. These salivary proteins have good prospects for validation in large sample cohort to develop clinical applications for oral cancers.

Keywords: oral cancer, salivary biomarkers

POS-01-112 Discovery of Lung Cancer Biomarkers by Differential Proteomes Analysis from Six Types of Pleural Effusions

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Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, which is the prominent cause of the death in the world. Because of organ proximal effects, pleural effusion (PE) would be a good source for biomarker discovery in NSCLC. Herein, we used GeLC-MS/MS to generate the most comprehensive PE proteome databases with 778 non-redundant proteins from six types of PEs, including malignant PE (MPE from lung, breast and gastric cancers), lung para-malignant PE (PMPE) and benign diseases (tuberculosis and pneumonia). The spectrum counting method was used for comparative proteome analysis. We then set several criteria to select the candidates for further verification, including the differentially expressed proteins with more than two-fold changes between malignant and non-malignant PEs, comparison of ONCOMINE datasets, functional classification, novelty and the availability of commercialized ELISA kits. Currently, the PE levels of three potential biomarkers (PE-001~003) were validated by ELISA and Western blot. Both PE-001 and PE-002 levels were significantly increased in lung patients with MPE (n=109), compared to those with non-malignant PEs (n=172). Notably, the PE-001 level was higher in MPE with lung cancer as compared to those in gastric or breast cancer, suggesting PE-001 was positively associated with lung malignancy. The ROC curve revealed that PE level of PE-001 was able to effectively discriminate lung MPE from benign disease (AUC=0.845, $P<0.05$) or lung PMPE (AUC=0.845, $P<0.05$). Our results collectively demonstrated the utility of the labeled free quantitative proteomic approaches and provide the useful datasets for lung diseases biomarkers research.

Keywords: pleural effusion, non-small cell lung cancer, biomarkers

POS-01-113 An Improved Protocol for the Enrichment of Plasma Membrane Proteins Allows the Identification of Accessible Antibody Targets on Trastuzumab-Resistant Breast Cancer Cells

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Characterization of the cell surface proteome is of fundamental importance for the development of antibody-based therapies, as well as for the understanding of how cells interact with their environment. Biotinylation of cell surface proteins is one of the most frequently used procedures for their mass spectrometry-based characterization. Here, we present a study investigating the enrichment of membrane proteins of SK-BR-3 breast cancer cells by surface biotinylation performed alone or in combination with either ultracentrifugation or detergent-based fractionation. SK-BR-3 cells are a model system for HER2-positive breast cancer, clinically characterized by high metastatic potential and dismal prognosis. The anti-HER2 antibody Trastuzumab is frequently used for the treatment of these patients, but its clinical efficacy is limited by the emergence of resistance. To understand the changes in cell surface proteome, we have developed an SK-BR-3 cell line resistant to Trastuzumab-mediated growth inhibition and we have studied proteome changes by cell surface biotinylation and detergent-based fractionation. Thirteen membrane-associated proteins were found to be regulated between resistant and parental cell lines. Human monoclonal antibodies against most promising antigens, isolated from phage display libraries and conjugated to a highly potent cytotoxic agent, mediated cancer cell killing *in vitro*. In summary, the procedure described in this article allowed an efficient characterization of the cell surface proteome of closely related SK-BR-3 cell lines and led to the identification of three tumor-associated antigens which could be considered for the development of anti-breast cancer antibody products.

Keywords: cell-surface biotinylation, Trastuzumab resistance, antibody drug conjugates

POS-01-114 Comprehensive Proteomics of Gastric Cancer for Biomarker and Drug Target Discovery

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Gastric cancer is the 2nd leading cause of cancer related deaths killing close to 800,000 lives annually and about 1 million new cases are reported each year. The two key pillars to improve the outcomes of gastric cancer patients are early detection and individualized therapy. Our lab has been spending the past 5 years publishing papers on various subproteomic approaches including secretomics, membrane proteomics, phosphoproteomics and plasma proteomics to mine biomarkers and drug targets from a large panel of cell lines as well as body fluids from patients and xenograft model. In this presentation, I will i) demonstrate the improved resolution and sensitivity of comprehensive proteomics and highlight the implications for molecular imaging and cancer therapy; ii) ascertain the degree of congruence of the data obtained between comprehensive proteomics and DNA microarray; iii) show how complementary transcriptomics and subproteomics data can provide new insights into the cause of the molecular aberrations observed; iv) compare the effectiveness of different sample preparation methods and the use of different non-cancer cell lines as baselines for identifying differentially expressed proteins in gastric cancer; v) share some serendipitous findings that emphasize the importance of post-translational modifications and subcellular compartmentalization to the molecular etiology of cancer; vi) discuss the inadequacy of current blood-based proteomics, potential solutions and the challenges of using candidates mined from the blood proteome as specific cancer biomarkers; Finally, I will vii) draw your attention to the power of phosphoproteomics in unraveling novel oncogenes as potential drug targets for gastric cancer therapy.

Keywords: cancer, biomarkers, drug targets

POS-01-115 Basic Fetoprotein is Homologous with G6PI/AMF/NLK/MF/PGI/PHI/SA-36 by the Identification of 99% AA Sequence for BFP Using MALDI-MS

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Basic fetoprotein (BFP), which is found in serum, gastro-intestinal tract and brain tissue of human fetuses, is widely used as a serum and urinary tumor marker, but its structure has not yet been investigated. Hence, we investigated the amino acid (AA) sequence of BFP, derived from the human hepatocellular carcinoma tissues grafted into nude mouse, using matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) (AXIMA Performance and AXIMA Resonance; Shimadzu/Kratos). By employing a wide variety of protease digestion (Trypsin, Lys-C, Lys-N, Asp-N, Arg-C, Trypsin & V8, Trypsin & Asp-N, Lys-C & Asp-N), selection of matrix system (3AQ/CHCA as matrix, MDPNA as additive), and MALDI-MS, we have successfully sequenced up to 99% of BFP without pre-fractionation. As a result, BFP tumor marker was found to be homologous to glucose-6-phosphate isomerase (G6PI), which revealed that BFP is also homologous to autocrine motility factor (AMF) / neuroleukin (NLK) / maturation factor (MF) / phosphoglucose isomerase (PGI) / phosphohexose isomerase (PHI) / sperm antigen (SA-36).
In conclusion, we showed that the BFP tumor marker is homologous to human G6PI protein by the identification of 99% AA sequence of BFP using MALDI-MS. Results demonstrated that the analytical system using MALDI-MS can serve as a very powerful tool for protein sequencing. Here in this session we describe details of this work.

Keywords: sequence coverage, biomarker, PTMs analysis

POS-01-116 Renal Metabolic Profiling of Early Renal Injury and Renoprotective Effects of Poria Cocos Epidermis Using UPLC Q-TOF/HSMS/MS^E

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Poria cocos epidermis is one of ancient traditional Chinese medicines (TCMs), which is usually used for the treatment of chronic kidney disease (CKD) for thousands of years in China. A metabonomic approach based on ultra performance liquid chromatography coupled with quadrupole time-of-flight high-sensitivity mass spectrometry (UPLC Q-TOF/HSMS) and a mass spectrometry$^{Elevated\ Energy}$ (MS^E) data collection technique was developed to obtained a systematic view of the development and progression of chronic kidney disease (CKD) and mechanism studies of the surface layer of *poria cocos* (Fu-Ling-Pi, FLP). By partial least squares-discriminate analysis analyses, 19 metabolites were identified as potential biomarkers of CKD. Among the 19 biomarkers, 10 biomarkers including eicosapentaenoic acid, docosahexaenoic acid, lysoPC (20:4), lysoPC (18:2), lysoPC (15:0), lysoPE (20:0/0:0), indoxyl sulfate, hippuric acid, p-cresol sulfate and allantoin were reversed to the control level in FLP-treated groups. The study indicates that FLP treatment can ameliorate CKD by intervening in some dominating metabolic pathways, such as fatty acid metabolism, phospholipid metabolism, purine metabolism and tryptophan metabolism. This work was for the first time to investigate the FLP therapeutic effect based on metabonomics technology, which is a potentially powerful tool to study the TCM.

Keywords: chronic kidney disease, poria cocos epidermis, metabonomics

POS-01-117 Using an Isolated Rat Kidney Model to Identify Kidney Origin Proteins in the Urine

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Using target proteomics for the identification of urinary biomarkers of kidney disease in urine can at least partially avoid the interference of serum origin proteins. It may provide better sample throughput, higher sensitivity, and specificity. Knowing the urinary proteins to target is essential. By analyzing the urine from perfused isolated rat kidneys, 990 kidney origin proteins were identified in the urine, which have human orthologs. Of these proteins, 128 were not found in normal human urine and may become biomarkers with zero background. A total of 297 proteins were not found in normal human plasma. These proteins will not be influenced by other normal organs and be kidney specific. The levels of 33 proteins were increased when perfused with oxygen-deficient solution compared with that from the kidneys that were perfused with oxygen. The 75 proteins in the perfusion-driven urine have a significantly increased abundance ranking comparing with their ranking in the normal human urine. Importantly, when compared with existing candidate biomarkers, over ninety percent of the kidney origin proteins in the urine identified in this study have not been examined as candidate biomarkers of kidney diseases

Keywords: kidney origin proteins in the urine, target proteomics, kidney diseases biomarkers

POS-01-118 The Expression and Function of GP73 in Hepatocellular Carcinoma (HCC)

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Golgi protein 73 (GP73) is a resident Golgi-specific membrane protein and it becomes a newly identified candidate marker for liver diseases. However, the function of GP73 protein in HCC remains unknown. In this study, the protein expression of GP73 was found to be higher in metastatic HCC cell lines (MHCC97L, MHCC97H, and HCCLM3) than that of the low and non-metastatic cell lines (L02, HepG2, Huh7, SMMC7721). Elevated expression of GP73 was also detected by Western Blot in HCC tissues compared with that of the adjacent non-tumor tissues. The immunohistochemistry result indicated that the expression level of GP73 was higher in metastatic HCC than those in non-metastatic HCC. We also found that shRNA-mediated down-regulation of GP73 in MHCC97H and HCCLM3 cells could decrease their migration and invasion abilities by transwell experiments. The results from Western blot and cell immunoflourescence analyses showed the reduction of N-cadherin expression and induction of E-cadherin expression which may correlate to epithelial-mesenchymal transition (EMT). In conclusion, our study indicated that GP73 was associated with HCC metastasis and may serve as a novel prognostic biomarker and therapeutic target.

Keywords: GP73, hepatocellular carcinoma(HCC), epithelial-mesenchymal transition (EMT)

POS-01-119 The PI3K Inhibitor, Wortmannin Ameliorates STZ-Induced Diabetic Nephropathy

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Diabetic nephropathy (DN) is a progressive kidney disease that is caused by injury to kidney glomeruli. Podocytes are glomerular epithelial cells and play critical roles in the glomerular filtration barrier. Recent studies have shown the importance of regulating the podocyte actin cytoskeleton in early DN. Although many studies are now in progress, the mechanism of injury in DN is poorly understood. The phosphoinositide 3-kinase (PI3K) inhibitor, wortmannin simultaneously regulates Rac1 and Cdc42, which destabilize the podocyte actin cytoskeleton during early DN. The main objective of this study is administered wortmannin, the other PI3K inhibitor to streptozotocin (STZ, 70 mg/kg)-induced DN Sprague-Dawley (SD) rats *in vivo* and confirm that whether there'll be improvement effect of DN. We administered wortmannin (1 mg/kg) to STZ-induced DN rats every day during 8 weeks. To demonstrate reno-protective role of PI3K inhibitor on STZ-induced DN rats, we measured albuminuria and albumin to creatinine ratio (ACR) level using ELISA kit and pathological changes by Periodic acid-Schiff (PAS) staining were examined. Expression level changes of nephrin, podocin and Rac1/Cdc42 by wortmannin administration were confirmed with Western blotting. As a results, expression level of nephrin, podocin and Rac1/Cdc42 of wortmannin group were increased when compared with diabetes group. As a result of immunofluorescence, expression and localization of podocyte marker proteins, nephrin, podocin and desmin were normalized by administration of wortmannin. Therefore, the wortmannin have an effective on treatment of DN through reno-protection.

Keywords: diabetic nephropathy, podocyte, wortmannin

POS-01-120 Serum Peptides, Represented by Complement 3f Des-Arginine, Are Useful for Prediction of the Response to Pegylated Interferon-α Plus Ribavirin in Patients with Chronic Hepatitis C

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Aim: Biomarkers predicting sustained virological response (SVR) to pegylated interferon-α plus ribavirin (PEG-IFNα/RBV) were investigated.

Methods: Peptides in pretreatment sera from 107 patients with hepatitis C virus (HCV) genotype 1 were comprehensively analyzed by mass spectrometry. Ion intensity of the peptides was used to generate discriminant models between the responders who achieved SVR (Rs) and the non-responders (NRs) to PEG-IFNa/RBV.

Results: In total, 107 peptides were detected in a training set (n=23). A discriminant model using a peptide, complement 3f des-arginine (C3f-dR), showed sensitivity of 35% and specificity of 94% for SVR prediction in a testing set (n=68). In all the Rs and NRs (n=96), area under the receiver operating characteristic curve (AUROC) of 0.64 in the C3f-dR model was increased to 0.78 by addition of platelet (PLT) counts (C3f-dR/PLT model). Another model using the 107 peptides (AUROC, 0.77) also showed higher AUROC (0.79) by addition of hemoglobin (Hb), body mass index (BMI), and age (107P/Hb/BMI/Age model). The sensitivity and specificity of the C3f-dR/PLT model were 59% and 88%, and those of the 107P/Hb/BMI/Age model were 70% and 92%, respectively. The C3f-dR/PLT model showed high AUROC (0.82) similar to that of IL-28B rs8099917 genotype analysis (0.86) in the 45 tested patients. Prediction by the combination of the C3f-dR/PLT model, the 107P/Hb/BMI/Age model, and the rs8099917 genotype analysis was accurate in 44 out of the 45 patients (AUROC, 0.95).

Conclusions: Serum peptides, especially C3f-dR, would be useful predictors for SVR to PEG-IFNα/RBV. The complements may be involved in the HCV elimination.

Keywords: chronic hepatitis C, pegylated interferon-α plus ribavirin, biomarkers

POS-01-121 Multi-Omic and Functional Network Analysis of Paediatric Urine from Patients Diagnosed with Idiopathic Nephrotic Syndrome

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Idiopathic nephrotic syndrome (INS) is the most prevalent glomerular disease in children. In spite of some progress, its pathogenesis is still unknown and the therapy options are confined to gross immune modulation. A variety of methods for diagnostic and treatment purposes are available for the patients; however, the lack of understanding regarding the pathogenic mechanisms underlying INS can lead to poor therapeutic response and adverse side-effects. Here, we describe quantitative proteomic approach to reveal new molecular factors involved in pathogenesis of INS with potential diagnostic and therapeutic significance. Urine samples were collected from 10 children diagnosed with INS receiving no therapy and 10 healthy children. All samples were purified using spin filters followed by affinity depletion of albumin. The purified proteins were recovered and digested with trypsin overnight. Label-free protein expression data were acquired with Synapt G2 using an ion mobility data independent approach. The acquired data was processed and searched against a human database, which was amended to account for N-terminal processed peptides. Normalized label-free quantitation results were generated using TransOmics software. In a similar fashion the diluted neat urine samples were analysed using a small molecule profiling approach. The resulting data was also analyzed using TransOmics. A significant number of proteins to be over-expressed in the urine from INS patients including a high percentage of glycosylated proteins. Combining both datasets through pathway analysis of the complimentary datasets strongly suggests correlation with the neuronal system disorders network, specifically acute fatigue.

Keywords: biomarker, multi-omics, label-free quantitation

POS-01-122 Proteomic Analysis of Non-Alcoholic Steatohepatitis in Mice

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Background: Non-alcoholic steatohepatitis (NASH) is a common cause of chronic liver disease that can progress to cirrhosis and hepatocellular carcinoma. Liver biopsy still remains the gold standard for diagnosis of this disorder. To identify novel biomarkers for NASH, we employed a proteomics approach to search for differentially expressed proteins in liver tissues from STAM® mice, a model of NASH induced using streptozotocin (STZ) and a high-fat diet (HFD).

Methods: We compared protein expression levels of the liver tissues in the three groups [normal diet (ND) without STZ, ND with STZ, and HFD with STZ (STAM)] using one- dimensional gel electrophoresis coupled with nano-liquid chromatography-tandem mass spectrometry. The data were processed on a Spectrum Mill MS Proteomics Workbench.

Results and Conclusion: By analyzing protein bands, which were differently expressed on the gel electrophoresis images among the groups, 93 proteins were identified uniquely in the ND without STZ group, 43 in the ND with STZ group, and 59 in the STAM group. We also identified 73 and 116 proteins showing more than a 2-fold difference in expression between the ND without STZ group versus the STAM group and between the ND with STZ group versus the STAM group, respectively. Hepatic proteins that were dysregulated in the STAM group functioned in a wide variety of pathways, including fatty acid oxidation, glycolysis, the urea cycle, oxidative stress, endoplasmic reticulum stress, and apoptosis. These results will be further test in human samples. The proteins identified in this proteomic analysis may be promising biomarkers for NASH.

Keywords: non-alcoholic steatohepatitis, biomarker

POS-01-123 Angiotensin-Converting Enzyme Inhibition Curbs Tyrosine Nitration of Mitochondrial Proteins in the Renal Cortex During the Early Stage of Diabetes Mellitus in Rats

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Background: During the early stage of diabetes mellitus (DM), renal cortical production of nitric oxide (NO) and superoxide anion (O_2^-) is increased, leading to peroxynitrite formation. This oxidant enhances nitration of protein tyrosine residues to form 3-nitrotyrosine (NT), a process that may contribute to development of diabetic nephropathy. Objective: The first goal was to evaluate the hypothesis that angiotensin-converting enzyme (ACE) inhibition (enalapril, renoprotection) suppresses the increase in NT production evident in the rat renal cortex during DM. The second goal was to identify renal cortical proteins exhibiting reduced DM-induced nitration during ACE inhibition. Methods: Enalapril was administered for 2 weeks to subsets of streptozotocin (STZ) -induced DM and vehicle-treated sham rats. O_2^- and nitrate+nitrite (NO_x) levels were measured in the media bathing renal cortical slices after 90 min incubation *in vitro*. Superoxide dismutase (SOD) activity and NT content were measured in the renal cortex homogenate. Renal cortical nitrated protein was identified by proteomic analysis. Results: Renal cortical production of O_2^- and NT was increased in DM rats; however, enalapril suppressed these changes. DM rats also exhibited elevated renal cortical NO_x production and SOD activity, and these changes were magnified by enalapril-treatment. Two-dimensional gel electrophoresis based Western blotting revealed more than 20 spots with positive NT immunoreactivity in the renal cortex of DM rats. Enalapril-treatment blunted the DM-induced increase in tyrosine nitration of three proteins aconitase 2, glutamate dehydrogenase 1 and methylmalonate-semialdehyde dehydrogenase, each of which resides in mitochondria. Conclusions: These data are consistent with enalapril preventing DM-induced tyrosine nitration of mitochondrial proteins by a mechanism involving suppression of oxidant production and enhancement of antioxidant capacity, including SOD activation.

Keywords: protein tyrosine nitration, Angiotensin-converting enzyme inhibitor, Diabetic nephropathy

POS-01-124 High Confident Human Glomerulus Proteome Identified Both by Mass Spectrometry- and Antibody-Based Proteomics

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Reality of the presence of proteins identified by MS may be somewhat uncertain. In addition, MS is neither good at localizing proteins in the tissue nor getting spatial information of each protein. To solve these problems, the antibody (Ab)-based proteomics has been introduced as the other tool of proteomics.
In this study we analyzed human kidney glomerulus proteomes by MS and Ab-based proteomics to build a more significant glomerulus proteome database, in which each protein are identified by both proteomics.
Human glomerular proteins were analyzed by LC-MS, and identified by Spectrum Mill with low confidence criteria and by Mascot with high confidence criteria. Localization of human proteins in the glomerulus was searched in the Human Protein Atlas and the intensity of IHC staining evaluated as negative, weak, moderate and strong is scored from 0 to 3.
By MS, 6,686 proteins (2,741 genes) and 1,878 proteins (1,439 genes) were identified as low and high confident proteins, respectively. IHC identified 3,164 gene-derived proteins with high confidence since multiple antibodies confirmed their glomerulus localization. On the other hand, 10,584 gene-derived proteins were identified with low confidence by using a single antibody each.
By comparing these datasets, the largest number (2,741 genes) and the highest proportion (14.6% of all proteins either by MS or Ab-based proteomics) of proteins were identified by both MS- and Ab-based proteomics when the MS-Spectrum Mill proteome dataset and the Ab-based proteome dataset using all antibodies (13,748 genes).
In conclusion, we created a high confident glomerulus proteome database, in which all proteins were identified by both MS- and Ab-based proteomics. Sample preparation for MS was important for identification of glomerular proteins by MS.

Keywords: glomerulus, kidney, Human Protein Atlas

POS-01-125 Phospho-Profiling of mTOR Inhibitor-Treated Renal Cell Carcinoma (RCC) Cell Line and Its Application to Drug Response-Efficacy Biomarkers

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Mammalian target of rapamycin (mTOR) is an evolutionally conserved serine/threonine kinase that integrates signals from multiple pathways, including nutrients, growth factors, hormones, and stresses to regulate a wide variety of eukaryotic cellular functions, such as translation, transcription, cell growth, differentiation, cell survival, metabolism, and stress response. mTOR pathway plays a crucial role in tumorigenesis as hyper-activation of the mTOR pathway is observed in most human cancer cells. Thus, mTOR pathway is of considerable interest in view of its potential as an anti-cancer drug target. In fact, two derivatives of rapamycin that is a specific inhibitor of mTOR, everolimus and temsirolimus (rapalog) successfully improved overall survival of renal cell carcinoma (RCC) patients. However, exactly how rapamycin and rapalog perturb mTOR function is not completely understood even though mTOR inhibitors affect well-known marker such as phosphorylation of the mTOR substrates. Thus, there is a clear need for the identification and validation of biomarker sets to predict and monitor responses to mTOR inhibitors, which will lead to patient selection and clinical trial design in the treatment.
As a part of effort to overcome such problems, we take advantages of the use of mass spectrometry to uncover novel biomarkers for the mTOR inhibitors using mTOR-inhibitors treated cancer cell lines. In this study, we perform global phospho-proteomics and selected reaction monitoring (SRM)-based targeted quantitative proteomics and believe that the introduction of such innovative technique is expected to lead to breakthroughs in discovering therapeutic target and biomarker to treat RCC patients with mTOR inhibitors.

Keywords: mTOR, biomarker, phospho-proteomics

POS-01-126 Localization of Normal Human Glomerulus Proteins Detected by Mass Spectrometry and Immunohistochemistry in the Kidney

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Normal glomerulus proteins were analyzed by LC-MS and by searching in the Human Protein Atlas (HPA). Extra-glomerular distribution of these proteins detected by both methods was further analyzed to find glomerulus-specific proteins.
When two or more antibodies were existed for one protein in HPA, intensity or localization of proteins was evaluated by the results from one antibody which showed highest intensity and largest number of localization sites.
Results:
1. A total of 1271 proteins were detected in normal human glomerulus by both LC-MS and HPA. Out of these proteins, 15 proteins were uniquely detected in only glomerulus, and 1256 proteins were present not only in the glomerulus but also in other sites of the kidney.
2. Ten out of the 15 glomerulus-unique proteins were localized in the glomerular epithelial cells, podocytes.
3. A total of 458 proteins were detected in a close association with glomerular and extra-glomerular capillaries. Among them, 196 proteins had been identified as plasma proteins by the HUPO Plasma Proteome Project. Fourteen proteins were observed uniquely in vascular endothelial cells of capillaries.
4. Most of the glomerular proteins (1230 proteins, 96.8%) were co-localized in other parts in the kidney (proximal tubule, distal tubule, collecting duct or interstitium).
In conclusion, 15 (1.2%) and 14 (1.1%) of 1271 glomerulus proteins identified by LC-MS were verified as unique glomerular proteins and vascular endothelial proteins, respectively in the kidney by HPA immunohistochemistry.

Keywords: human glomerulus, kidney, Human Protein Atlas

POS-01-127 Proteomes of Normal Human Glomerulus Identified by Mass Spectrometry (MS) and Immunohistochemistry: Origin of Proteins Identified Only by MS

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In this study, human glomerulus proteome identified by MS were compared with that by antibody-based identification (immunohistochiemistry, IHC) and the proteins, which were only identified by MS, were further analyzed.
Human glomeruli were isolated by the sieving method from human kidneys removed for cancer therapy. Glomerular proteins were separated by 2D-gel electrophoresis and analyzed by MS. On the other hand, glomerular proteins were also identified by antibody (Ab)-based IHC done by Human Protein Atlas (HPA: http://www. proteinatlas.org/).
Comparison of the MS-based and Ab-based proteome elucidated that 920 proteins were detected only by MS but not by IHC of HPA. Among them, 217 proteins had been demonstrated in the glomerulus by IHC using different antibodies in HPA. Localization and immunostaining intensity of the remaining 703 proteins in the kidneys were examined by viewing HPA IHC images. These proteins were localized at glomerulus, proximal tubule, distal tubule, collecting duct and other parts, and intensity of IHC was scored from 0 (negative) to 3 (strong intensity).
As the result, about 76% of proteins were observed in the proximal tubules, distal tubules or collecting duct, but not in the glomerulus. It was suggested that a possibility that extraglomeular tissues were contaminated in the glomerulus sample for MS separated by the sieving method. On the other hand, 24% of the proteins detected only by MS were negative by IHC in the kidney.Several reasons were considered for the MS-positive, IHC-negative proteins: identification error of MS, reactivity of Ab or contamination of plasma proteins in the MS samples.
In conclusion, a higher-precision glomerulus proteome database can be built by combining MS- and Ab-based analysis. It is also important to take care of sample preparation for MS and reactivity of antibodies.
Keywords: human glomerulus, kidney, Human Protein Atlas

POS-01-128 Is Urine a Better Biomarker Source Than Blood?

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So far biomarker discovery is not as fruitful as we would like to see. Is blood the best place to look for biomarkers? For all the cells to survive and function, blood is supposed to be homeostatic. It tends to minimize any changes introduced into it. Those changes are what we are looking for as biomarkers. We are playing hide-and seek with blood. That is why it is hard to find a biomarker. Unlike blood, urine is supposed to be dumped as waste. It has no mechanism to be homeostatic. It tolerates all the changes the whole body gives it. Is this the better place to look for changes or what we call biomarkers? The changes in the urine may be too complicated to sort out to a particular pathophysiological condition at the beginning. But as analyzing technology and bioinformatics develop, eventually we will sort them all out. After all the changes are there, not hidden by the body. Because the close relationship between urine and secretory system, the first group of biomarkers may be established in nephrology field. Large number of samples are needed for validation. Storage of large amounts of samples can be simple and economical if storing urinary proteins on the membrane dry in a vacuum bag is proved to be successful. Since there is less ethical concerns to acquire urine than blood, the only difficulty in the field may be for labs to refocus the attention from blood to urine.
Keywords: biomarker, urine, blood

POS-01-129 Human Urine Proteome Changes Induced by Space Flight

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In long duration exploration-class missions crew must be able to function adequately in a hypo-gravity or zero gravity environment, and then be able to return safely to the Earth. The urine proteome for sixteen Russian cosmonauts (male, aged of 35 up to 51) performed long flight missions and varied from 169 up to 199 days on the International Space Station (ISS) were analyzed versus the control group, six back-up crew, in the same timeline. We used proteomic techniques to obtain data and bioinformatics to perform the analysis. From the total number of identified proteins (238) in our data set, 129 were associated with a known tissue origin. Preflight samples contained 92 tissue-specific proteins, samples obtained on Day 1 after landing had 90 such proteins, while Day 7 samples contained 95 tissue-specific proteins. Analysis showed that consistently present proteins in urine (under physiological conditions and after space flight) are cubilin, epidermal growth factor, kallikrein-1, kininogen-1, megalin, osteopontin, vitamin K-dependent protein Z, uromodulin. Variably present proteins consists of: Na (+) / K (+) ATPase subunit gamma, beta-defensin-1, dipeptidyl peptidase 4, maltasa-glucoamilasa, cadherin-like protein, neutral endopeptidase and vascular cell adhesion protein 1. Only two renal proteins are related to the space flight influence, not being represented in the urine of cosmonauts before flight and in samples of back-up crew: AFAM (afamin), AMPE (aminopeptidase A). In several persons AQP2 (aquaporin-2) was over-represented. This fact has been related with physiological readaptation of water-salt balance. The proteomic analysis of urine samples in different phases of space missions using homemade bioinformatic tools of passway identification provides new data on kidney functioning biochemistry after space flight
Keywords: international space station, urine proteome, kidney

POS-01-130 Autosomal Dominant Polycystic Kidney Disease Patients Urine Proteome

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Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder affecting 1 in 1000 people and is responsible for 10% cases of end staged renal disease. To describe the ADPKD urine proteome in details, we detected all proteins larger than 10 kD from 30 patients of both sex, and widely differentiated according to age. The control group consisted of 30 healthy people stratified according to sex and age. We applied a new approach to minimalize the urine proteins degradation in the bladder via collecting second urine in the day, not the first one retaining in the bladder overnight. Pooled control and ADPKD samples were analyzed during three LC-MS/MS experiments of 4-plex iTRAQ labeled samples. A protein was regarded as confidently identified if at least two peptides of this protein were found. The statistical analysis of the quantitative results of the polling iTRAQ experiment revealed 157 proteins that were differently populated in the urine of ADPKD patients as compared to healthy controls. It is worth to stress, that urine content of proteins representing complement system (7 proteins) and apolipoproteins (6 proteins) were significantly changed in ADPKD patients. The urine proteome of ADPKD patients differs significantly from the urine proteome of healthy and become the clinical tool for early diagnosis of the disease. The pathophysiological information obtained in presented study may become basis for the development of new therapies.
Keywords: proteomics, kidney

POS-01-131 Subtractive Proteomic Analysis Determines Lipid Droplet Associated Protein (LDP) and Unravels Differential Liver LDP Profiling in Fatty Liver Disease

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Eukaryotic cells store neutral lipids in cytoplasmic lipid droplets enclosed in a monolayer of phospholipids and associated proteins. Growing evidence has demonstrated that LD associated proteins play important roles in the pathogenesis and development of fatty liver disease, which features abnormal LD accumulation in hepatocytes. However, it is remarkably unclear about liver LDP profiles and their alterations in fatty liver diseases. In this study, we compared liver proteome and lipid droplet-associated sub-proteome to identify LDP, and to quantify their changes in a diet-induced-obesity (DIO) fatty liver. Among 5000 quantified proteins, 101 of which were enriched greater than ten folds in LD. Differential profiling of LDP proteome demonstrated altered expression profiles in whole proteome and LDP sub-proteome in DIO mice fatty liver. The function of altered LDP candidates were further validated *in vivo* with adenovirus-mediated gene silencing. S100A10 Knockdown accelerates liver steatosis induced by high fat diet. This study has identified LDP from the sub-proteome of liver for the first time and shed light on the discovering of the potential targets for fatty liver disease therapy.

Keywords: lipid droplet protein, proteomics, iTRAQ

POS-01-132 Urine Peptidomics and Exosome Profiling in Liver Diseases

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Urine samples are ideal for diagnostic purposes because of the ease of sample collection using standardized protocols without the need for invasive methods, and the ease of manipulation and storage. We explored this biofluid as a source to identify makers for liver pathologies by using two different approaches: exosome proteomics and peptidome profiling.

We have fractioned and studied by proteomics different sub-populations of vesicles including exosomes that are present in normal urine as starting point to standardize methodology for liver biomarker discovery using this urinary compartment.

We have also studied the urinary peptidome in different liver-damaging conditions. Peptidome and low molecular weight proteome profiling is a technique that allows the recognition of distinctive patterns and differentiation among diverse physiopathological conditions.

We evaluated the utility of peptide/small protein profiling using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) coupled with WCX magnetic bead-based solid phase extraction (SPE) as a screening tool for drug toxicity assessment in mice urine samples. Given that drug-induced injury is primarily reflected in liver, three different, well-described hepatotoxic drugs were chosen for this work. These were: carbon tetrachloride (CCl4) which induces liver fibrosis, D (+)-galactosamine (GalN) as a model for acute liver injury, and *Escherichia coli*-derived lipopolysaccharide (LPS) to study the damage caused by endotoxins. The profiles obtained with a correct clustering analysis show that this methodology can be used as a non-invasive and straightforward approach to test for potential drug toxicity.

Keywords: liver proteomics, biomarker, drug toxicity, urine

POS-01-133 Changes of the *O*-GlcNAc Modification of Proteins Accompanied with Diabetic Nephropathy

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Increased flux through the hexosamine biosynthesis pathway promotes the modification of proteins by *O*-linked *N*-acetylglucosamine (*O*-GlcNAc), which is a unique posttranslational modification of nuclear and cytoplasmic proteins and has been implicated in the development of insulin resistance and diabetes complications. In our previous immunohistochemical study, we demonstrated that the *O*-GlcNAcylation level increased in various tissues including kidney from diabetic GK rats, which is an animal model of non-insulin dependent type (type 2) diabetes.

To identify marker proteins that change in their extent of *O*-GlcNAcylation in the diabetic kidney from GK rats, we separated total kidney proteins by two-dimensional gel electrophoresis. *O*-GlcNAcylated proteins were detected by the immunoblot using anti-*O*-GlcNAc antibody. Selected proteins that changed markedly in the *O*-GlcNAc level were identified by Mass Spectrometry analysis. The localization and the quantity of these *O*-GlcNAcylated-proteins were analyzed by *in situ* Proximity Ligation Assay (PLA). *O*-GlcNAcylated proteins that changed significantly in the degree of *O*-GlcNAcylation were identified as cytoskeletal proteins (α-actin, α-tubulin, α-actinin 4, myosin) and mitochondrial proteins (ATP synthase, pyruvate carboxylase). Results of immunoprecipitation and immunoblot studies, as well as *in situ* PLA demonstrated that the extent of *O*-GlcNAcylation of the above proteins increased in the diabetic kidney. Immunoelectron microscopy revealed that α-actinin 4 increased in the foot process of podocytes and the proximal tubules. To further examine the changes of the *O*-GlcNAc modification of glomerular proteins accompanied with diabetic nephropathy, we isolated glomeruli from kidney and performed proteomic analysis.

Keywords: O-GlcNAc modification, diabetes, kidney

POS-01-134 Application of Glycoproteomics for Development of Serum Biomarker of Liver Disease

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Identification of new glycobiomarkers predicting the liver disease progression and the presence of hepatocellular carcinoma (HCC) has been attempted. An accurate method for monitoring the progression of liver fibrosis, predicting liver cirrhosis (LC) and detecting HCC is urgently needed. We previously proposed a large number of candidate glycoproteins for monitoring liver disease progression which were identified by Lectin-IGOT-LC/MS, an LC/MS-based glycoproteomic approach.

In this study, we found liver disease-associated glycan profile on a candidate protein, which pointed out the presence of markedly developed HCC or progressed LC in the patients with HCV-/HBV- infected chronic liver disease. In order to clarify the relationship between the presence of the candidate molecule with aberrant glycosylation and the prevalence of progressed liver diseases, we analyzed this candidate marker in a clinical verification study. As a result, the increased amount of the candidate molecule with the aberrant glycosylation was more relevant to poor outcome of LC than hepatic carcinogenesis of the patients. While our result suggests that the biomarker is feasible to be used in individualization of poor prognostic patients with a risk of LC progression, further clinical studies would yield more precious information.

This work was supported by a grant from New Energy and Industrial Technology Development Organization (NEDO) in Japan.

Keywords: IGOT, biomarker, liver

POS-01-135 Processed B-Type Natriuretic Peptide is a Biomarker of Post-Interventional Restenosis in Ischemic Heart Disease

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Background: Restenosis, a condition in which re-narrowing of the lesion vessel is seen after a coronary intervention procedure, remains a limitation in management. A surrogate biomarker for risk stratification of restenosis would be welcomed. B-type natriuretic peptide (BNP) is secreted in response to pathologic stress from the heart. Its use as a biomarker of heart failure is well known; however, its diagnostic potential in ischemic heart disease has remained less explored. Recently, it has been reported that processed forms of BNP exist in the circulation. We hypothesized that circulating processed forms of BNP might be a biomarker of ischemic heart disease.

Methods: We characterized processed forms of BNP by a newly developed mass spectrometry-based detection method combined with immuno-capture using commercially used anti-BNP antibodies.

Results: Measurements of processed forms of BNP by this assay were found to be strongly associated with presence of restenosis. Reduced levels of the amino-terminal processed peptide, BNP (5-32), relative to BNP (3-32) (as the index parameter BNP [5-32] / BNP [3-32] ratio), were seen in patients with restenosis (restenosis, 1.19 [median]; interquartile range [IQR], 1.11-1.34; n = 22 vs. without restenosis, 1.43; IQR, 1.22-1.61; n = 83; $P < 0.001$) in a cross-sectional study in 105 patients undergoing follow-up coronary angiography. A sensitivity of 100% allowing for 'rule-out' for presence of restenosis was attained at a ratio of 1.52.

Conclusions: Measurement of processed forms of BNP may serve as a viable potential biomarker to 'rule-out' restenosis.

Keywords: biomarker, cardiovascular, restenosis

POS-01-136 Deciphering the Proteome and Secretome of Human Coronary Atherosclerotic Arteries

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The aim of this work is twofold:

1) To study the proteome of intima and media layer of human atherosclerotic coronary arteries compared to preatherosclerotic ones.

2) To analyze human arterial tissue secretome and the quantitative comparison of preatherosclerotic and atherosclerotic secretome to discover proteins with a key role in this pathology.

We analyzed the intima and media layer from human atherosclerotic coronaries isolated by laser microdissection and compared with those from preatherosclerotic coronary and radial arteries using a saturation labeling DIGE approach. Secretomes from atherosclerotic coronary arteries, preatherosclerotic coronaries and mammaries were quantified by label-free LC-MS/MS. Alterations observed were further analyzed by immunohistochemistry.

Results have pointed out 13 and 12 proteins to be altered from atherosclerotic intima and media, respectively. Differential proteins from intima layer are implicated in the migrative capacity of vascular smooth muscle cells, extracellular matrix composition, coagulation, apoptosis, heat shock response and intraplaque hemorrhage deposition. A deregulation of cytoskeleton was evident in VSMCs from atherosclerotic media layer, which indicated their switch to a secretory phenotype. In the secretome, 64 proteins were identified in 3 replicates of at least one of the 3 groups and 14 of them have not been previously reported in plasma. Gelsolin, vinculin, lamin A/C and phosphoglucomutase-5 were oversecreted in mammary arteries.

A comprehensive study of the proteome and secretome of the human atherosclerotic coronary has allowed us to enlighten the molecular mechanisms underlying this pathology and provide a repository of proteins released to the blood with potential clinical use in atherosclerosis.

Keywords: coronary atherosclerosis, laser microdissection, secretome

POS-01-137 Proteomics and Metabolomics: New Tools to Study Albuminuria in Patients with Arterial Hypertension

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Albuminuria is defined as a urinary excretion of albumin above normal levels, as a consequence of renal damage in patients with arterial hypertension. The presence of albuminuria has been shown to be a potent predictor for future development of cardiovascular and renal events in the general population. It is reported that patients with chronic suppression of renin-angiotensin-aldosterone system (RAAS) present a high prevalence of albuminuria and even in a high percentage of normoalbuminuric patients, albuminuria appears *de novo*.

The aim of this study is to compare plasma proteins and metabolites from albuminuric patients with respect to patients with arterial hypertension without albuminuria, in order to identify novel biomarkers with potential prognostic value.

For protein analysis, plasma samples were depleted of the 14 most-abundant proteins and analyzed by 2D-DIGE, seeking to identify low dynamic range proteins involved in the pathology process. Metabolomic analysis was performed by GC-MS/MS using a gas chromatograph 6890N coupled to a single quadrupole mass detector 5975C (Agilent technologies).

With this approach, we found 70 spots differentially expressed (average ratio ≥1.5 or <1.5; *pvalue*<0.05) by proteomic analysis. Identifications were performed by MALDI-TOF/TOF. In addition, 23 metabolites with differential expression were identified in albuminuric patients and classified as follows: 38% fatty acids, 25% carbohydrates, 21% organic acids, 8% aminometabolites and 8% aromatic/steroids.

The study of circulating proteins and metabolites from these patients provided an overview of the altered metabolic pathways and gave us a better understanding of the mechanisms associated to the development of albuminuria.

Keywords: metabolomics, hypertension, albuminuria

POS-01-138 N-Glycan Profiling of Surfactant Protein D (SP-D) by MALDI Mass Spectrometry

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Surfactant Protein D (SP-D) is a lung specific glycoprotein which is widely used as a marker for interstitial pneumonia and is considered to be a candidate biomarker for Chronic Obstructive Pulmonary Disease (COPD). SP-D contains one possible attachment site of N-glycosylation but its glycan structure is unknown because SP-D easily makes a complex with IgG in the presence of Ca^{+} and is hard to purify to homogeneity. In the present study, we employed several chromatographies such as a maltose agarose column and an antibody affinity column, and purified SP-D to apparent homogeneity from 300 ml of normal human serum. A protein band with 43 KD was found to be monomer of SP-D. By the high-sensitive method for the analysis of N-glycans developed by Kaneshiro et al, [(1)] we obtained mass spectrum of 3-aminoquinoline (3-AQ) labeled N-glycans derived from serum SP-D. The most of glycans contains fucosylated glycans. To confirm this result, purified serum SP-D was also analyzed as tryptic glycopeptides and three peaks of glycopeptides, which have the peptide containing the possible attachment site of N-glycosylation, were obtained. The glycans of these glycopeptides may contain core fucose in the structures. Detailed analysis is still under consideration. Based on this study, we would like to find a novel serum biomarker candidates for diagnosis of COPD.

Reference

(1) K. Kaneshiro *et al*., Anal. Chem., **83**, 3663-3667 (2011)

Keywords: surfactant protein D, MALDI mass spectrometry

POS-01-139 Systematic Vascular Proteomics in Vascular Remodeling Model

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Restenosis is well recognized after cardiovascular angioplasty. Analyses at the organ, subcellular, and molecular levels have revealed dynamic, complex, and subtle intracellular processes associated with restenosis. In this study, we performed systematic proteomic analyses using subcellular fractions of carotid arterial vessels obtained after balloon injury. The 44 differentially-expressed proteins were identified by the combination of 2D-DIGE and LC-MS/MS spectrometry, among which the *in vitro* regulatory function of 27 proteins were validated for proliferation and migration of human aortic SMCs. The analysis of protein-protein interaction network indicated that 25 out of 27 validated proteins were associated with the intracellular signaling pathways converging into receptor tyrosine kinase and actin cytoskeleton. In particular, OLR-1 played key roles in neointimal hyperplasia by promoting the PDGF-induced SMC proliferation/migration and by mediating oxidized LDL-induced monocyte adhesion to SMCs via NF-κB activation. Consequently, this study reveals a set of potential biomarker/target genes relevant to vascular remodeling.

This study was supported by Bio & Medical Development Program (2011-0019696) and 21C Frontier Functional Protemics Project (FPR08-B1-190)

Keywords: proteomics, vascular remodeling, smooth muscle cells

POS-01-141 Discovery and Verification of Novel Biomarkers Predicting the Response of Antidepressants Using Mass Spectrometry-Based Proteomic Approach

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Clinical response to antidepressantdrug therapy is quite different among patients with depression. Genetic variations and protein biomarkers to predict the efficacy of antidepressants have been suggested, buttheir clinical utilities has not been clearly definedand large-scale clinical studies of biomarker are still lacking. Therefore, we performed the mass spectrometry (MS)-based global serum proteome profiling in depressed patients and healthy individuals. We performed quantitative proteomics technologies for verification of protein candidates selected.

To identify plasma proteome, nine pooled plasma were used as follows; abundant proteins in plasma samples were removed by MARS and subjected to In-solution trypsin digestion. Tryptic peptide mixtures were fractionated into 24 fractions by OFFGel fractionator. Plasma proteome data were acquired using Agilent HPLC-chip/MS interface with Agilent accurate mass Q-TOF. MS-based quantification on MRM mode was achieved on 90 clinical samples using Q-TRAP 5500. Western blotting was carried out to investigate the altered expression of biomarker candidate interest. The MS/MS data was searched using Spectrum Mill search database algorithm (Agilent Technologie) with the human database from NCBI for peptide identification. Thus, we identified a total of 288 proteins (FDR, <1%), of them we chose the differentially expressed proteins (fold change, 2≤) compared with healthy donor. We developed multiplex biomarker verification strategy including DB-based search and MS based proteome profiling. We screened 95 candidate proteins acquired from DB search and carryed out MRM assay to verify these markers. 16 marker candidates were used for the further verification phase by MRM and Western blotting.

Keywords: biomarker, selective serotonin-reuptake inhibitor (SSRI), multiple reaction monitoring (MRM)

POS-01-142 Identification of Biomarkers Predictive of Chronic Lung Allograft Dysfunction (CLAD) After Lung Transplantation by Proteomic Technologies

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With more than 32,000 procedures performed around the world in the least 30 years, lung transplantation (LT) has become the standard of care for selected patients with advanced lung diseases. Unfortunately, LT procedures are often at risk of chronic lung allograft dysfunction (CLAD) leading to two most common clinical symptoms such as a bronchiolitis obliterans syndrome (BOS) and a restrictive allograft syndrome (RAS). Almost 50% of LT recipients will develop CLAD within 5 years post LT, rising to 75% after 10 years. The median survival is about 2 years from the time of diagnosis, which is based of the irreversible decline of the forced expiratory volume in one second (FEV1). However that decrease occurs late in CLAD development and no early symptoms of this pathology are currently known. Thereby, identification of biomarkers at early stage is really required to allow the use of adequate treatments for patients that will develop BOS or RAS syndromes. The goal of our study was to identify at 6 and 12 months after LT biomarkers that will predict CLAD occurrence 3 years later. We used for this purpose patient samples from a large cohort of 500 lung transplantation recipients (LTR) from France, Belgium and Switzerland, whom 100 reached 3 years follow up at the time of the study. Proteome of plasma and bronchiolo-alveolar lavages withdrawn at 6 and 12 months post-transplantation were analysed by SELDI-TOF method and profiles of LTR developing a CLAD were compared to those of stable patients, allowing characterization of several proteins differentially expressed between both groups. Identification of these candidate biomarkers will be performed by a bottom-up approach. These results will further be integrated into a prediction computational model of CLAD based on principles of systems biology developed from all the clinical and experimental data (environment, phenotype, microbiome, biology, omics) of donors and recipients.

Keywords: predictive biomarkers, chronic lung allograft dysfunction, SELDI-TOF

POS-01-143 Proteome Profiling of Epidermal Differentiation

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Epidermal differentiation is an important process for maintenance of skin homeostasis. Abnormality in epidermal differentiation causes various skin disorders. Although there are many reports concerning about signal and/or marker molecules of the differentiation, detailed mechanisms of the epidermal differentiation have not been cleared sufficiently. Therefore, we comprehensively analyzed and profiled the change in proteins during epidermal differentiation. We analyzed protein profiles of the human epidermal keratinocyte cell line HaCaT and 3D epidermal model during *in vitro* differentiation. Proteins were extracted using a powerful detergent, C7BzO, and were digested by trypsin at various stages of differentiation. As the result of proteomic analysis by LC-MS/MS, we detected totally 3,808 proteins in HaCaT, and totally 1,423 proteins in 3D epidermal model. Furthermore, we identified many differentiation-related proteins in both examinations. In addition, we collected stratum corneum samples from human skin by tape stripping technique, and profiled stratum corneum proteins. As results, we detected totally 445 proteins in the stratum corneum of the skin. According to the correlation analysis between stratum corneum proteins and functions of stratum corneum, we found several proteins which involved in the maintenance and formation of stratum corneum functions. In this presentation, we will discuss the biomarkers for the epidermal differentiation.

Keywords: epidermal differentiation, HaCaT, 3D epidermal model

POS-01-144 Direct Brain Omics-Study of Alzheimer's Disease Using UPLC-MS Assay for the Early Diagnosis

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Alzheimer's disease (AD) is an irreversible, progressive brain disease that is the most common cause of dementia among older people. AD can be definitively diagnosed after death with a direct examination of senile plaques and neurofibrillay tangles in several brain regions. Inevitably, it is for something to transmute various compounds in specific brain region that potential prime knowledge would be linking of pathological change in AD for the early diagnosis. Here we proposed the direct brain omics-study using ultra performance liquid chromatography electrospray time-of-flight mass spectrometer assay (UPLC-ESI/TOF/MS). These brain tissues were selected from patients confirmed at autopsy to be either AD (n=10) and non-AD control groups (n=10) using pathological findings, and at genotype of ApoE 3/3. Based on these pathologically brain tissue, the multivariate data analysis was utilized to identify pathologic compounds based on UPLC-ESI/TOF/MS data. Acquired data were subjected to principal components analysis for differentiating the frontal and parietal lobes of AD/Control groups. Potential factors were screened by using S-plot and were identified by the accurate mass and isotopic pattern. Significant differences in the levels of spermine and spermidine could be identified using metabolomics-databases and standard matching. Based on the investigation of polyamine metabolite pathway, this data establish that the downstream metabolites of ornitihine are increased and suggested to the key of AD process with possible implication of ornithine decarboxylase activity. This study proved that first evidence is novel AD process for the early diagnosis.

Keywords: brain, omics-study, AD

POS-01-146 Proteomic Analysis of Posttraumatic Stress Disorder Mouse Model Reveals Nucleus Accumbens Affected Pathways

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Posttraumatic stress disorder (PTSD) is caused by exposure to a traumatic event. Individuals diagnosed for PTSD not only experience significant functional impairments but also have higher rates of physical morbidity and mortality. The neurobiological pathways involved in the development of PTSD remain obscure. Using a PTSD mouse model, we have established a proteomic platform to study affected molecular mechanism. In addition, we have investigated the effects of chronic treatment with fluoxetine, an antidepressant used in PTSD therapy. The PTSD mouse model is generated by one electric foot shock to emulate an aversive encounter. Shocked mice develop PTSD-like symptoms, including hyperarousal and conditioned fear following 28 days of incubation. PTSD-like symptoms in shocked mice were ameliorated after 4 weeks of fluoxetine treatment. Nucleus accumbens (NAc), located in the limbic brain area, plays an important role in fear conditioning, memory, reward and motivational aspects. Punched NAc tissue specimens from several mice were pooled and membrane associated proteins were subjected to mass spectrometry analysis using stable isotope metabolically labeled reference material to analyse protein expression level differences. Pathway analyses indicate a significant down-regulation of the citrate cycle in the NAc of PTSD mice. Other pathways affected include synaptic transmission, neurite outgrowth, synaptic vesicle recycling and neurotransmitter secretion. Chronic fluoxetine treatment of PTSD mice reversed the downregulated expression of proteins that are part of these pathways. The proteomic data delineate PTSD dysfunctional pathways with the ultimate goal to improve diagnosis and treatment.

Keywords: posttraumatic stress disorder, biomarker

POS-01-145 Proteome Analysis of Aortic Aneurysm Based on Stage Classification Utilizing Protein Profiles

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Thoracic aortic aneurysm (TAA), principally caused by atherosclerosis, is a life-threatening cardiovascular disease associated with sudden death. There are few insights into its pathogenic mechanism and there is no clinical treatment to prevent the disease progression. The purpose of this study is to identify novel clinical biomarkers and elucidate the pathogenic mechanism by comprehensively analyzing proteins increased or decreased in the TAA progression. Proteome analysis was performed by pulverization of frozen tissue, denaturation, tryptic digestion of TAA tissues (aortic media) followed by nano-LC/MS/MS and quantitative differential analysis with 2DICAL software. Approximately 1000 proteins were identified by proteome analysis, of which 250 proteins showed significant changes between pathologically normal and diseased areas. Gene ontology analysis of these altered proteins suggested disruption of elastic fibers by inflammation-induced upregulation of proteases, and resulting fibrosis and calcification in the lesion. However, most of the proteins were thought to reflect a terminal phenotype of advanced TAA, suggesting that molecularly based classification is crucial to study proteome changes. Thus, we studied expression of major proteins that were dynamically changed in the TAA progression to separate intermediate and advanced stages of TAA, and found a set of proteins showing disrupted smooth muscle structure. We are investigating more subtle proteome changes between the initial and the intermediate stages. Several proteins involved in inflammation, oxidative stress and lipid metabolism showed slight alterations, which may be used to differentiate the initial from the intermediate stage. Upcoming data will make it possible to identify promising candidates for biomarkers which can detect TAA at an initial stage and help elucidate its pathogenic mechanism.

Keywords: biomarker, aneurysm, classification

POS-01-148 Establishing Apolipoprotein J as a Potential Candidate for Alzheimer's Disease Biomarker Panel: Australian Imaging, Biomarker and Lifestyle (AIBL) Flagship Study of Ageing

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Apolipoprotein J (apoJ) is a multifunctional protein, functions as a chaperone of misfolded extracellular proteins. It participates in functions like cell proliferation and apoptosis. Its levels are increased in the CSF of patients with AD. The plasma levels have been correlated to brain atrophy, baseline disease severity and clinical progression in AD. The present study includes longitudinal plasma measurement of apoJ in order to establish it as a potential analyte to be included in AD biomarker panel of AIBL.

apoJ protein levels were measured in the baseline and 18-month follow-up plasma samples from 833 individuals participating in the Australian Imaging, Biomarkers and Lifestyle (AIBL) Longitudinal Study of Ageing. It was assayed by a commercially available ELISA kit (Human Clusterin ELISA, R and D systems). apoJ plasma levels were then compared between healthy controls (HC), mild cognitively impaired (MCI) individuals and Alzheimer's disease (AD) patients. The data was further compared against other collected demographics including but not limited to cerebral amyloid load as measured by positron emission tomography (PET), hippocampal volume, *APOE* genotype and neuropsychological scores.

MCI and AD had significantly higher levels of apoJ at baseline and 18-month data. MCI group had highest percentage change between the two time points viz. 2% (HC), 7% (MCI), 0.5% (AD). Significant correlation was observed with PiB-PET SUVR and hippocampal volumes. Increased levels of plasma apoJ correlate with cognitive decline at both baseline and 18 months. apoJ levels were significantly higher in apoE4 carriers, as compared to the non apoE4 carriers at both the time points. Further analysis will determine the relationship between plasma apoJ and other potential biomarkers such as apoE as potential candidates for AD biomarker panel.

Keywords: apolipoprotein J, Alzheimer's disease, biomarker

POS-01-149 Proteomic Analysis of Stratum Corneum of Human Scalp

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Scalp is exposed to a unique environment compared to other skin. For example, it has thick hairs, many sweat and sebaceous glands, and high relative humidity. These circumstances may involve scalp in diseases, such as seborrhoeic dermatitis, dandruff, and itching. Although the detailed onset mechanisms of these scalp diseases are still unclear, there are several reports that an aberration of keratinocyte differentiation may be involved in the diseases. Therefore, to clarify the characteristics of the cornification process of the scalp, and to search for biomarkers for the scalp diseases, we carried out the comprehensive, comparative analysis of the stratum corneum proteins of the scalp and other skin part. Twenty-nine volunteers were participated in this study. Stratum corneum functions of the scalp and the upper-arm skin were measured, and the scalp condition was graded according to dandruff score. Stratum corneum samples from both skins were collected by tape stripping technique. Proteins were extracted using a powerful detergent, C7BzO, and were digested by trypsin. As the result of proteomic analysis by LC-MS/MS, totally more than 1,000 proteins could be identified and approximately 500 proteins were quantified commonly in both samples. According to the comparative analysis of protein profile, the amount of several keratins and enzymes, which were related in the skin structure and the cornification process, was different between the scalp and the upper-arm skin. In addition, we found several stratum corneum proteins correlated to the grade of dandruff.

Keywords: scalp, stratum corneum

POS-01-150 Identification of Proteins Involved in Thoracic Aortic Aneurysm

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Aortic aneurysms occur in the largest vessel of our body and our understanding of the genesis and progression has been limited. Aneurysm begins as a localized structural reorganization in the aorta and leads to the weakening of the aortic wall and progressive dilatation. The core of the reorganization involves alterations of the cells and also the extracellular matrix. During reorganization also named as tissue remodeling/expansion smooth muscle cells are lost due to cytocine and Fas activated apoptosis which originates from the ROS/RNS generated oxidative stress. Oxidative stress also triggers matrix metalloproteinases which destroys the elastin in the extracellular matrix that gives the vessel its elasticity which eventually lead to aortic rupture. During this project we aimed to elucidate the mechanism of thoracic aortic aneuryms and in total we have analyzed 36 patient and 8 control samples. The Media section of the aorta was obtained through laser capture microdissection and tryiptic peptides were obtained via filter aided sample preparation protocol. A label-free differential data independent acquisition method was used for the LC-MS/MS analysis. 352 proteins were identified in the aortic wall and 42 of them were calculated to be statistically significant. Differentially expressed proteins are mainly related to cell adhesion, cytoskeletal, defense, enzyme modulator, extracellular matrix and signalling pathways. The findings will be discussed in the context of aneurysm genesis and progression.

Keywords: Label-free proteomics, thoracic aortic aneurysm, biomarker

POS-01-151 Mass Spectrometry Methods for Surrogate Biomarker Discovery in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is one of the most common and severe form of childhood muscular dystrophies affecting about 1 in 3,500 boys. The need to define surrogate biomarkers to monitor DMD progression and response to treatments is becoming crucial as promising treatment strategies for DMD are entering phase II and III clinical trials. The most commonly used surrogate endpoint for DMD to date is the 6 minute walk test. However, this test seems to be not sensitive enough for clinical trials with a short a period of time and also impractical for DMD patients who lost ambulation. In this study we have initiated proteome and metabolome profiling on serum samples from both DMD patients and dystrophin deficient mouse model (mdx). Using nano-hydrogel particle proteome profiling on serum samples from DMD donors (n = 10) and age matched healthy controls (n = 10) we identified 11 candidate biomarkers that were highly elevated (p value < 0.05) in serum of DMD patients relative to controls. Whole serum proteome profiling of mdx (n = 3) and wild type mouse (n= 3) using SILAC mouse spike-in strategy led to the identification of an additional 19 candidate biomarkers that were associated with dystrophin deficiency. Of these 8 were validated in human serum samples. Furthermore metabolomics analysis of DMD serum (n = 5) versus healthy control serum (n = 5) revealed dramatic alterations in the levels of specific amino acids and lipids. Biomarkers associated with disease progression were evaluated and validated in the mouse model.

Keywords: Duchenne muscular dystrophy, serum, surrogate biomarkers

POS-01-152 Relative Quantification of Endogenous Peptides in Cerebrospinal Fluid Using TMT 6-plex to Identify New Biomarkers for Alzheimer's Disease

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Background

The protein and peptide composition in cerebrospinal fluid (CSF) reflects ongoing processes in the brain, thus making it a valuable source for biomarkers. We have developed a method for relative quantification of endogenous peptides in CSF using isobaric labeling with TMT 6-plex for biomarker discovery.

Methods

A CSF pool was used to evaluate labeling efficiency and reproducibility of the reporter ion intensities when labeled with TMT 6-plex. A study consisting of 8 patients with Alzheimer's disease (AD) and 8 non demented controls was then performed. The CSF samples (100 μl) were reduced, alkylated, labeled with TMT 6-plex, and combined. The endogenous peptides were separated from larger proteins by 30 kDa molecular weight cut-off filtration and desalted on C_{18} columns. The samples were analyzed using a Q-exactive and an UltrafleXtreme MALDI-MS.

Preliminary results/Conclusions

Labeling efficiency was complete and the reporter ion intensities when the same CSF sample was used in all six TMT channels showed a mean ratio close to 1. Around 400 endogenous CSF peptides were identified and quantified. Several significantly altered endogenous peptides were found between the AD and control group, many derived from proteins known to be involved in AD related processes e.g. Neurosecretory protein VGF, Secretogranins, Metallothionein 3, Superoxide dismutase 1 and Integral membrane protein 2B. This is the first time isobaric labeling for quantification of endogenous peptides in CSF has been used. This application provides a useful tool for identifying new biomarkers for diseases affecting the brain.

Keywords: Alzheimer's disease, Cerebrospinal fluid, Peptidomics

POS-01-153 Proteomics Biomarkers for Human African Trypanosomiasis

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Human African trypanosomiasis (HAT) is a neglected parasitic disease affecting the poorest communities in sub-Saharan Africa. HAT is caused by *Trypanosoma brucei gambiense* and *T.b. rhodesiense* parasites, both transmitted to humans by tsetse flies. HAT evolves through two stages: the first is due to parasite proliferation in blood and lymph, while the second occurs after invasion of the central nervous system. A correct stage determination, based on CSF investigation, is essential to safely and properly treat patients. After treatment patients need to be followed for two years by blood and CSF examinations to detect relapses. Recently, we proposed powerful CSF staging and test-of-cure markers; however the elimination of invasive lumbar punctures is still a priority to further improve patients' care.
We here investigated plasma samples taken before treatment, after treatment and at the time of the relapse from *T.b. gambiense* relapsing patients using quantitative proteomics and 6-plex TMT isobaric labeling. Eleven proteins were found over-expressed at the time of the relapse in both patients and the significant over-expression of lumican, a small leucine-rich repeat proteoglycan, was further confirmed by an immuno-based approach on a larger number of patients.
This pilot study shows, for the first time, the possibility of having plasma HAT test-of-cure markers and paves the way for lumican validation in large multicenter cohort studies. The same quantitative proteomics approach was also applied to explore other important issues associated to HAT, such as differences between *T.b. gambiense* and *rhodesiense* infections. These analyses will further help in finding new targets for the development of rapid diagnostic tests and in understanding the physiopathology of HAT.
Keywords: HAT, biomarkers, quantitative proteomics

POS-01-154 Comparative Proteomic Analysis of Neutrophils from Patients with Microscopic Polyangiitis and Granulomatosis with Polyangiitis

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Both microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA) belong to ANCA-associated vasculitis (AAV), in which neutrophils play a key role in their pathology. In this study, in order to discriminate between MPA and GPA, protein profiles of peripheral blood polymorphonuclear cells (PMNs) of 11 MPA patients and 9 GPA patients and 10 healthy controls (HC) were analyzed by 2D-DIGE. In all the 864 spots detected, intensity of 55 spots was significantly different ($p < 0.05$) among the three groups by ANOVA. 31 out of the 55 spots were identified by mass spectrometry. Orthogonal partial-least-squares-discriminate analysis revealed that the abundance profile of the protein spots discriminated the AAV group from the HC group, and the MPA group from the GPA group completely. 13 protein spots were considered as biomarker candidates to distinguish between MPA and GPA. In those, spots whose intensity was higher in MPA than in GPA included actin with various pI values, while a considerable part of spots whose intensity was higher in GPA were proteins related with the activity of neutrophils. Among the candidate proteins, ROC analysis showed that a combination of neutrophil gelatinase-associated lipocalin and a-kinase anchor protein 7 isoforms beta had a high diagnostic potential.
Keywords: biomarker, vasculitis, neutrophil

POS-01-155 Proteomic and Transcriptomic Analysis of Triglyceride Deposit Cardiomyovasculopathy for Discovery of Novel Biomarkers

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Triglyceride Deposit Cardiomyovasculopthy (TGCV) is characterized by triacylglycerol deposition in multiple tissues. Mutation in ATGL genes are associated with TGCV, although pathogenic mechanism remains unknown. Here we examined proteomic and transcriptomic profiles of fibroblasts derived from patients and disease-model mice to find biomarker for TGCV. Fibroblasts derived from healthy volunteer and patients were labeled by stable isotope labeling by amino acids in cell culture (SILAC). Protein extracts of labeled cells were mixed, fractionated by one-dimensional gel electrophoresis and fragmented by in-gel trypsin digestion, followed by nanoLC-MS/MS analysis. Transciptome analysis was also performed using Affymetrix GeneChip Array. Differentially expressed genes and proteins were verified by quantitative PCR and selected reaction monitoring, respectively. Protein extracts of hearts from wild-type and ATGL KO mice were digested by trypsin. Then, each sample was labeled with iTRAQ reagents, fractionated with a strong-cation exchange column and quantitatively identified by nanoLC-MS/MS.Our multiomics analysis identified several biomarker candidates for TGCV, which include proteins involved in triacylglycerol metabolism such as adipophilin, CGI-58 and G0S2. Also a protein called filaggrin, an intermediate filament-associated protein, was upregulated in the fibroblast of TGCV patients. We are currently validating our results and investigating functions of these candidates.
Keywords: SILAC, iTRAQ

POS-01-156 Biomarker Discovery from Low Abundance Proteins in Bovine Serum; Proteomics for Developing Beef Production Systems

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Introduction:
For establishment of optimum beef production systems, farm animal sciences are aimed at understanding biological mechanisms concerning with the production of beef cattle with high economic value such as high intramuscular fat content. Diagnosis by detecting specific biomarkers for high economic meat quality traits during the early period of fattening could be advantageous to production of beef cattle. However, biomarker discovery in serum is often complicated by the wide dynamic range. In this study, in order to discover the biomarkers, we performed proteomic analysis of major and minor fractions of bovine serum proteins.
Methods:
Serum samples were collected sequentially from Japanese Black beef cattle during the fattening period. Proteins in the serum were fractionated into two groups; major and minor protein groups using ProteoMiner Protein Enrichment kits. Proteins in the fractions were then separated by 2DE, and their expression levels were evaluated with image analysis software, Progenesis PG220. Proteins in the gel were extensively identified using mass spectrometry. We investigated the correlation between the carcass or meat quality trait and the quantitative expression values of each protein.
Results:
Total of 228 and 399 spots were detected on the 2DE gels of the major and the minor protein fractions, respectively. In analysis of the serum at the end of fattening period (n=100), we identified 71 spots (24 protein) that were differentially expressed between high and low group of carcass or meat quality traits such as carcass weight and beef marbling standard (BMS) number. Among the 24 proteins, one of the minority protein A, which is involved in lipid transport, showed more than three times higher expression in high BMS group (n=4) than low BMS group (n=3) in the middle period of the fattening. The protein could be used as a serumbiomarker to monitor the accumulation of intramuscular fat during the fattening.
Keywords: serum, biomarker, 2DE

POS-01-157 Identification of Proteins Related to Accumulation of Intramuscular Fat in Japanese Black by Proteomic Analysis

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[INTRODUCTION]
In Japan, high accumulation of intramuscular fat (IMF) is strongly associated with the economic value of beef cattle. In this study, to identify protein biomarker candidates related to this trait, we performed proteomic analysis of the trapezius muscle in the beef cattle using two-dimensional electrophoresis (2-DE) and mass spectrometry (MS).
[METHOD]
Quantitative data of 141 carcass traits from 57 Japanese Black / Limousine Crossbred (F2) steers were subjected to principal component analysis (PCA). Based on the PCA, individuals with high and low IMF content were selected (high IMF group: n=10, low IMF group: n =8). Proteins extracted from trapezius muscle samples of the 18 animals were separated by 2-DE. Proteins in the 2-DE gel were identified using MALDI-TOF/TOF MS. The protein expression levels were compared between the two groups.
[RESULTS]
A total of 502 protein spots were detected on each gel, and 155 spots were identified. Among the identified spots, 49 spots (20 proteins) showed significantly different expression between the high and low IMF content groups. The expression level of proteins related to aerobic respiration, response to oxidative stress, and muscle contraction was higher in the high IMF content group (12 proteins), while those related to anaerobic respiration were higher in the low IMF content group (6 proteins). The high expression of the proteins related to the response to oxidative stress in the high IMF content group was confirmed by western blot.
[CONCLUSION]
We found 49 protein spots (20 proteins) related to accumulation of IMF in Japanese Black by 2-DE analysis of muscle tissue. Especially, the expression level of proteins involved in response to oxidative stress was also significantly higher in high IMF group in western blot analysis, indicating that oxidative stress is increased in high IMF muscle.
Keywords: proteomics, 2DE, biomarker

POS-01-158 The Regulatory Effects of the Tau Protein on Triose Phosphate Isomerase (TPI) in Brain Cells for the Normal and Diseased States are Different

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Alzheimer's disease (AD), a neurodegenerative disease which ultimately leads to death is characterized by progressive loss of memory and cognitive skills as well as the eventual loss of the ability to carry out the simplest of tasks. AD is caused by the accumulation of Tau tangles in the brain. Tau is a microtubule-associated protein which is mainly expressed in neurons, where it enhances neuronal transport by the stabilization of microtubules. In addition it is known to confer resistance against apoptosis. Aside these beneficiary roles, Tau has also been found to enhance oxidative stress. In this study, we show the controversial effect of Tau on the activity and quantity of triose phosphate isomerase (TPI) in the normal and diseased state. An oxidative stress-induced decrease in the activity of TPI was attenuated in Tau-overexpressing cells, indicating that Tau protects TPI against oxidative damage. On the contrary, the activity of TPI was decreased in Tau-transgenic (Tg) mice compared to non-Tg (NTg) mice while protein levels remained unchanged in both groups. The decrease in the activity of TPI in the Tg mice could be explained by the presence of some TPIs on the paired helical filament (PHF). These findings suggest that Tau binds to TPI and protects TPI under normal conditions while in the diseased state, tau reduces the activity of TPI.
Keywords: Alzheimer's disease, Tau protein, triose phosphate isomerase

POS-01-159 Proteomic Analysis of Saliva Identifies Potential Biomarkers in Physiological State

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Saliva, contains an abundance of proteins, offers an easy, inexpensive, safe and non-invasive approach, and then possesses a high potential to investigate the stress. At present, alpha-amylase and chromogranin A are well known proteins as stress markers in saliva. However, the studies on these proteins were limited to the effect of acute stress. Little is known about the effect of relaxing treatment after long-term stress. Therefore, the proteomic analysis of saliva before and after relaxing treatment is promising to find new biomarkers in physiological state such as unstresses. First, we examined the effect of doing Tai Chi. Saliva samples were collected before and after treatment, and the activity of alpha-amylase of each sample was determined immediately. Samples taken after doing Tai Chi showed a decrease in the activity, salivary proteins were resolved using two-dimensional gel electrophoresis over a pH range of 3-10, and the resulting proteome profiles were compared. Differentially expressed protein spots were then identified by MALDI-TOF/TOF tandem mass spectrometry. Many spots identified as the alpha-amylase, therefore, western blotting analysis was performed. Results showed these spots having the immunoreactivity with antibody of alpha-amylase. Next, samples taken after healing treatment such as listening to the relaxing music, and drinking tea were studies. Similarly, the proteins cross-linked with alpha-amylase antibody were appeared after treatment. The alpha-amylase might have isoforms and/or be degraded. As alpha-amylase was glycoprotein, lectin-blotting analysis was performed on sodium dodesyl sulfate-poly acrylamide gel electrophoresis. Taken together, the alpha-amylase might be altered on sugar components. Now, we examined further analyses about alpha-amylase and other protein spots to elucidate the biomarkers in unstresses.
Keywords: human saliva, stress marker, alpha-amylase

POS-01-160 Implications of Methylglyoxal Modification of Peroxiredoxin 6 for Impaired Healing in Diabetic Wound

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In the streptozotocin (STZ)-induced diabetic mice, a significant delay in gastric ulcer healing was observed. Methylglyoxal (MGO) is a highly reactive dicarbonyl compound derived from triose phosphates during glycolysis pathway, which modifies arginine, lysine, and cysteine residues in proteins. By using a proteomics approach, an antioxidant protein peroxiredoxin 6 (Prx6) was found as the major MGO-adducted protein in the diabetic ulcer mice. Prx6 functions in antioxidant defense mainly by facilitating repair of damaged cell membranes via reduction of peroxides. Peroxidase activity of recombinant Prx6 was inhibited not only by oxidation but also by MGO modification. Prx6 contains one conserved reactive cysteine residue Cys-47 in the active site. The decrease in the number of free thiols upon incubation with MGO was observed. Mass spectrometry analysis of peptide fragments of MGO-modified recombinant Prx6 identified residues Arg-24, Arg-41, Arg-106, Arg-132, Arg-219 and Lys-63 as the modification site in the protein. N(delta)-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine was generated at Arg-132 by MGO modification, which is situated in the catalytic center of peroxidase activity of Prx6. Our results suggest that the decrease in peroxidase activity is due to sulfhydryl modification of Cys-47 and guanidino modification of Arg-132. Other MGO adducts may also contribute to the loss of enzyme activity. *In vivo*, MGO-modified Prx6 was detected in red blood cells from diabetic patients. Levels of MGO-modified Prx6 in erythrocyte proteins correlated with both blood glucose and glycosylated hemoglobin (HbA1c) levels. These results suggest that MGO modification of antioxidant proteins including Prx6 is involved in the delayed wound-healing process in diabetes.
Keywords: methylglyoxal, peroxiredoxin 6, diabetes mellitus

POS-01-161 Proteomic Study of Biomarkers for Amyotrophic Lateral Sclerosis in Human Muscle Biopsies

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Amyotrophic lateral sclerosis (ALS) is a serious neurodegenerative disorder characterized by progressive loss of motor neurons causing muscle atrophy. Many research groups looked for ALS protein biomarkers applying proteomic and genomic approaches, using different tissues and body fluids, animal models and real patients. In present study we used muscle biopsies from patients diagnosed with ALS in order to find the altered proteins specific for this disease. We compared three groups of patients: ALS diagnosed, control objects, and patients with other diseases causing denervation (one of them is post-polio syndrome). Our gel-free proteomic approach involved detergent-based protein extraction from the muscle tissue, in-solution digestion of proteins, and isotope dimethyl labelling of tryptic peptides, followed by protein identification and relative quantification by nano-liquid chromatography - high resolution mass spectrometry (nanoLC-MS/MS, Fourier-transform ion cyclotron resonance mass spectrometer, FT-ICR, has been used). We found an array of protein candidates which were: (i) downregulated exclusively in ALS patients' biopsies, e.g. FHL1, Four and a half LIM domains protein 1, (ii) upregulated exclusively in ALS patients' biopsies, e.g. PADI2, Protein-arginine deiminase type-2, (iii) altered in biopsies of patients with other diseases, but not changed in ALS patients' biopsies. These proteins, individually or in combination, could be used as disease biomarkers revealing molecular mechanisms of ALS development. Importantly, after additional verification, these potential biomarkers can be used for diagnosis of ALS from muscle biopsy without a risk to confuse ALS with other denervation (presently ALS cannot be diagnosed though muscle biopsy).

Keywords: ALS, muscle, proteomics

POS-01-162 Proteomics and Biomarker Discovery in Bipolar Disorder

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Few psychiatric proteome-wide studies of potential biomarkers in cerebrospinal fluid (CSF) have been conducted although CSF contains a large number of endogenous proteins and peptides which may have a great potential value as biomarkers for central nervous system disorders. We hypothesize that this study will lead to the identification of new biomarkers for diagnosis, better possibilities to monitor the effect of drug treatments, and provide clues to the underlying molecular mechanisms of bipolar disorder. In the current study we are presenting a proteomic 2-step approach using first a discovery nano-liquid chromatography mass spectrometry technology in a limited sample of patients with bipolar disorder (to date, N=159) from which CSF is available from both patients and matched controls. High-resolution mass spectrometry on a Q-Exactive instrument in combination with stable isotope labeling with Tandem Mass Tag (TMT) has greatly improved the ability to perform quantitative proteomic analyses. To date, approximately 400 CSF proteins can be semi-quantified with this technique without any prior sample fractionation step. Identified candidate biomarkers will further be compared against the Swedish Psychiatric quality registers to search for correlations to e.g. suicide attempts and/or relapses and also to evaluate the effect of different treatment regimes. In the second validation step, Selected Reaction Monitoring (SRM) will be used, allowing detection of ions at a specific mass to charge ratio and thereby detecting specific pre-determined fragment ions from the target molecules. By using stable-isotope labeled synthetic peptides corresponding to the peptides formed from the target molecule as internal standards, a fast and sensitive quantification can be performed. The identified candidate biomarkers will further be analyzed quantitatively in the whole bipolar cohort using high-throughput serum immunoassays (N=5000).

Keywords: TMT Proteomics, bipolar disorder, cerebrospinal fluid

POS-01-163 Proteomics of Axons during Presynapse Formation Using the Novel Culture Method "Neuron Ball Culture"

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Elucidating whole process of synapse formation is important for understanding brain function. Recently, several transmembrane proteins have been identified to induce pre- and post-synaptic structures. However, the precise molecular basis on pre- and post-synaptic differentiation remains unclear. To investigate whole picture of pre- and post-synaptic differentiation on the molecular basis, we have started to analyze proteomics during formation of presynaptic terminals as the first step. To do this, it is important to prepare postsynase-free axons with presynapses. For this purpose, we have established "neuron ball culture" to obtain the axonal fraction with high yield and purity. Furthermore, we applied the method to induce presynaptic differentiation using beads coated with leucine-rich repeat transmembrane protein 2 (LRRTM2), which is the postsynaptic protein to interact with presynaptic neurexins to form synapses. The LRRTM2-coated beads promoted functional excitatory presynapses on axons in neuron ball culture, judged by accumulation of vesicular glutamate transporter and uptake of FM1-43 dye. Proteomics using isobaric Tag for Relative and Absolute Quantitation (iTRAQ) revealed that expression ratio under LRRTM2 stimulation compared to control (iTRAQ ratio) in axons were distributed more broadly than those in cell bodies, especially towards higher iTRAQ ratio. These data suggest that LRRTM2 induce to increase some proteins in axons. We have identified a set of proteins that change their expression level in axons during presynaptic differentiation using proteomics. This work will provide new insight into understanding synaptogenesis on the molecular basis.

Keywords: iTRAQ, synapse, neuron

POS-01-164 Quantitative Mass Spectrometry for Proteomic Screening of Potential Biomarkers in Alzheimers Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia, characterized by cognitive impairments and memory loss. AD is confirmed by the presence of extracellular plaques and neurofibrillary tangles in the cortex, but the accuracy of the early antemortem diagnosis is not ideal. Thus, there is a need for the discovery of early diagnostic and prognostic biomarkers. Mass spectrometry-based proteomics has emerged as a powerful technology for biomarker discovery. The major aim was to study the neuroproteomic profiles of AD and healthy human brain tissue. Shotgun approach based on label free quantification mass spectrometry (MS) was used to follow changes in protein expression associated with AD. Proteins were extracted from homogenized human brain biopsy of temporal neocortex using previously optimized detergent n-Octyl-β-D-glucoside extraction protocol. Extracted proteins were delipidated and tryptically digested on 3kDa spin filters and analyzed LC-MS/MS using a 7 T hybrid LTQ-FT.

In total, we were able to identify and relatively quantify more than 600 unique proteins per sample and the percentage of identified membrane proteins was approximately 29% which is at the reported genome expression levels of ~30%. By applying a label free quantification approach, a large number of peaks were found that showed differential expression throughout the elapsed AD. Our preliminary protein reports reveal a number of proteins with altered expression between AD patient samples and healthy controls candidates. Cluster analysis followed by protein-protein interaction analysis revealed a decrease in expression in mitochondrial proteins. Several of significantly regulated identified proteins are directly involved in the onset of mitochondrial apoptosis. Our findings may provide knowledge for the understanding of disease pathogenesis as well as for the discovery of potential biological markers.

Keywords: neurodegeneration, mass spectrometry, label free quantification

POS-01-165 ATP Accessibility Screening (AAS), A High-Throughput and High-Resolution Kinase Analysis Platform for Signaling Research

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Phosphoproteome analysis is now widely used for various signaling research. It is possible to quantitate phosphorylation level of thousands or more phosphosites. However, deep phosphoproteome analysis is low-throughput. Due to this disadvantage, it is very difficult to use phosphoproteome analysis for experiments which need high-throughput fashion, such as chemical library screening or genome-wide knockdown experiment. Phosphoproteome analysis is useful to monitor kinase activity, when target kinase has a phosphorylation site which represents its kinase activity. However, a kinase which activity is not regulated by phosphorylation, such as constitutive active kinase, cannot be monitored by phosphoproteome analysis. In that case, we might predict kinase activity from phosphosites (substrate) data using bioinformatics analysis, but such kind of analysis is low-resolution (for example, it is difficult to distinguish AKT1 and AKT2 activation). In order to overcome these challenges, we developed a high-throughput and high-resolution proteomic analysis platform, ATP Accessibility Screening (AAS). AAS is a kind of activity-based proteomic analysis targeted on ATP accessibility of kinases. We employed amine-reactive ATP-biotin tag to label lysines conserved in ATP binding pocket. After labeling, protein was digested, enriched by streptavidin beads and applied to LC-MS/MS. About 15% of identified proteins were kinases. Thus we constructed an inclusion list contains *m/z* and retention time information on peptides from 249 kinases. Using this inclusion list, we can quantified ~130 kinases in 1 hour run from 4 mg SILAC labeled Hela-S3 lysate. We will also report the effects of ionized radiation at different doses and at different time after irradiation on ATP accessibility.

Keywords: ATP accesibility, kinase, screening

POS-01-167 Autophagonizer Induces Autophagy via Endocytosis Inhibition

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Autophagonizer, a novel autophagy inducing small molecule, was discovered from HCS with phenotypic screening for autophagy inducers. Autophagonizer induces autophagy and apoptosis independent cell death, but its activity is not inhibited by known autophagy inhibitors. In order to reveal the mode of action behind autophagonizer, the ORFeome technology was applied. ORFeome is the collection of the entire overexpressed open reading frames (ORFs) of *Schizosaccharomyces pombe*. By measuring the growth of overexpressed strains after autophagonizer treatment, genes affected by autophagonizer were identified. As a result, genes related to endocytosis were hypersensitive to autophagonizer. Validation with yeast and mammalian cells resulted in endocytosis inhibition by autophagonizer, in accordance to the ORFeome results. Furthermore, the Drug Affinity Responsive Target Stability (DARTS) assay was applied for the identification of direct binding protein of autophagonizer. Cell lysate was incubated with autophagonizer and exposed to pronase treatment. Proteins bound to autophagonizer were resistant to pronase degradation, and identified through MS analysis. DARTS assay also resulted in identification of proteins related to membrane proteins and ATPase activity. Collectively, autophagonizer induces autophagy by inhibiting endocytosis as revealed by ORFeome assay, and binds to membrane related proteins as revealed by DARTS assay. These results imply that autophagy could be induced by endocytosis inhibition as done by autophagonizer.

Keywords: Autophagonizer, ORFeome technology, DARTS

POS-01-166 Replenishment of Recombinant UQCRB Protein, A Terpestacin-Binding Mitochondrial Protein, Enhances Angiogenesis *In Vitro* and *In Vivo*

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Ubiquinol-cytochrome c reductase binding protein (UQCRB), one of subunits of mitochondrial Complex III, is a specific cellular binding protein of anti-angiogenic natural small molecule, terpestacin. Mitochondrial Complex III (cytochrome bc_1 complex) has been reported as a crucial regulator in hypoxia-induced angiogenesis through mitochondria-derived reactive oxygen species (ROS) involved oxygen sensing. Here, cell permeable recombinant UQCRB protein is generated using protein transduction domain (PTD), a small peptide transferring its binding partner into the cell, to uncover the biological role of UQCRB. Consequently, PTD-UQCRB transduction enhances generation of mitochondrial ROS and HIF-1α stability. Also, trans-membrane delivery of PTD-UQCRB induces vascular endothelial growth factor (VEGF) expression and invasion of human umbilical vascular endothelial cells (HUVECs) *in vitro*. Furthermore, PTD-UQCRB treatment enhances wound healing *in vivo*. These results imply new insights into the function of PTD-UQCRB in angiogenesis via mitochondria-mediated ROS generation and also open new basis on application of PTD-UQCRB as a pro-angiogenic agent via regulating mitochondrial function.

Keywords: angiogenesis, UQCRB, mitochondria

POS-01-168 Phage Display-Based High Throughput Screening to Identify Cell-Internalizing Monoclonal Antibodies for Antibody-Drug Conjugates

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Monoclonal antibodies (mAbs) that are internalized into cells are a current focus in the development of antibody-drug conjugates (ADCs). mAbs are considered rare in the antibody repertoire, however, and therefore a robust screening system is required to isolate potent cell-internalizing mAbs. Here we describe a phage display-based high-throughput screening system using protein synthesis inhibitory factor (PSIF) as an efficient method for screening cell-internalizing mAbs. We simultaneously examined the cell-internalizing activities of several hundred independent mAbs, and successfully isolated cell-internalizing mAbs against the tumor endothelial markers Roundabout homolog 4 (Robo4) and vascular endothelial growth factor receptor 2 (VEGFR2). Flow cytometry analysis revealed that the cell-internalizing activity was fully retained even if the mAb format was altered (single chain Fv [scFv], dimerized scFv [dscFv], and fullbody [IgG]). Tumor accumulation of mAbs with high cell-internalizing activity was significantly higher than that of mAbs with low cell-internalizing activity. Furthermore, the anti-tumor effects of ADCs comprising mAbs with high cell-internalizing activity were significantly stronger than those of mAbs with low cell-internalizing activity. While anti-VEGFR2 therapy led to a significant body weight loss, anti-Robo4 therapy did not. These findings indicate that cell-internalizing activity has an important role in the biodistribution and therapeutic effects of ADCs. Further, Robo4 can be an effective marker for targeting tumor vasculature. This technology will be useful for developing ADCs against various membrane-associated biomarkers that are discovered by proteomic techniques, such as chemical proteomics.

Reference Mukai Y *et.al.*, Robo4 is an effective tumor endothelial marker for antibody-drug conjugates based on the rapid isolation of the anti-Robo4 cell-internalizing antibody., *Blood.* 2013 in press.

Keywords: monoclonal antibody, cell-internalizing antibody, phage display

POS-01-169 Target Identification of A Novel IL-12p40 Production Inhibitor by Chemical Proteomics

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The functional inhibition of pro-inflammatory cytokines such as IL-12 and IL-23 has been considered to be an effective therapeutic strategy for various inflammatory diseases. We have performed phenotypic screening using RAW264.7 cells and generated potent inhibitors for IL-12p40, a subunit of IL-23, production. In order to identify the molecular target of these inhibitors, affinity purification was performed using an active analogue of these compounds. As a result of chemical proteomics approach, a lipid kinase was identified as a target of the inhibitors. These results indicated that the lipid kinase and its related molecules should be the candidates of drug target for the treatment of inflammatory diseases.

Keywords: chemical proteomics, target identification

POS-01-170 A Chemical Proteomics Strategy for the Discovery of Targets for Antibody-Based Therapy

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Therapeutic antibodies represent the largest and fastest growing class of pharmaceutical biotechnology products, with yearly sales exceeding 30 billion US$. The efficacy of antibody-based therapeutics is mainly determined by the availability and accessibility of antigens at the site of disease. The identification of bloodstream-accessible antigens specifically up-regulated at sites of disease is complicated by the low abundance of plasma membrane proteins.

In collaboration with ETH Zurich, Philochem developed the *in vivo* biotinylation methodology for the identification of bloodstream-accessible proteins in animal models of disease. This methodology is based on the vascular perfusion of organs or whole animals with an active ester derivative of biotin. Proteins accessible from the bloodstream are labeled with biotin via their primary amines, purified from total organ or tissue extracts, digested on resin and the resulting proteolytic peptides are analyzed by liquid chromatography coupled to mass spectrometry. The *in vivo* biotinylation methodology was applied to a variety of different diseases including cancer, Alzheimer's disease, diabetic nephropathy and rheumatoid arthritis. Next to identifying markers of disease, the biotinylation strategy also generates "Atlases" of accessible proteins in healthy and diseased tissues. In comparison with popular protein localization/distribution repositories such as Proteinatlas, this methodology has the advantage of identifying bloodstream-accessible markers only. The availability of such atlases will be of great value for the development of antibody based therapeutics since it facilitates the selection of high quality tissue specific and bloodstream-accessible vascular antigens.

The broadly applicable *in vivo* biotinylation technology provides "Atlases" of accessible antigens for antibody-based therapeutic intervention.

Keywords: chemical proteomics, In vivo biotinylation, target discovery

POS-01-171 Application of Proteomics to Drug Discovery Research

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Understanding precise mechanisms of disease and drugs is a key challenge in drug discovery research, and for that purpose proteomics has become an indispensable tool.

To understand a mechanism of disease, proteome comparison between patients and controls is a standard approach. The comparison is also useful in identifying disease biomarkers.To understand a mechanism of drug action, proteomics analysis, such as protein-protein interaction, subcellular location and post-translational modification, is essential, which is difficult to be done with other techniques like genomics and transcriptomics. In the case of a compound discovered in a cell-based assay, a chemical proteomics approach to target identification becomes one of the highlighted topics in drug discovery research.

We have performed proteomics studies to find disease biomarkers using proteome comparison and to elucidate mechanisms of drug action using chemical proteomics. From those studies, we have learned that proteomics approaches are hypothesis-making, and the following validation steps are critical, and often rate-limiting, especially in chemical proteomics.Therefore, it is important to integrate appropriate validation techniques into proteomics research and to exclude as much as possible non-related molecules by state-of art proteomic technologies, which will contribute to efficient validation works and increase success rates of projects.

Keywords: chemical proteomics, drug discovery, urinary proteomics

POS-01-172 Application of Various Types of Linkers for Photoaffinity Biotin-Tagged Chemical Probes to Identify Drug Targets

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Target identification of drugs discovered in cell-based assays becomes very important in drug discovery, which is often performed by a chemical proteomics approach. Recently, a photoaffinity biotin-tagged chemical tri-functional probe composed of a compound-binding, a photoreactive and a biotin-tagged parts has been used for that purpose (1,2). In this approach, it is critical that the linker structure between the compound-binding and the photoreactive parts should not interfere with the binding between the compound and its target protein. To study the effect of the linker structure, we prepared tri-functional probes with various type of linkers and compared the quantities of the captured proteins by label-free quantitative proteomic analysis. The results showed that the amounts of the target and non-specific proteins were varied with each linker, and suggested that it would be helpful for efficient target identification to use tri-functional probes with various kinds of linkers. References (1) J. Fisher et al, J. Proteome Res., 2010, 9, 806-817. (2) S. Michaelis et al, J. Med. Chem., 2012, 55, 3934-3944.

Keywords: target identification, chemical probe

POS-01-173 Proteomic Analysis of Two Lung Cancer Cell Lines Treated with Umbelliprenin

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Background: Umbelliprenin is a prenylated compound belonging to the class of sesquiterpene coumarins. Recently, umbelliprenin has been attracted attention for its antitumor activities. The aim was to determine the protein targets of umbelliprenin in two human lung cancer cells.
Methods: QU-DB and A549 cells were cultured in RPMI 1640 and 10% BSA contained umbelliprenin at 31 μM (IC30) or 0.15 % DMSO under similar conditions until 60-70% confluency. Cells were washed, lysed, and proteins were separated using 18-cm IPG strips (PH 3-10 NL) and 12.5 % polyacrylamide gels. Three gels were run for each group. Silver staining method was used and images were analyzed using Prodigy software. Differentially expressed proteins spots were successfully picked up and were analyzed by LC-MS/MS.
Results: Nipsnap1 and glycine-tRNA ligase were up-regulated in umbelliprenin treated QU-DB cells, while Stathmin, alpha-actin-1 and 2, GAPDH, calreticulin, GRP78 and AHA1 were overexpressed in A549 cells. Umbelliprenin down-regulates the production of HSP90, HSP27, p97/VCP, vimentin, SF3a3, importin-α2, impotrin-β1, NDUFS3, GRP94, hnRNP C1/C2, FKBP4 and tubulin α-1B in QU-DB cells, and DDAH-2, MST, Keratin-1, Annexin A4, Proteasome α-1, VHR, APRT, Cyclophilin B and Prohibitin in A549 cells in comparison with DMSO treated control cells.
Conclusion: This is the first report of potential umbelliprenin's protein targets in lung cancer cell lines. Some of these proteins were already suggested as lung cancer biomarkers such as calreticulin, stathmin, HSP27 and vimentin. Most of these potential protein targets may involve in cytotoxic and anti-proliferative effects of umbelliprenin on QU-DB and A549 cell lines.
Keywords: Umbelliprenin, lung cancer cells

POS-01-174 Identification of Prostasin Serine Protease Against Hepatitis C Virus Replication

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Hepatitis C viruses (HCV) infection is considered as a major causative factor associated with chronic hepatitis and formation of hepatocellular carcinoma (HCC). By using pharmacoproteomic approach, we first identify a prostasin protein (PRSS8), a trypsin-like serine protease, associated with HCV replication. In this study, we find that overexpression of prostasin inhibits HCV protein synthesis and RNA replication in a concentration-dependent manner in HCV subgenomic replicon and infectious systems. The antiviral activity is confirmed by using dominant-negative prostasin mutant and silencing of prostasin. On the contrary, HCV infection down-regulates prostasin expression, suggesting prostasin acts as a negative factor limiting viral replication. To investigate molecular mechanism by which prostasin exerts its anti-HCV activity, we first perform several reporter assays measuring HCV IRES, NS3/4A protease and NS5B polymerase activity upon prostasin overexpression and find that HCV NS3/4A protease activity is slightly down-regulated by prostasin. In addition, prostasin significantly suppresses cyclooxygenase 2 (COX-2) expression which is required for HCV replication. We further evaluate the upstream signaling mediators by which prostasin down-regulates HCV-induced COX-2 expression and find that mitogen-activated protein kinases (MAPKs) involved in its regulation. In conclusion, we first discover the host factor prostasin protease involved in viral replication, which may provide an opportunity to develop therapeutic drug for treatment of HCV infection.
Keywords: hepatitis C virus, prostasin, cyclooxygenase 2

POS-01-175 Functional Proteomics Approaches for High-Throughput Determination of Small Molecules Interactions on cKIT

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cKIT is a trans-membrane receptor with tyrosin-kinase activity. Aberrant levels of cKIT activity have been associated with several hematopoietic disorders and gastrointestinal stromal tumors (GIST). Several point mutations of cKIT have been described in a cohort of 150 patients, mostly of them are related to tyrosine-kinase activity and interactions with Stem Cell Factor (SCF). As a consequence, more than 75% of the patients are resistant to conventional treatments, so new ones are neede ed. Here, we have designed and developed a Nucleic Acids Programmable Array (NAPPA), which content wild-type cKIT and all clinical mutations, in order to discover new inhibitors of cKIT activity and deep into cKIT-SCF interaction. By this approach, novel molecules have been discovered as useful for inhibition of aberrant cKIT activity.
Keywords: drug discovery, functional proteomics, protein arrays, tyrosine-kinase

POS-01-176 Target Identification of Novel Anti-Inflammatory Compound Using Chemical Proteomics Approach with Bait Compound

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Interleukine-12 (IL-12) and IL-23 are pro-inflammatory cytokines which induce serious pathological conditions in diverse of inflammatory diseases such as inflammatory bowel disease (IBD), psoriasis and rheumatoid arthritis. The therapeutic potential of IL-12/23 inhibition has been validated in clinical by Ustekinumab which is an approved monoclonal antibody neutralizing IL-12/23. Through the cell-based phenotypic screening and extensive medicinal chemistry campaign, we generated APY0201 as a potent and unique inhibitor for IL-12/23 production from activated macrophages, possessing significant selectivity over other cytokines including TNF-α. As a result of chemical proteomics approach using a bait compound equipped with FLAG peptide, PIKfyve kinase was identified as a biological target of our promising IL-12/23 production inhibitor. APY0201 is a potent, highly selective and ATP-competitive PIKfyve kinase inhibitor, which ameliorated inflammation in experimental model of colitis. We will disclose the design of bait compound equipped with FLAG peptide, strategy of target identification, SAR, and unique character of this novel drug target, to treat autoimmune and inflammatory diseases.
Keywords: target identification, PIKfyve, IL-12/23

POS-01-177 Andrographolide Analogue AL-1 Reciprocally Exerts Cytotoxic and Protective Effects on Cells by Inducing ROS Generation

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Andrographolide-lipoic acid conjugate (AL-1) is a new in-house synthesized chemical entity, which was derived by covalently linking andrographolide with lipoic acid. AL-1 can induce ROS generation to exert either cytotoxic or protective effect on cells depending on the concentrations of AL-1 used in treatments. By performing proteomics analyses and follow-up functional studies, we found that AL-1 with high concentrations (5, 10, 15 μM) provokes the generation of high concentration cellular ROS, resulting in oxidative DNA damage and subsequent G2/M arrest and mitochondrial-mediated apoptosis in leukemia K562 cells. However, AL-1 with low concentrations (0.01, 0.1 μM) induces low concentration NADPH oxidase-dependent ROS in pancreatic β-cells, serving as signaling molecules to activate AKT1 and ERK1/2 signaling pathways. As a consequence, the expressions of antioxidant proteins were upregulated to protect β-cells from H_2O_2-induced oxidative injury. AL-1 probably worked as a "vaccinum" to activate the cellular antioxidant system by inducing the generation of low concentration ROS which then reciprocally protected β-cells from oxidative damage caused by high-level ROS from H_2O_2. This is the first comprehensive proteomic analysis illustrating a novel molecular mechanism for an andrographolide-lipoic acid conjugate in both the cytotoxic and protective effects on cells.

Keywords: ROS signaling, Cytotocixity, Drug-action mechanism

POS-01-178 Cell Permeable Conjugates for Identification of Small Molecule - Protein Interactions

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The field of chemoproteomics has dramatically expanded as the interest in discovering novel interactions between proteins and small molecules has increased. This interest primarily stems from two disparate sources: the deconvolution of hits from phenotypic screens and the identification of off-target effects of pharmaceutically active compounds. This poster describes a chemoproteomics approach to solving this problem based on HaloTag® technology. A key feature of this approach is that the small and chemically inert chloroalkane required for reaction with the HaloTag® protein can be attached to pharmaceutically active small molecules without dramatically abrogating their potency or cell permeability, unlike many other commonly used tags. This unique tag allows direct confirmation that the chloroalkylated probe compound can recapitulate the biological activity of the parent compound in living cells prior to running the capture experiment, increasing confidence in the biological relevance of the captured proteins. In addition, the fast, covalent capture of the probe compound by immobilized HaloTag® protein allows capture of both strong and relatively weak protein interactors.

Keywords: Chemoproteomics, Drug-protein Interaction, Target Identification

POS-01-179 Synthesis of Mannosylated Lipopeptides as Targets for the Mannose Receptor

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Mannose receptors located on the antigen presenting cells are one of the key components of carbohydrate rich antigen uptake and an important part of an immune response. The aim of this research was to synthesize mannosylated peptides to target mannose receptors which are found on human antigen presenting cells for use in synthetic vaccines. This work describes the synthesis of the *O*-mannosylated amino acid building block followed by synthesis of fluorescein-labeled *O*-mannosylated lipidated peptides using a combination of manual or microwave assisted solid phase peptide synthesis. Lipids included on these constructs provide self-adjuvanting and cell penetrating properties A library of dendritic structures containing one or more mannosylated moieties separated by a variable-length linker containing the OVA peptide SINFEKEL was synthesized. In a two-step reaction, mannose was per-*O*-acetylated using acetic anhydride and DMAP in a quantitative yield. A Lewis Acid reaction with Fmoc-Serine-OH gave the final amino acid building block, Fmoc-Serine(per-*O*-acetylated mannose)-OH, suitable for use in SPPS. The peptides were labeled with 5(6) carboxyfluorescein to enable visualization inside cells. Crude peptides were purified using RP-HPLC and characterized by mass spectrometry and analytical RP-HPLC. Studies into the molecules self-assembly properties will also be assessed. We were able to successfully synthesize and characterize the *O*-mannosylated amino acid building block and subsequently prepare a library of fluorescein-labeled *O*-mannosylated lipidated peptides containing one or more *O*-mannosylated building blocks in milligram quantities. Synthetic strategies and preliminary self-assembly data will be presented.

Keywords: O-mannosylated amino acid

POS-01-180 Synthesis of Lipid LHRH Peptides for Targeted Drug Delivery

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Owing to the high expression of LHRH receptors on cancer cells, as compared to normal cells, LHRH can be used as a targeting ligand. A library of asymmetric fluorescent lipid peptides were synthesized using Solid Phase Peptide synthesis to test their self-assembly, stability and sterical rearrangement properties. Cell internalisation studies were also investigated. Lipids included on these constructs provide self-adjuvanting, self-assembly and cell penetrating properties.

A library of dendritic structures consisting of poly-lysine cores or lipid peptide cores (LCPs) containing the LHRH epitope, p-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 was synthesized. The C12-lipoamino acid was synthesized and N α-protected with 1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl (Dde). The LHRH peptide, poly-lysine and LCP cores were synthesized on Rink Amide MBHA resin using standard Fmoc solid phase peptide synthesis (SPPS). A fluorescent label, 5(6)-carboxyfluorescein (5(6)-FAM), was coupled to each peptide using 2.4 eq DIC and 2.5 eq HOBt in DMF overnight. Removal of the Fmoc amino acid protecting group was achieved using 20% piperidine in DMF solution (2 x 15 min). The Dde lipid protecting group was removed by 2% hydrazine treatment (2 x 30 min). Cleavage from the resin was achieved using TFA:TIPS:H_2O (95:2.5:2.5). Crude peptides were purified using RP-HPLC and characterized by mass spectrometry and analytical RP-HPLC. Studies into the molecules self-assembly, sterical rearrangement and cell internalization properties will be assessed.

We were able to successfully synthesize, purify and characterize fluorescently-labeled lipid modified LHRH peptides in milligram quantities. Preliminary self-assembly and cell internalization data of these nanoparticles will be presented.

Keywords: LHRH, Targeting, Solid Phase Peptide Synthesis

POS-01-181 Relative Quantification of Intact Hemoglobin A2 with an Ion Trap Mass Spectrometer

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Introducion: The main diagnostic parameter for beta thalassemia trait is an increase of hemoglobin (Hb) A2. In normal adult blood, HbA ($\alpha 2\beta 2$), HbA2 ($\alpha 2\delta 2$), and HbF ($\alpha 2\gamma 2$) represent the total hemoglobin content. While reference HbA2 levels are between 2.5% and 3.5% of total Hb, beta thalassemia trait is characterized by HbA2 levels above 3.7%. There is no reference measurement procedure currently approved for the measurement of HbA2. To precisely measure HbA2 levels from the perspective of a clinically relevant application, we developed an MS-based assay for the quantification of the intact HbA2 delta chain in a standard commercial ion trap MS.

Methods: EDTA blood specimens were obtained from a donor with normal HbA2 levels (3%, measured with a Bio-Rad Variant II, Bio-Rad). A Hb solution was obtained by washing and lysing red blood cells. Purified HbA2 was spiked at six different concentrations (0-15%) in this control solution. Direct infusion analyses were performed with an ion trap MS (Amazon Speed ETD, Bruker Daltonics). The α and δ chains were isolated at specific charge states and ejected without activation. Areas of corresponding isolated precursor ions were used to calculate δ to α ratios. Three quantitative series of analyses were performed at seven different days.

Results: Standard curves fitted linearly (R2=0.9982) and allowed quantification of HbA2 over a concentration range from 3% to 18% of total Hb. Interday CVs ranged from 3.3% to 6.6%. The precision was enough to determine if HbA2 levels were below 3.5% or above 3.7%. Parameters such as LOD and LLOQ will be further assessed. Importantly, the use of a robust and reproducible ion trap MS makes this method potentially exportable to the clinical laboratory. In conclusion, our method reaches precision requirements for the determination of HbA2 levels in beta thalassemia trait diagnosis.

Keywords: mass spectrometry, hemoglobin, intact protein quantification

POS-01-182 Analysis of PTH Variants by Immunocapture with High Affinity N and C Terminal Monoclonal Antibodies

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Parathyroid hormone (PTH) is fundamental for calcium homeostasis and its receptor (PTH1R) is present in several tissues, with special role in renal tubules and bone cells. The classical biological activity of PTH, mediated by PTHR1 activation, is dependent on the presence of an intact amino terminal sequence, mainly the first amino acids. Yet PTH present in circulation is very heterogeneous, and this heterogeneity is the consequence of a complex metabolism that starts in the parathyroid cells and continues in other tissues, mainly in the kidneys and liver.This complex metabolism results in several circulating parathyroid hormone molecular forms. These variants show different biological activities and currently immunoassays methods tend to fail to detect them and/or distinguish them from the intact hormone. Here we describe an assay using immunocapture-based on two high affinity (N and C terminal) monoclonal antibodies to capture PTH variants from parathyroid cyst fluid and serum samples. Monoclonal antibodies were conjugated with protein A/G magnetic beads and incubated with parathyroid cyst fluid during 16 hours. After magnetic capture, beads were washed twice with wash buffer and eluted with acetate buffer. Eluted fractions were separated by size exclusion and reverse phase chromatography, digested by trypsin and analyzed by nanochromatography coupled to high resolution tandem mass spectrometry. PTH, PTH prehormone and other 4 variants were identified in the cyst fluid using MS^E and DDA acquisitions. A targeted MS/MS method on a QTOF (pseudo-MRM) was developed to detect the PTH variants in serum. The characterization of PTH secretion profiles in different clinical conditions can reveal important information for prognosis and treatment.

Keywords: PTH, LC-MS/MS

POS-01-183 Glycol Chitosan-Conjugated ApoA1 Protein Complex Regulation of Silica-Induced IPF

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Pulmonary fibrosis occurs as a wound-healing process after many forms of pulmonary injury that is induced by a variety of factors. However, chronic inflammation and pulmonary fibrosis can be developed without any known causes in certain cases. To regenerate the damaged tissues, there are try to treat IPF using stem cell therapies and is increasing experimental evidence for reprogram injured cells by using biomaterials.

We reported that apolipoprotein (Apo) A1 is one of the candidate molecules for IPF. In this respect, we try to examine the possible mechanism of pulmonary restoration using glycol chitosan (GC) -conjugated ApoA1 treatment.

To determine whether GC-conjugated ApoA1 protein contributes SiO_2-induced IPF, we treated GC-conjugated ApoA1 protein in SiO_2-treated BL/6j through intratrachea of mice in the SiO_2-induced IPF. Using proteomic, histological, immunohistochemistry analyses, we estimate pathogenic changes in IPF mice treated GC-conjugated ApoA1.

Mice treated with SiO_2 were taken IPF. GC-conjugated ApoA1 restricted the extent of lung pulmonary inflammation reaction to SiO_2. In case of SiO_2-treated mice, 0.73 pmol treatment of ApoA1 didn't regulate fibrosis accumulation, inflammation. However, GC-conjugated ApoA1 treatment decreased fibrosis markedly in mice. Besides, we observed a small quantity of GC-conjugated ApoA1 was needed to treat fibrosis.

The deficiency of ApoA1 increased collagen and SMA-α, contrariwise, the increase of ApoA1 protein by using GC-conjugated ApoA1 effectively decreased collagen and SMA-α in SiO_2-treated mice.

These results suggest that GC plays a role of delivering protein as carrier and limits the extend of lung fibrosis. ApoA1 also contributes repairing lung response in SiO_2-induced fibrosis.

Keywords: idiopathic pulmonary fibrosis, apolipoprotein A-1, glycol chitosan

POS-01-184 Proteomic Analysis on the Mechanism of LDL Apheresis Therapy in the Steroid-Resistant Nephrotic Syndrome

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[Background] Steroid-resistant nephrotic syndrome is intractable kidney disorder often associated with the progression to end stage renal diseases CKD. To treat steroid-resitant nephrotic syndrome LDL-apheresis (LDL-A) has been instituted and its efficacy is reported to be about 50%. In the present study the mechanism whereby of LDL-A does or does not induce the remission of steroid-resitant nephrotic syndrome was investigated using the proteomic analysis of the adsorbed plasma proteins from the patients.

[Method] The effect of LDL-A was assessed by the clinical indicators such as proteinuria and serum albumin and the patients were grouped as responder (n=4) and non-responder (n=4). The adsorbed plasma was obtained at the first and the last sessions of the apheresis. Following the removal of albumin and gamma-globulin, the samples were separated by two-dimensional differential in-gel electrophoretic analysis (2-D DIGE). All spots were picked and subjected for in-gel digestion with trypsin followed by peptide analysis by MALDI-TOF/MS. [Results] Since 2D patterns of the adsorbed proteins in non-responder group were almost identical between the first and the last sessions of the apheresis, we focused on the difference of 2D patterns in the first and the last session in responder group. 16 proteins varied in their amounts between the first and the last sessions. Among them apolipoprotein B-100, complement component 3, etc decreased in the last, indicating the association with nephrotic condition. On the other hand, complement component 9, apoprotein E increased probably suggesting of the association with clinical remission. Of interest is that apolipoprotein E and serum amyloid P were high in both the first and last session. Moreover, serum apolipoprotein E was also high in a no-responder group.

Keywords: nephrotic syndrome, LDL-apheresis, 2-D DIGE

POS-01-185 Discovery of New Therapeutic Targets in Epithelioid Sarcoma Using Proteomic Analysis

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Background: Epithelioid sarcoma (ES) is a malignant soft tissue tumor, usually occurring in the distal extremities in young adults. Surgery is the mainstay of treatment for ES, however, high rates of local recurrence and distant metastasis generally result in poor patient outcomes. Therefore, there is critical need for effective systemic treatment of ES. Recently, targeted therapy which interferes with specific molecules involved in tumor development is widely expected to improve clinical outcome in anti-cancer treatment. However, the disease is poorly known at the molecular level probably because the incidence of ES is extremely rare. Purpose: We aimed to identify the proteins related to tumorigenesis in ES. Such proteins should be good candidates of targeted therapy. Materials and methods: This study included frozen tumor tissue and non-tumor tissue specimens from twelve ES patients after surgery. Protein expression profiles were created using two-dimensional difference gel electrophoresis (2D-DIGE). Protein samples were labeled by CyDye DIGE flour saturation dye and separated by our original large format gel. The reproducible proteome data were obtained by using internal control samples. We compared the protein expression profiles between tumor tissues and non-tumor ones. Results: Proteomic analysis resulted in the identification of proteins unique to tumor tissue samples. Those proteins were functionally involved in the molecular pathways which were previously reported in other malignancies. Conclusions: We identified proteins associated with oncogenesis in ES. The utility of such proteins as therapeutic target are worth investigating in the further functional study *in vitro*.

Keywords: epithelioid sarcoma, therapeutic target, 2D-DIGE

POS-01-186 Comparative Proteomic Analysis of Extremely Low and Moderate Parasitemic Vivax Malaria for Identification of Potential Early Diagnostic and Prognostic Serum Biomarkers

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Vivax malaria poses considerably huge burden on global health and economy. The present study has been conducted to perform an in-depth comparative proteomic analysis of different clinicopathological parameters and serum proteome profiles of extremely low (LPVM: n = 23) and moderate parasitemic (MPVM: n = 38) *vivax* malaria patients and healthy controls (HC: n = 40) to identify early diagnostic and prognostic protein markers for *P. vivax* infection. Different hematological and liver function parameters were measured in malaria patients (LPVM: parasite count < 200/μL and MPVM: parasite count > 2000/μL) and controls for comparative statistical analysis. Comparative serum proteome profiling was performed using gel-based 2D-DIGE and gel-free iTRAQ techniques. Hemoglobin level and platelet count was found to be significantly lower ($p < 0.01$) in malaria patients (both LPVM and MPVM). Differential proteomic analysis revealed significant modulation of 18 proteins ($p < 0.05$; > 1.5-fold change) in LPVM patients compared to the healthy controls; while 20 serum proteins exhibited differential expression ($p < 0.05$; > 1.5-fold change) between LPVM and MPVM. Differential expression of serum amyloid A, haptoglobin, apolipoprotein A-1, apolipoprotein E, hemopexin, and RBP4 proteins was validated by ELISA. ROC curve analysis demonstrated SAA, Apo E and Haptoglobin as efficient predictor proteins (AUC > 0.80) for low-parasitemic *vivax* malaria detection. These three serum proteins also exhibited good correlation with parasite count (r >0.5; $p < 0.001$). Proteins like SAA and haptoglobin, which exhibited sequential increase with respect to the parasite loads, can be considered as potential disease monitoring/ prognostic markers.

Keywords: Vivax malaria, Prognostic markers

POS-01-187 Calcyclin Levels Determined by High-Throughput SRM in Serum Samples of Pre-Eclampsia Patients

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Pre-eclampsia (PE) is a pregnancy-specific disease that complicates 2-8% of all pregnancies. It is associated with serious perinatal and maternal morbidity and mortality. Endovascular remodeling and invasion of the spiral arteries is impaired which results to reduced and irregular placental perfusion. An SRM assay was developed for quantitative measurements of calcyclin in serum using two stable isotopic labeled peptides. We compared sera obtained from preterm pregnant controls (n=10), not-pregnant controls (n=10), and sera from pre-eclamptic patients (n=10). Trypsin-digested sera were fractionated by SCX followed by SRM using a nanoACQUITY LC equipped with Triazic (150 μm ID) directly connected to a Xevo TQ-S triple quadrupole mass spectrometer. The linearity for both stable isotopic labeled peptides LMEDLDR and LQDAEIAR was determined by creating linear calibration curves with concentration ranges of 0 - 5 fmol/μL to quantify calcyclin levels. The linear correlation coefficients (R^2) for the stable isotopic labeled peptides were ≥ 0.99 with LODs of 0.36 and 0.70 ng/ml serum for both peptides, respectively. The concentration of calcyclin was different between sera from pregnant versus non-pregnant for both peptides at 30 week gestation. Calcyclin did not show at week 34 of gestation a significant difference between preterm pregnant control samples and pre-eclampsia. The average concentration of calcyclin in pre-eclamptic serum was for both peptides 113 ± 104 and 66 ± 74 ng/ml. Preterm pregnant control serum was 212 ± 252 and 150 ± 160 ng/ml for both peptides, respectively, whereas non-pregnant controls showed values of 37 ± 12 and 47 ± 14 ng/ml.

Keywords: pre-eclampsia, SRM, triple quadrupole

POS-01-188 Proteomic Study Discovering Talin 1 Fragment Related to HIV Infection

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The human immunodeficiency virus type 1 (HIV-1) pandemic has continued unabated for nearly 30 years. To better understand the influence of virus on host cells, we performed the differential proteome research of peripheral blood mononuclear cells (PBMCs) from HIV-positive patients and healthy controls. 26 protein spots with more than 1.5-fold difference were detected in two dimensional electrophoresis (2DE) gels. Of which, a fragment of talin 1 (38KDa) was found to be up-regulated in HIV-positive patients. This fragment can be detected after HIV infection for 2 hours to 8 hours. This fragment is negatively related to HIV load and not caused by apoptosis. Further, *in vitro* study showed this fragment could decrease HIV infection. In order to discover the mechanism of talin 1 fragment to HIV infection, we overexpressed talin 1 fragment and knocked down talin 1 in Tzm-b1cell line, followed by proteomic study. In all, in this study, we found talin 1 fragment through proteomic study and the interaction between HIV and talin 1 fragment were intensively study.

Keywords: HIV, proteomics, talin 1

POS-01-189 PlasmaQBaby: Quantification of Plasma Proteins in Preterm Infants Using Heavy Protein Standards and Peptide Group-Specific Immunoenrichment

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The current capacity for monitoring large numbers of plasma proteins from premature babies, born before 37 completed weeks of pregnancy is very limited. Low total blood volumes (typically 50-150 ml), severely restrict the amount of blood that can be sampled, thus rendering the diagnostic methods commonly used for adults inapplicable. As such, information concerning the concentration of even the abundant plasma proteins in preterm infants is scarce, and intensive blood sampling for the repeat analysis of plasma proteins in intensive care impossible.
Quantitative immunoenrichment and mass spectrometry-based screening methods allow highly sensitive and selective protein-detection in plasma droplets. However, these methods are often limited to the detection of a few proteins per analysis at most, preventing fast, cost-efficient quantitative screening for relevant biomarkers.
Here we present the development of a quantitative antibody based immunoenrichtment mass spectrometry strategy for the screening of 50 biomarkers relevant to coagulation, organ maturity, organ integrity, and inflammation status from as little as 5 μl plasma. TXP-antibodies allow the selective enrichment of multiple protein relevant signature peptides by peptide group-specific immunoenrichment. In addition, spiking plasma samples with isotopically labeled recombinant protein standards permits accurate identification and quantification of biomarker-relevant signature peptides.
We will demonstrate the efficacy (dynamic range, LOD, LOQ, reproducibility) of our quantitative immunoenrichtment mass spectrometry workflow for biomarker-screening from 5 μl plasma samples. Additionally, experiments demonstrating the maximum multiplexing capacity of our assay with a view to increasing throughput whilst reducing screening costs will be discussed.

Keywords: heavy labelled protein standards, immunoaffinty MS, clinical assay

POS-01-190 Production and Characterization of a Set of Mouse Monoclonal Anti-Peptide Antibodies Against Selected Oral Cancer Marker Candidates for SISCAPA-MRM and -MALDI Assay Development

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Oral cancer, one of the common cancers in Southern Asia including Taiwan, has become a growing burden of the health care system in this area. Although visual inspection and visually guided biopsy in outpatient clinics can help screen early oral cancer, this experience-based protocol may cause high false-negative results. Currently, there are no any biomarkers approved by official health agents in the endemic areas for aiding oral cancer management. Thus, development of the evidence-based molecular tools, such as non-invasive protein markers, should greatly help detect and manage oral cancer. Although numerous potential oral cancer biomarkers have been discovered in the past decades, very few of them have been verified and validated in parallel to compare their clinical utility. To address this issue, we thought to develop a high-throughput, multiplexed SISCAPA-MRM/MALDI assay for quantifying target peptides from 61 selected biomarker candidates (prioritized from published literatures and our in-house database) in saliva and plasma samples. As the first step of this verification study, we produced and characterized mouse mAbs against 76 peptide antigens. More than 800 clones of mAbs that passed the ELISA-based screening criteria were further evaluated regarding their binding affinity to peptide antigens (using Biacore 3000 SPR system) and immuno-capture capability (using SISCAPA-MS assay). Those well characterized mAbs with acceptable binding affinity and immuno-capture capability to peptide antigens represent valuable reagents for further development of useful SISCAPA-MRM/MALDI assays. Factors and protocols that affect the efficacy and efficiency of producing and characterizing these mAbs are presented and discussed.

Keywords: monoclonal anti-peptide antibodies, oral cancer biomarker, SISCAPA-MRM/MALDI assay

POS-01-191 Validation of Pancreatic Disease Markers in Secretin-Stimulated Duodenal Juice Using Selected Reaction Monitoring

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Introduction CEL-MODY (Carboxyl-ester lipase-Maturity Onset Diabetes of the Young; MODY8) is a monogenetic subtype of diabetes and pancreatic exocrine dysfunction where affected individuals have disease-causing sequence variants in the carboxyl ester lipase (CEL) gene. Using isobaric labelling (TMT, tandem mass tag), we have previously identified a subset of MAPK-signaling related proteins, which were significantly more abundant in secretin-stimulated duodenal juice of selected CEL-MODY patients as compared to controls. The aim of this study is to assess the validity of the identified candidate proteins using selected reaction monitoring (SRM). **Result and Conclusion** First, in order to validate the method of choice we included peptides for several high abundant enzymes in the SRM assay, in particular digestive enzymes from healthy controls. The SRM intensity of these peptides was correlated to enzyme activities as measured by the Phadebas colorimetric assay and provided a strong correlation (R2 value of 0.76 (N=37)), reflecting that SRM was able to robustly quantify these proteins. Next, we validated the upregulated proteins in CEL-MODY patients from the discovery study in 37 patients using SRM. 16 of the proteins were successfully validated and show a clear difference between CEL-MODY and control (p-value< 0.01).

Keywords: SRM, secretin-stimulated, duodenal juice

POS-01-192 A Proteomic Portrait of Colon Cancer-Associated Fibroblasts Identifies Novel Prognostic Markers

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Cancer-associated fibroblasts (CAFs) or myofibroblasts are essential components of the cancer stroma that play a critical role in cancer invasion. This study aimed to identify novel colorectal cancer (CRC) CAFs markers that might contribute to the invasion and the prognosis of CRC. The azoxymethane/dextran sodium sulfate mouse model of sporadic colon cancer represents an adequate source for the isolation of CAFs and normal fibroblasts (NFs). By using the explants technique we isolated and purified enriched populations of CAFs and NFs from colon tissues. Whole cell extracts and supernatants were subjected to in-depth quantitative proteomic analysis by tandem mass spectrometry. Further validations were carried out by chemokine microarray analysis and immunohistochemistry of tissue arrays. Using a fold-change ≥1.4, we found 132 and 119 differentially-expressed proteins. We found CAFs-associated pro-inflammatory and desmoplastic signatures. The pro-inflammatory signature was composed of several cytokines. The desmoplastic signature associated to CAFs was composed of 41 secreted proteins. In human samples, expression of FSTL1 was significantly expressed in the tumoral stroma without significant expression in the cancer epithelial cells. On the other hand, the combination of Calumenin and Cadherin-11 stromal expression showed a significant association to disease-free survival and poor prognosis. Among others, we have identified FSTL1 as a selective biomarker of colon cancer stroma and Calumenin and Cadherin-11 as candidate biomarkers of prognostic significance in colon cancer. The new panel of stromal biomarkers will facilitate future studies with CAFs to study their relevance in cancer progression, invasion and prognosis.

Keywords: cancer-associated fibroblasts, prognostic biomarkers, colon cancer

POS-01-193 Differences in Proteome between Luminal A and Luminal B HER2 Negative Breast Cancers

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Treatment of breast cancer had changed dramatically by discovery of "intrinsic" subtypes using cDNA microarray analyses. Breast oncologists have decided therapeutic strategy depending on the subtype. However, it is still difficult to detect subtype with accuracy. Since subtype classification shows variable results with different microarray analyses and/or immunohistochemistry (IHC), especially in luminal breast cancer, it is considerably difficult for accurate subtyping. The purpose of this study was to clear the qualitative and quantitative differences in proteomes between luminal A and luminal B HER2 negative (n-luminal B) breast cancers. Total 6 patients (3 luminal A and 3 n-luminal B breast cancers) were analyzed. They were pathologically diagnosed as invasive ductal carcinoma and at Stage I/II, and corresponding subtype was determined using IHC: luminal A or n-luminal B was determined based on Ki-67 staining score, and the staining score of estrogen receptor (ER) and other markers were similar in both groups. Samples of surgical specimens were stored as FFPE blocks. The tumor tissues were laser-microdissected, trypsinized, and analyzed by mass spectrometry using an LTQ-Orbitrap XL under similar conditions. Protein identification was done using Mascot against UniProt_human database. Quantitative analysis was performed using Progenesis-LC-MS. Total 1,838 proteins were identified in luminal A and 2,143 proteins in n-luminal B. Qualitative comparison clearly revealed that 6 proteins were unique in luminal A, and 19 proteins in n-luminal B. Label-free quantification analysis showed that 226 peptide features were significantly different (p <0.01) in the two groups, demonstrating that 6 proteins were up-regulated in luminal A, and 21 proteins were up-regulated in n-luminal B. Luminal A and n-luminal B breast cancers have considerable difference in proteome. The difference could explain the diversity in results with microarrays and IHC.

Keywords: luminal breast cancer, label-free quantification, FFPE

POS-01-194 Quantitative Proteomics Characterization of Pancreatic Tumor Tissues: New Insights and Challenges

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Pancreatic cancer is an almost uniformly lethal disease, and has the highest mortality rate of all major solid cancers. Over the past decade, quantitative proteomics offers a wide range of opportunities to investigate malignancy-associated molecular alterations at the protein level and has stimulated great interest in applying the technology to the study of pancreatic cancer. We have applied a variety of quantitative proteomics approaches in order to 1) investigate the molecular events associated with pancreatic cancer development and progression, and 2) develop biomarkers and therapeutic targets for improving diagnosis and treatment. The studies involved clinically relevant, well characterized tissue specimens, including pancreatic tissues from patients with pancreatic ductal adenocarcinoma, pancreatic intraepithelial neoplasia (pre-cancer), chronic pancreatitis and healthy controls. Through these studies, we have discovered protein groups and aberrant glycosylation patterns that were involved in protein-driven interactions between the ductal epithelium and the extracellular matrix. The protein interactions between the epithelium and the surrounding stromal cells and matrix orchestrate tumor growth, migration, angiogenesis, invasion, metastasis, and immunologic escape, underscoring the importance of tumor microenvironment in promoting pancreatic cancer progression. The results have revealed common molecular features shared between pancreatic cancer and chronic pancreatitis - a chronic inflammatory disorder of pancreas. Our studies illustrate the benefits and challenges of current proteomics technology and demonstrate the important role of proteomics, especially quantitative proteomics, in clinical translational research.

Keywords: quantitative proteomics, pancreatic cancer, mass spectrometry

POS-01-195 Generation and Characterization of New Bispecific Antibodies to EphA10 and CD3 as Candidate Drugs Against Breast Cancer

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【Background】 Monoclonal antibodies (mAbs) are clinically used to treat several types of cancer due to their high specificity and potential to activate immune systems. The therapeutic effects of mAbs are unsatisfactory, however, in cases of aggressive cancer or low target-protein expression. Bispecific antibodies (BsAb) are a highly promising form of mAbs. A BsAb that simultaneously targets the antigens on cancer cells and activated T-cells reportedly has the potential to kill cancer cells more effectively than conventional mAbs. Here, to develop a new BsAb drug against breast cancer, we attempted to generate a BsAb targeting both T-cell CD3 antigen and Eph receptor A10 (EphA10), which we have identified by proteome analysis as novel biomarker proteins of breast cancer.

【Methods】 We constructed two types of expression vectors that contain either a 6xHis tag or FLAG, and encode the single chain Fv (scFv) derived from the anti-EphA10 antibody or two different types of anti-CD3 antibodies. Expression of these scFvs in *E.coli* was analyzed by SDS-PAGE and Western blot. Binding activities were evaluated by flow cytometry.

【Results】 The scFvs could all be produced in *E. coli*, but their expression levels differed depending on the type of antibody or tag. The purification efficiency of the scFv with the His tag was higher than that with the FLAG tag. All scFvs showed almost full binding activity compared to conventional mAbs. We are now preparing the two BsAbs (EphA10xCD3) and evaluating their function.

Keywords: bispecific antibody, breast cancer, Eph receptor A10

POS-01-196 Serum Fibrinogen Alpha C-Chain 5.9 kDa Fragment (FIC 5.9) as a Biomarker for Early Detection of Hepatic Fibrosis Related to Hepatitis C Virus

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We previously identified a fragment of the fibrinogen alpha C chain (FIC5.9) as a possible biomarker of excessive drinking by SELDI and MALDI -TOF MS analyses (1, 2). We developed ELISA system for serum FIC5.9 levels (3). In this study, we performed a prospective study in patients with chronic hepatitis C and liver cirrhosis.

A total of 88 serum samples obtained from patients with chronic hepatitis C (stage F1 to F3), liver cirrhosis (F4) and 181 samples of apparently healthy subjects were analyzed. All study participants gave written informed consent. The area under the ROC (AUROC) for FIC5.9 was compared with those of the conventional markers for hepatic fibrosis.

Serum levels of FIC5.9 in healthy subjects were 15.2 ± 3.4 μg/ml (Mean± SD). In contrast, the levels in HCV- related chronic liver diseases with fibrosis stages F1, F2, F3 and F4 (cirrhosis) were 5.6 ± 2.8 μg/ml, 5.6 ± 2.7 μg/ml, 4.9 ± 1.7 μg/ml, 3.6 ± 2.3 μg/ml respectively. In the F1 stage, the AUROC for FIC5.9 was significantly greater than those for the other markers.

Measurement of serum FIC5.9 levels could be an early indicator for detection of hepatic fibrosis in HCV- related chronic liver diseases. This study provides an example of a pipeline from biomarker discovery by proteome analysis to assay optimization and preliminary clinical validation.

(1) Proteomics 2004; 4: 1187-1194 (2). Proteomics Clin Appl. 2009; 3: 821-828 (3). Proteomics Clin Appl. 2013; 4.

Keywords: fibrinogen alpha C chain, Liver disease, biomarker

POS-01-197 A SILAC-Based Approach Defines an Angiotensin II- Regulated Proteome in Primary Human Kidney Cells

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Angiotensin II (AngII), the major effector of the renin-angiotensin system, mediates kidney disease progression by signalling through AT-1 receptor (AT-1R), but there are no specific measures of renal AngII activity. Accordingly, we sought to define an AngII-regulated proteome in primary human proximal tubular cells (PTEC) in order to identify potential markers of AngII activity in the kidney. We utilized stable isotope labelling with amino acids (SILAC) in PTECs to compare proteomes of AngII-treated and control cells. Of 4618 quantified proteins, 83 were differentially regulated in 4 replicates. SILAC ratios for 18 candidates were confirmed by Selected Reaction Monitoring (SRM) assays. Both SILAC and SRM revealed heme oxygenase-1 (HO-1) as the most significantly upregulated protein in response to AngII stimulation. AngII-dependent regulation of HO-1 gene and protein was further confirmed by qRT-PCR and ELISA in PTECs. In order to extend these *in vitro* observations, we overlaid a network of significantly enriched gene ontology (GO) terms from our AngII-regulated proteins with a dataset of differentially expressed kidney genes from AngII-treated wild type mice and AT-1R knock-out mice. Five GO terms were enriched in both datasets and included HO-1. Furthermore, HO-1 kidney expression and urinary excretion were reduced in AngII-treated mice with PTEC-specific AT-1R deletion compared to AngII-treated wild-type mice, thus confirming AT-1R-mediated regulation of HO-1. In summary, our *in vitro* approach identified novel molecular markers of AngII activity and the animal studies demonstrated that these markers are relevant *in vivo*. These interesting proteins hold promise as specific markers of renal AngII activity in patients and in experimental models.

Keywords: renin angiotensin system, proximal tubular kidney cells, SILAC

POS-01-198 Efficient High Throughput Proteomic Workflow for Clinical Biopsy Samples

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Background: For well understanding human disease mechanisms by proteomic approaches, analysis of pathological tissues is very important. Clinical biopsy samples may be obtained at different stages of human diseases. In this study, an efficient high throughput workflow of proteomic analysis for clinical biopsy samples was established by combining two new technics, On-site Direct Digestion (OSDD) and SWATH methods.
Method: From fixed and paraffin embedded human kidney biopsy specimens of IgA nephropathy (IgAN), glomerular sections were collected by laser microdissection (LMD). Peptides were extracted from the glomerular sections by using OSDD method and purified by C18 tip. A data independent MS acquisition method (SWATH) was used to collect information of peptides and their fragments as much as possible within a limited retention time and amount of peptides. OpenSWATH software was employed to analyze all of the SWATH data by searching in an optimized human kidney peptides library.
Results: Targeted proteomic analysis of SWATH data provided over 2800 protein identification hits with mProphet score less than 0.01 (FDR < 1%), using peptides extracted from IgAN glomerulus sections of 10 μm thickness and 1 mm^2 area. A total of 9837 peptides had quantification valves, indicating practical reality of large scale, quantitative analysis. This workflow will be applicable to obtain significant information from clinical biopsy samples in a short period and with low cost, and can be considered as a powerful examination of biopsy samples not only for diagnosis but also for personalized medicine in the future.

Keywords: clinical biopsy, IgA nephopathy, SWATH

POS-01-199 Potential Plasma Markers of Hypertrophic Cardiomyopathy - Targeted Proteomic Analysis of Soluble Fibronectin

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Introduction Hypertrophic cardiomyopathy (HCM) is the most prevalent inherited disease of the myocardium characterized by otherwise unexplained left ventricular hypertrophy. Its phenotypic expression may vary among individuals. Early pathological changes in HCM are associated with impaired collagen metabolism which is closely related to various mediators, where fibronectin (FN) plays crucial role. Selected reaction monitoring (SRM) has become an important method for protein detection and quantification in complex samples with high sensitivity, selectivity and good reproducibility.
Methods Samples: Study group included 17 HCM patients and 17 healthy control subjects with similar age and sex characteristics. **ELISA:** Plasma concentration of soluble fibronectin was determined by commercial ELISA kit. GraphPad Prism software was used for all statistical analyses. **SRM:** Trypsin digested and stable isotope spiked samples were analyzed on QTRAP mass spectrometer. Absolute quantification of FN peptides was performed in Skyline software.
Results: Plasma concentrations of soluble FN assayed by ELISA were designated as significantly different ($p<0.0001$) between HCM and control group. Plasma concentrations of soluble FN measured using SRM assay were significantly different for peptides STTPDITGYR and VDVIPVNLPGEHGQR. Linear correlation of both peptide levels was graphed (r=0.98; $p<0.0001$). Subsequently, linear correlation of ELISA results and individual peptide abundances were calculated (r=0.61; $p<0.0002$). We successfully used the SRM approach for quantification of putative HCM marker. **Innovative aspects** SRM method has been developed for quantification of soluble FN - a new putative marker of HCM.

Acknowledgements Grant of Ministry of Health, Czech Rep., NT/13721 and the European Social Fund and the state budget of the Czech Rep., CZ.1.07/2.3.00/30.0022.
Keywords: fibronectin, biomarker, targeted proteomic analysis

POS-01-200 Biomarkers for Duchenne Muscle Dystrophy

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Duchenne muscular dystrophy (DMD), which is caused by the absence of dystrophin, is the most frequently inherited childhood neuromuscular disease. To extend our breadth of knowledge about the molecular processes involved in DMD disease, the proteomes of primary human skeletal muscle cells from four patients aged between 3 months and 6 years were compared with those of four controls. In the first step for relative comparison of the above samples 2D-PAGE was used. For quantitative analysis iTRAQ-labelling was applied. After cleavage and labelling of tryptic peptides with iTRAQÔ reagents, proteins were analysed by mass spectrometry. In this study, it was found that 101 proteins were differentially expressed in DMD patients compared to controls. Ten proteins: ALDH1A1 desmin, FABP3, AHSG-FetuA, PEBP1, EDPR1, CALD1, SFPQ1, TAGLN and YBX1, showed greater than 5-fold changes in expression as well as age-dependent changes. The upregulation of ALDH1A1 and desmin and the downregulation of FABP3 in 3-month-old and 4-year-old patients seem to indicate a process compensating for muscle loss through high a reconstitution capacity. In contrast, the reversal of these protein levels in a 6-year-old patient appears to mark the initiation of adipogenesis. These three proteins could act as markers indicating the critical turning point from myogenesis to adipogenesis, a step which is important for the success of therapies such as myoblast transplantation.

Keywords: ITRAQ, muscle dystrophy, mass spectrometry, 2D- electrophoresis

POS-01-201 Over-Expressed Calponin3 by Sub-Sonic Vibration Induces Neural Differentiation of hUC-MSCs by Regulating the Ionotropic Glutamate Receptor

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Mechanical vibration has been used for medical purposes; however, little is known regarding the mechanism of these positive effects. In this study, we used proteomics to investigate the effects of sub-sonic vibration (SSV) on mesenchymal stem cells derived from human umbilical cords (hUC-MSCs) during neural differentiation to understand how SSV enhances neural differentiation of hUC-MSCs. We investigated the mRNA levels of genes related to neural differentiation after 3 or 5 days in a group treated with 40 Hz SSV. Differentiation of hUC-MSCs into neuronal cells was further examined through immunofluorescence studies. In addition, protein expression patterns were compared between the control and the 40 Hz SSV-treated hUC-MSC groups via a proteomic approach. Among these proteins, calponin3 (CNN3) was confirmed to have 299% higher expression in the 40 Hz SSV stimulated hUC-MSCs group than that in the control by Western blotting. Notably, over-expression of CNN3-GFP in CHO-K1 cells had positive effects on the stability and reorganization of F-actin compared with that in GFP-transfected cells. Moreover, CNN3 changed the morphology of the cells by making a neurite-like form. After being subjected to SSV, mRNA levels of glutamate receptors such as PSD95, GluR1, and NR1 as well as intracellular calcium levels were up-regulated. These results suggest that the activity of glutamate receptors increased because of CNN3 characteristics. Taken together, these results demonstrate that over-expressed CNN3 during SSV increases expression of glutamate receptors and promotes functional neural differentiation of hUC-MSCs.

Keywords: sub-sonic vibration, hUC-MSC, neural differentiation

POS-01-202 Genome-Wide Protein Parts List of Human Placenta Tissues for the Chromosome-Centric Human Proteome Project

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Initial goals of the Chromosome-Centric Human Proteome Project (C-HPP) are to identify at least one representative protein encoded by each of the approximately 20,300 human genes and to organize these data in accordance with chromosomal gene locations. As a starting point, we have established protein identification, PTM analysis and quantitative analysis strategies using the paired human placenta tissues from healthy and preeclampsia patients. These tissues contain the largest number of genes of any organ, and perform many functions including exchanges of gases, nutrients, and electrolytes. An initial analysis of central chorionic parts of placenta was performed using a high-accurate LTQ-Orbitrap ETD. Here, total of 4,239 unique proteins were identified with high confidence (FDR < 1%) from the initial profiling of human placenta tissues, 71 were identified as chromosome 13-encoded proteins. Additional 78 chromosome 13-encoded proteins were identified from human brain tissues, demonstrating that combining datasets of collaborating teams from different tissues will help identify chromosome 13-encoded proteins (total 149 chromosome 13 encoded proteins). We also identified a total of 219 unique N-linked glycopeptides and 591 unique phosphopeptides. Moreover, we identified 28 proteins exhibiting differentially expressed preeclampsia-specific proteins. Our data represent the highest number of proteins identified in the placenta and will be useful for annotating and mapping all proteins encoded in the human genome. [*This study was supported by a grant from the National Project for Personalized Genomic Medicine (A111218-11 to Y.K.P.), and World Class University (WCU) grant (R31-2008-000-10086-0)*].

Keywords: C-HPP, placenta, preeclampsia

POS-01-203 Systematic Characterization of Human Platelets in Arterial Vascular Disorders by Quantitative Proteomics

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Anti-platelet treatment is of fundamental importance in combatting functions/dysfunction of platelets in the pathogenesis of cardiovascular and inflammatory diseases. Dysfunction of anucleate platelets is likely to be completely attributable to alterations in protein expression patterns and post-translational modifications. Combining elaborate protocols for platelet isolation from fresh blood donations in conjunction with quantitative mass spectrometry, we created the first comprehensive and quantitative proteome of highly pure human platelets, comprising almost 4,000 unique proteins with copy number estimates for ~3,700 of those and relatively quantified ~1,900 proteins between four different healthy donors - with negligible contamination by leukocytes, erythrocytes and plasma, respectively. For the first time, our data allow for a systematic and weighted appraisal of protein networks and pathways in human platelets, and indicate the feasibility of differential and comprehensive proteome analysis from small blood donations. Since 85% of the platelet proteome show no variation between healthy donors, this study represents the starting point for disease-driven platelet proteomics. These findings allow for correlation to genome-wide association studies which identified in a retrospective manner a set of chromosomal regions affecting the risk of cardiovascular diseases. While respective gene products could be identified in platelets, a comprehensive and quantitative comparison of protein patterns between patients and relevant controls such as relatives and spouses to validate risk factors is still missing. In order to improve cardiovascular risk management, genomic and proteomic analyses of respective corresponding gene loci and proteins using next generation sequencing and targeted MS strategies are applied with the final goal to characterize valuable biomarkers for biomedical screenings.

Keywords: platelets, quantitative proteomics, posttranslational modification

POS-01-204 Application of Quantitative Proteomic Analysis Using Tandem Mass Tags for Discovery and Identification of Novel Biomarkers in Periodontal Disease

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Periodontal disease is characterized by destruction of hard tissue and soft connective tissue constituents of the periodontium. As a fluid lying in close proximity to periodontal tissue, gingival crevicular fluid (GCF) is the principal target in biomarker search for periodontal diseases. Analysis of biochemical markers in GCF, which predict the progression of periodontal disease, may facilitate disease diagnosis. However, no useful GCF biochemical markers with high sensitivity for detecting periodontal disease have been identified. Thus, the search for biochemical markers of periodontal disease is of continued interest in experimental and clinical periodontal disease research. Using tandem mass tag (TMT) labeling, we analyzed GCF samples from healthy subjects and patients with periodontal disease, and identified a total of 619 GCF proteins based on proteomic analysis. Of these, we focused on two proteins, MMP-9 and LCN2, which are involved in the progression of periodontal disease. Western blot analysis revealed that the levels of MMP-9 and LCN2 were significantly higher in patients with periodontal disease than in healthy subjects. In addition, ELISA also detected significantly higher levels of LCN2 in patients with periodontal disease than in healthy subjects. Thus, LC-MS/MS analyses of GCF using TMT labeling led to the identification of LCN2, which may be a promising GCF biomarker for the detection of periodontal disease.

Keywords: periodontal disease, gingival crevicular fluid

POS-01-205 Analysis of Amniotic Fluid Proteome and Peptidome in PPROM Pregnancies with Inflammatory Complications

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Introduction

Preterm prelabor rupture of membranes (PPROM) complicated by microbial invasion of the amniotic cavity followed by histological chorioamnionitis (MIAC/HCA) is responsible for serious neonatal morbidity. Hence, reliable markers are required for improving clinical management of such pregnancies. Our recent proteomic study has revealed large number of amniotic fluid proteins potentially associated with MIAC/HCA, such as myeloperoxidase, lipocalin-2, and several distinct proteases. Dysregulation in proteolytic enzymes due to MIAC/HCA suggests that characterization of amniotic fluid peptidome may reveal molecules with diagnostic potential in MIAC/HCA too. In the present work, we sought to validate our pilot findings regarding myeloperoxidase and lipocalin-2. In addition, our effort was to highlight the alterations of amniotic fluid peptidome due to MIAC/HCA.

Method

ELISA assays for myeloperoxidase and lipocalin-2 were used to assess their levels in prospective PPROM cohorts. The enrichment of amniotic fluid native peptides was performed using denaturing ultrafiltration. The protocol performance was characterized by means of SDS electrophoresis and LC-MALDI analysis.

Results

Altered abundance of myeloperoxidase ($p<0.001$) and lipocalin-2 ($p=0.008$) was successfully validated in prospective PPROM cohort. Initial LC-MALDI experiment revealed differences in peptidome between MIAC/HCA positive and negative samples. A pilot iTRAQ LC-MS/MS study to create a list of potential MIAC/HCA associated peptide markers is under way. The most auspicious findings will be confirmed using LC-SRM.

Acknowledgement

This work was supported by Internal Grant Agency of Ministry of Health of the Czech Republic (NT/13599). Travel costs were covered by Long-Term Organization Development Plan 1011.

Keywords: MIAC/HCA, amniotic fluid, peptide markers

POS-01-207 A Proteomics Approach to the Identification of Biomarkers for Psoriasis Utilising Keratome Biopsy

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The treatment of psoriasis and other inflammatory skin disorders still poses a challenge for the medical profession. Plasma biomarkers for such disorders may aid disease grading and monitoring of treatment response. We have developed a proteomics/mass spectrometry based workflow which enables the discovery of biomarkers and we describe its application to the investigation of psoriasis vulgaris. Keratome epidermal biopsy was used as a sampling method, which results in a reduced cellular complexity compared to those gathered by standard punch biopsy of the whole skin (epidermis and dermis). Furthermore, we implemented a short term culturing approach to access a "secretome" from the keratome, rich in proteins which have the potential to drain into plasma. Combining these two sample preparation techniques we obtain a sub-proteome which is both reflective of the disease and enriched in potential plasma biomarkers.We performed a quantitative proteomics screen of four patients using stable isotope dimethyl labelling, identifying over 50 proteins consistently altered in abundance in psoriasis skin. This includes canonical psoriasis related proteins (e.g. S100A7 [Psoriasin] and FABP5 [Epidermal Fatty Acid Binding Protein]).Additionally, more than 30 novel alterations in protein abundance were discovered. From this disease localised dataset we assessed several proteins as a potential biomarkers in the plasma of patients with psoriasis and healthy controls utilising selected reaction monitoring mass spectrometry (SRM-MS/MS).

Keywords: psoriasis, SRM, biomarker

POS-01-206 Proteomic Analysis of Cerebrospinal Fluid Gives Insight Into the Pain Relief Through Spinal Cord Stimulation

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Neuropathic pain is caused by a lesion or disease of the somatosensory nervous system affecting approximately 6% of the population and 17-52% of all diagnosed pain patients. Current pharmacological treatments are ineffective for more than 50 % of the patients and often give adverse effects. Spinal cord stimulation (SCS) is an alternative cost-effective treatment with high efficacy, prolonged pain relief, few side effects and minimized risk of drug abuse. We have compared the cerebrospinal fluid (CSF) proteomes from neuropathic pain patients during pain relief induced by SCS and during pain sensation to gain further insights into the mechanisms behind the obtained analgesia.

Paired CSF samples were taken from SCS-responsive neuropathic pain patients after the SCS had been turned off for 48 hours and when the SCS had been used normally for three weeks. Thus, each patient acted as their own control. The corresponding proteomes for each patient were relatively quantified using a mass spectrometry based shotgun approach. In total, 419 unique proteins were simultaneously identified and relatively quantified. A panel consisting of seven proteins, 5 up-regulated and 2 down, were found to be significantly regulated by SCS in two complementary statistical tests (P<0.01). The most up-regulated protein in the SCS linked panel is a known modulator of nicotinic acetylcholine (ACh) receptor activity. Interestingly, it has a striking tertiary structural similarity and biological functionality as pain modulating neurotoxins found in snake venoms. Our findings reveal possible insights into the mechanism of spinal cord stimulation and the obtained pain relief.

Keywords: neuropathic pain, cerebrospinal fluid, mass spectrometry

POS-01-208 Discovery, Development, and Verification of Fatty Acid Binding Protein 5 as a Serological Marker of Crohns Disease

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BACKGROUND: Crohn's Disease (CD) is a chronic intestinal inflammation that affects a heterogeneous population. We report novel stepwise proteomic studies in developing a CD biomarker candidate from discovery to verification in case-control and longitudinal cohorts.

METHODS: CD, ulcerative colitis (UC), rheumatoid arthritis (RA), and healthy subjects were recruited from Concord and Bankstown Hospitals, Australia. A global low-mass (≤25kDa) serum proteome analysis was performed using in-solution electrophoresis and an LTQ Orbitrap. Multiple Reaction Monitoring (MRM) quantitation of a biomarker candidate was performed on a 4000 QTrap.

RESULTS: 79 CD, 25 UC, 13 RA, and 13 healthy serums were analysed. 231 proteins exhibited abundance patterns that correlated with aggressive CD by global analysis (ANOVA P≤0.05, FDR q≤0.01). Of note, Fatty Acid Binding Protein 5 (FABP5) was increased in aggressive CD (4709.1 ± 1645.8 ion intensity) compared to less aggressive (1324.1 ± 452) (P<0.01, q<0.01). By MRM, A FABP5 proteotypic peptide (FABP5P) was increased in aggressive CD (152797 ± 118954 peak area) compared to less aggressive (400.3 ± 315.2, P<0.01), UC (24174 ± 934.9, P=0.05), RA (1613.4 ± 3066.1, P<0.01), and healthy (2493.4 ± 2524.0, P<0.01). A CD and UC patient were followed longitudinally. FABP5P was increased in the CD patient from moderate disease (14734.6 ± 9952.0) to severe (63544.6 ± 27883.5) (Paired T-test P=0.01).

CONCLUSIONS: Verifying biomarker candidates in clinically relevant groups is essential before using isotopic labels. We preliminarily demonstrate correlation of serum FABP5 with CD. FABP5 is an endothelial protein that may be shed from the intestinal barrier during active CD.

Keywords: biomarker, inflammatory bowel disease, quantitative proteomics

POS-01-209 Separation of Mycobacterial Cell Wall Extracts Responsible for the Treatment of Bladder Tumor

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Bacillus Calmette-Guerin (BCG) application is gold standard treatment for some type of bladder tumors. However, lethal side effects of BCG limits its usage. We aimed to to find less toxic and more potent therapeutic agents which can be used instead of BCG. Experiments with *Mycobacterium phlei* and *Mycobacterium smegmatis* indicates that cell wall components cause activation of tumor necrosis factor-alpha (TNF-alpha) and interleukin-12 (IL-12). We investigated the cell wall extracts of several nonpathogenic mycobacteria strains for TNF-alpha and IL-12 stimulating activity. *Mycobacterium brumae* extracts which showed significant immunostimulating activity were analyzed further with HPLC. Bacteria were sonicated after heat treatment. The supernatants were fractionated by successive C18 and SCX columns. Protein and sugar concentrations of the fractions were measured, and immunostimulating activities were determined by ELISA technique. The MALDI-TOF/MS spectra of the fractions were also taken. As a result a ca. 1800 Da component and a ca. 3600 Da component were detected as candidates for the immunostimulating activity. These molecules have a potential for development of new drugs for the treatment of superficial bladder carcinoma.
This study was supported by Hacettepe University Research Unit, (Project no: HUBAB 03G31) and the Ministry of Industry and Trade through the Scientific and Technical Research Council of Turkey -TUBITAK- (Project No. SBAG-SANTEZ-5-105S361).
Keywords: mycobacterium cell wall, immunostimulating activity, bladder tumor

POS-01-210 Identification of Biomarkers Related to Capacetibine Response in Solid Tumors by Nucleic Acids Programmable Protein Arrays (NAPPA), iFISH, and SNPs Approaches

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Biomarkers, particularly those with strong positive and negative predictive value, have many potential uses in diagnosis and treatment of cancer, including monitoring treatment success, indicating disease progression and detecting early disease. Antibodies to tumor antigens have been detected as early as several years before the clinical appearance of cancer. Although the specificity for these responses is high, typically only <20%of patients demonstrate a response to any given antigen, which has limited the usefulness of single antigen responses as biomarkers. The recent development of protein microarrays may offer an ideal tool for screening for immune response to tumor antigens. These arrays offer the advantage that hundreds to thousands of different proteins can be printed and screened simultaneously and only require a few microliters of serum per assay. A novel method for producing protein microarrays, called Nucleic Acids Programmable Protein Array, has been developed. Here, we propose adapting the NAPPA protein microarray technology for use in the rapid and efficient screening of sera from cancer patients for antibodies to 6000 known and potential tumor antigens in a multiplex format in order to better characterize and identify new biomarkers related to drug treatment. For this purpose, patient's sampels pre- and post- chemotherapy have been included. A set of novel biomarkers suggest that approximately 30% of the patienst show resistance against chemotherapy pre-surgery, as it has been reported previously. For the validation of the possible biomarkers found in 20 different patients (pre- & postchemotherapy), currently we are using iFISH and SNPs aproaches with the main goal to correlate genomics and functional proteomics.
Keywords: biomarker discovery, protein arrays, tumor antigens, personalized medicine

POS-01-211 Human Proteome Project in Cancer

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The central theme of B/D-HPP is the analysis of molecular networks as most complex diseases are not the result of a single protein/gene but rather the consequence of a multitude of factors that contribute incrementally to the etiology of disease. Overall, the B/D-HPP attempts to generate and disseminate the assays and resources to support the analysis of biological networks underlying biological processes and disease.
The use of emerging mass spectrometry (MS)-based platforms such as selected reaction monitoring (SRM) or targeted data extraction for candidate proteins from SWATH MS data has become an increasingly popular method for quantitative analysis of target proteins. It has been shown that the use of synthetic peptide standards and isotope dilution makes identification and accurate quantitation of proteins in a multiple laboratories possible. Therefore, the assays, once developed, can be easily transferred and used in other laboratories. However, unlike the development and the ability to make antibodies available, the development of MS-based assays is limited to a specific cancer type in each laboratory, the efforts are redundant in different laboratories and cancer type-specific information is not considered. Acceptance of high throughput MS assays for proteins or protein modifications has been limited due to the difficulty in establishing assays and the availability of the assays comparing to using traditional antibody based assays, here we propose an international effort to target cancer proteins in each cancer type. By working together, we can create a synergistic effort to work with a list of cancer protein targets, develop assays, and make assays available. We further discuss the procedure to accrue a list of target proteins from each cancer type, the strategy for assay development, quality control, and procedure and materials needed for transferring the established assays to a new laboratory.
Keywords: cancer

POS-01-212 Detection of Target Antigens for Anti-Endothelial Cell Antibodies in Patients with Kawasaki Disease

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Purpose: We tried to detect target antigens for anti-endothelial cell antibodies (AECA) comprehensively by proteomics and investigate clinical importance of them in patients with Kawasaki disease (KD). **Methods:** We separated proteins extracted from HUVEC and HeLa cells respectively by 2-dimensional electrophoresis (2DE) and then transferred them onto nitrocellulose membranes. By WB using serum samples from vasculitis patients, we detected antigens that were positive only in the HUVEC samples but not in the HeLa cell samples. We next identified the detected proteins by peptide mass finger-printing and characterized antigenicity by preparing recombinant antigens and antibodies to them. **Results:** 150 protein spots were detected specifically in 2DE-WB. 63 of the 150 protein spots recovered from 2DE gel were determined successfully. These included some proteins relevant to vasculitis such as annexins. In addition, previously reported target antigens for AECA such as heat shock proteins were also identified. One of the identified 63 proteins was found peroxiredoxin2 (Prx2), an anti-oxidative enzyme. IgG antibodies to Prx2 were detected in 60% (18/30) of the patients with KD, but not in controls. WB using cell lysate proved expression of Prx2 not only in HUVEC but also in other endothelial cells (ECs). The anti-Prx2 antibodies increased various inflammatory cytokine secretion significantly, such as IL-6, G-CSF and MCP-1. Anti-Prx2 antibodies also induced increased expression of adhesion molecule on ECs. **Conclusion:** IgG antibodies to Prx2 would be a useful marker for KD. Proteomic surveillance is an effective way to identify target antigens for AECA.
Keywords: anti-endothelial cell antibodies, Kawasaki disease, Peroxiredoxin2

POS-01-213 A Fast, Robust, and Reproducible Proteomic Solution to Convert Human Tissue into a Digital Data Set for Targeted Data Analysis

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Fast, quantitatively accurate, deep and reproducible proteomic analysis of complex biological samples remains a major hurdle in systems biology and systems medicine. Here, we propose a method to convert the proteome contained in small (biopsy) cell or tissue sample into a digital data set that contains signature fragment ion patterns for all peptides derived from the sample. These datasets can be then perpetually mined in silico. In essence the method converts the physical proteome in a (clinical) sample into a permanent digital representation. The method is built on pressure-cycling technology and SWATH-MS. The entire workflow can be completed within 12 hours. Subsequent targeted data analysis offers unprecedented performance in identifying and quantifying low-abundance signaling protein networks in human tissues in an extendable manner. We have applied this method to a kidney cancer cohort, and uncovered dis-regulated signaling protein networks.

Keywords: cancer biopsy tissue, SWATH-MS, pressure-cycling technology

POS-01-214 Towards the Use of Mass Spectrometric Immunoassay (MSIA) in Protein-Based Diagnostics

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Molecular diagnostics requires standardized and rigorously analytical technologies. Regarding proteins, such technologies must be; 1) Highly accurate, sensitive and reproducible, 2) Responsive to large concentration differences and disease-specific qualitative variations, 3) Employed at rates sufficient to economically accommodate large clinical sample sets, and 4) Sustainable into the indefinite future. Here discuss one such technology, mass spectrometric immunoassay (MSIA), which over the past 15-years has matured to the point of meeting these specifications. During this talk, we foremost recognize that qualitative differences in proteins that are detrimental to conventional assays are resolved with MSIA, and ultimately form the basis of molecular diagnostics based on disease-related protein microheterogeneity. Additionally, we will find that such mass spectrometry-based assays yield analytical metrics exceeding those of conventional clinical platforms, are capable of multiplexing for multiple proteins and can be operated at throughputs and prices points comparable with existing diagnostics platforms. The application of MSIA-based multi-analyte biosignatures used inside the healthy - type 2 diabetes - cardiovascular disease continuum will be used to illustrate.

Keywords: diabetes, immunoaffinity, proteoforms

POS-01-215 Protein C Inhibitor Proteotypic Peptide Quantitation by LC-Free SISCAPA-MALDI Mass Spectrometry Predicts Recurrence of Prostate Cancer After Radiotherapy

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Biomarker validation remains one of the most important constraints to development of new clinical assays. To address this challenge, we recently reported on the development of a SISCAPA-MALDI assay, which allows high throughput mass spectrometric quantitation of protein analytes in large sample sets. Here we describe the application of this assay to measure a surrogate peptide from protein C inhibitor (PCI) in sera from 51 prostate cancer patients to verify the use of the surrogate peptide as a biomarker for monitoring prostate cancer. A 2-plex SISCAPA-MALDI assay was developed for quantitation of surrogate peptides from PCI and soluble transferrin receptor (sTR) and was applied to 159 trypsin-digested sera collected from 51 prostate cancer patients. Corresponding Stable Isotope Standard peptides were produced with a label resulting in a mass shift of +10 Da, allowing quantitation using mass spectrometry. Patients who experienced biochemical recurrence of prostate cancer after treatment with androgenic hormones/radiation showed decreased levels of the PCI analyte within 18 months of treatment. Levels remained high in the plasma of patients who did not experience cancer recurrence. Prostate specific antigen (PSA) levels had no predictive value in the same time-period. Results from the study show that the high-throughput, LC-free SISCAPA-MALDI assay is capable of analyzing peptide analytes in clinical samples with accuracy and in a short period of time. The lower limit of detection for PCI in the assay was found to be 1 fmol, which is 150 times lower than the endogenous level of PCI in 10 μL of plasma. The CV at the endogenous concentration was 3.8%. Adoption of this assay will allow systematic, rapid validation of putative protein biomarkers using large sample sets. An even much larger cohort (perhaps more than 1000 patients) will be required to unequivocally validate the PCI analyte for clinical use.

Keywords: SISCAPA, MALDI-TOF, biomarker validation

POS-01-216 A Platform for Multiplexed Quantitative Measurements of Low-Abundant Proteins in an Assay Format by Combining Recombinant Single-Chain Antibody Fragments (scFv) with LC-SRM-MS

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Quantitative measurements of specifically targeted proteins in complex samples such as serum or plasma remain a tremendous challenge. The large-scale mapping of proteins using proteomic technologies has opened up for biomarker discovery. The potential of a protein as a functional biomarker needs to be reproducibly measured and verified in large sample cohorts and preferably in an easily accessible body fluid such as blood. This calls for high-capacity technologies allowing high-throughput measurements with high sensitivity and specificity. Immunoaffinity enrichment combined with mass spectrometry has proven successfully through the SISCAPA workflow. We have developed a novel immunoaffinity enrichment method denoted AFFIRM that combines protein enrichment from complex samples using single-chain antibody fragments (scFv) with selected reaction monitoring (SRM) mass spectrometry readout for sensitive and quantitative protein analysis. This automated and optimized platform allows multiplexed measurements in parallel in a 96-well format. Three proteins, Keratin19, BRCA1 and MUC1, were chosen as targets for a proof-of-concept study. Capture experiments were performed with individual scFv as well as in a multiplexed fashion targeting all three proteins in one analysis generating linear response curves for all. We are now expanding the number of targets and epitopes targeted. This integrated platform holds the capacity for large-scale parallel quantitative measurements of target proteins in complex samples with high sensitive and specificity.

POS-01-217 Disease Proteomics Reveals Modulation of Cell Microenvironment Due to Loss of Collagen VII

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Recessive dystrophic epidermolysis bullosa (RDEB), a genetic skin blistering disorder, was used as a model for identification of hitherto unknown proteins involved in its pathogenesis by a disease proteomics approach. RDEB is caused by loss of function mutations in the gene encoding collagen VII, a dermal-epidermal adhesion protein. A strategy using SILAC-based quantitative mass spectrometry, combined with bioinformatics data processing, was developed to assess quantitative differences in the microenvironment of skin fibroblasts derived from normal and pathologically altered skin. This global, unbiased approach revealed unanticipated differences in the extracellular matrix (ECM) proteome of RDEB fibroblasts compared to that of normal dermal fibroblasts. The loss of collagen VII was associated with the reduction of important basement membrane proteins, like collagen IV, nidogen-1/2 and laminin γ-1 and presumably intensifies the skin fragility. On the other hand, dermal matrix proteins like tenascin-C, thrombospondins and interstitial collagens were enriched in RDEB ECM, contributing to the extensive scarring seen in patients. Interestingly, although proteases are enriched in the RDEB ECM they are less active. This is likely due to abolished lysosomal turn over, inhibition by respective protease inhibitors and/or proteolytic processing of the active protease. Taken together, this comparative quantitative mass spectrometry-based proteomics study between RDEB ECM and normal ECM provides insights into the composition of the microenvironment of fibroblasts and shows how the loss of one particular ECM protein, collagen VII, leads to the remodeling of the complex extracellular meshwork, promoting negative concomitants of RDEB. These findings offer novel concepts for future therapies.

Keywords: disease proteomics, extracellular matrix, primary dermal human fibroblast

POS-01-218 Rapid Discrimination between Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus* Using MALDI-TOF Mass Spectrometry

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens responsible for nosocomial infection. The presence of MRSA in a hospital is very detrimental to patients and to hospital management. Thus, rapid identification of MRSA is needed. This study performed a prospective study of rapid discrimination of MSSA from MRSA using the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) system. We evaluated 305 clinical isolates of *S. aureus* using the MALDI-TOF MS system and support vector machine. The predictive model was trained using 100 *S. aureus* isolates (50 MSSA and 50 MRSA). The identification rates were 90.2% for MSSA and 78.6% for MRSA following the 10-fold cross-validation SVM. In the blind test sets, 205 *S. aureus* isolates (95 MSSA and 110 MRSA) were correctly classified. The identification rates were 95.8% for MSSA and 81.8% for MRSA. In conclusion, the method proposed in this study using a predictive model enables detection from one colony in 5 minutes, and thus is useful at clinical sites at which rapid discrimination of MRSA from MSSA is required.

Keywords: rapid discrimination, MSSA, MRSA, MALDI-TOF MS

POS-01-219 Mechanisms of Virulence in MSSA/MRSA *Staphylococcus aureus* Clinical Isolates

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S. aureus causes severe infection even if it is mainly a harmless bacterium. It exists in a oxacillin-resistant (meticillin-resistant *S.* aureus, MRSA) form that represents the most important cause of antibiotic-resistant healthcare-associated infections worldwide. Most MRSA strains contain Panton-Valentine leukocidin (PVL) genes, that encode for a citotoxin that is one of the essential components of the virulence mechanisms of *S. aureus*. Furthermore, the presence of PVL is also associated with increased virulence of certain strains (isolates) of *S. aureus*. Although clinicians are currently concerned primarily with MRSA infections, methicillin-susceptible *S. aureus* (MSSA) infections can be present with similar epidemiologic and clinical characteristics when are PVL positive. In this study, proteomics has been used to compare protein profiles of (PVL+) and (PVL-) MRSA and MSSA *S. aureus* in order to highlight protein differences related to PVL presence or absence. Bacterial samples were isolated from human infections and classified on the basis of PCR analysis and antibiotic susceptibility test. Quantitative two dimensional electrophoresis was performed on MSSA and MRSA samples further classified in PVL+ and PVL-. Experiments were done in triplicate using custom IPG strips pH 4-5.5 to optimize spot resolution and to visualize different isoforms on gels. Image and statistical analysis were performed with Progenesis SameSpots software (Nonlinear Dynamics), proteins differentially expressed (ANOVA $p<0.05$) were identified by MALDI-TOF/TOF for identification. Results indicated new efforts about mechanism virulence of MSSA with interesting findings about high expression of Alchol dehydrogenase in MSSA that can explain the high incidence of this strain in nosocomial context due to the ability of this pathogen to degrade alchool based disinfectants

Keywords: microbial proteomics, staphylococcus aureus, antibiotic resistance

POS-01-220 Analysis of HER2 Graded Breast Cancer FFPE Tissue Samples with Reverse Phase Protein Arrays

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Reverse-phase protein arrays (RPPA) is a sensitive tool for detection of proteins from cell lysates and body fluids. As the current standard method for tissue preservation in hospitals is formalin-fixation and paraffin-embedding (FFPE) it would be highly desirable if RPPA could also be applied to FFPE tissue. Two independent sample sets (19 and 27 patients) of FFPE breast cancer tissue samples were analyzed through RPPA for the expression level of human epidermal growth factor receptor 2 (HER2) and compared to the gold standard used in clinical practice, immunohistochemistry (IHC). We hypothesized that a successful recovery of HER2 from FFPE tissue should result in concordant HER2 classification between RPPA and IHC. The Qproteome FFPE Tissue Kit was used for the extraction of proteins from the breast cancer samples. RPPA technology was previously described (Pawlak M, 2002). The relationship between IHC HER2 score and RPPA HER2 signal intensity was modeled using ordinal multinomial logistic regression (OMLR). Here, it aims to predict the probability of association of a sample to be assigned to HER2 score based on RPPA fluorescence. The RPPA fluorescence for HER2 increases with higher IHC HER2 score. By assigning each sample the most probable HER2 score based on OMLR model, the concordance between IHC and RPPA was estimated in both sample sets as highly significant. Both sample sets show a good assignment of RPPA signals to IHC scores 2+ and 3+ whereas distinguishing between IHC scores 0 and 1+ by RPPA is error-prone. For validation, the HER2 scores of samples of set B were predicted based on the model parameters estimated in the set A and using the RPPA intensities of the set B. The concordance between prediction and IHC was highly significant. We demonstrated that HER2 can be extracted from FFPE tissue samples with high recovery and that the RPPA analysis of these samples delivers biologically meaningful results.

Keywords: RPPA, FFPE, breast cancer

POS-01-221 Phosphoproteomics of Human Liver Cancer Analyzed by 2-Dimensional Image-Converted Analysis of Liquid Chromatography and Mass Spectrometry (2DICAL)

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Two-dimensional image-converted analysis of liquid chromatography and mass spectrometry (2DICAL), which we developed originally, is a proteomic analysis system that compares individual peptide peaks of multiple samples and selects significant peptide peaks with statistical analyses in a label-free method. We applied 2DICAL in analyses of the phosphoproteomics of human liver cancer to search for specific phosphorylation changes in human liver cancer.
106 samples were collected from patients with liver cancer in an ethically approved process. Deep-frozen raw tissue (1mg) was fixed in 100% methanol and trypsinized in 1% sodium deoxycholate solution. Phosphopeptides were extracted by the HAMMOC method and desalted. Liquid chromotography-mass spectrometry and tandem mass spectrometry (MS-MS) spectra were acquired by Triple-TOF 5600. LC-MS-MS data (n = 212) were analyzed by 2DICAL, and the peptide peaks with fragment expectation below 0.05 by Mascot analysis were statistically analyzed in liver cancer and noncancerous liver tissue. The significant phosphopeptide peaks were selected and applied to the informatics investigation.
In the detected 44,990 peptide, 2,390 were phosphopeptides with fragment expectations below 0.05 by Mascot analysis. In the differential analysis between the liver cancer and noncancerous liver tissues, 173 phosphopeptides were increased more than two-fold and 145 were decreased by less than half in liver cancer tissue compared to noncancerous liver tissue. The altered phosphopeptides were referred to the derived proteins and classified by the Gene Ontology terms. There were several phosphopeptides that were not reported to change in human liver cancer. They were considered to be not only candidate biomarkers but also potential therapeutic targets for liver cancer. A phosphoproteomics analysis of 106 human liver tissues by 2DICAL revealed specific and systemic phosphorylation changes in liver cancer.
Keywords: 2DICAL, liver cancer, phosphoproteomics

POS-01-222 Synthesis of Galactose-Deficient IgA1 O-glycans by GalNAc-transferases: Implications for the Pathogenesis of IgA Nephropathy

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IgA1 with galactose (Gal)-deficient hinge-region (HR) *O*-glycans (Gd-IgA1) plays a key role in the pathogenesis of IgA nephropathy (IgAN). IgA1 HR has up to 6 of the 9 potential *O*-glycosylation sites occupied; some Gal-deficient glycans consist of terminal *N*-acetylgalactosamine (GalNAc). IgA1-producing cells derived from IgAN patients secrete more IgA1 with Gal-deficient *O*-glycans and higher content of GalNAc compared to IgA1 from cells of healthy controls (HC). IgA1 *O*-glycosylation is thought to be initiated by GalNAc-T2, but the expression of GalNAc-T2 does not differ between the cells from IgAN patients and HC. In contrast, expression of GalNAc-T14, a GalNAc-T with high structural similarity to GalNAc-T2, is elevated in the cells from patients. To determine potential contribution of these enzymes to Gd-IgA1 formation, we analyzed kinetics and site-specificities of GalNAc-T2 and -T14 for IgA1 HR using high-resolution mass spectrometry. A synthetic IgA1 HR peptide (sHR) and a panel of synthetic IgA1 HR glycopeptides (sGP) with a single GalNAc residue at different sites were used as acceptors. GalNAc-T2 had higher activity, *i.e.*, faster rate of glycosylation of sHR, than did GalNAc-T14. The sites of glycosylation in sHR catalyzed by GalNAc-T2 and -T14 were the same for the variants with up to 5 sites and appeared in a predominantly ordered fashion. Localization of GalNAc on sGP did not affect the kinetics of GalNAc-T2, but GalNAc-T14 more effectively glycosylated the sGP variant with a GalNAc at S9. In summary, GalNAc-T2 and -T14 have similar site-specificity for IgA1 HR, but differ in kinetics and in how their activity is affected by preexisting glycosylation. We speculate that the elevated expression of GalNAc-T14 could contribute to the production of Gd-IgA1 in IgAN.
Keywords: IgA nephropathy, IgA1 glycosylation, O-glycopeptides

POS-01-223 Population Based Omics ? Identification of Genetic Variants Influencing the Human Plasma Proteome

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Genetic variants influencing the transcriptome have been studied extensively. However, the impact of the genetic factors on protein expression and the proteome is largely unexplored, partly due to lack of suitable high-throughput quantitative methods. Here we present a unique set of identifications of genetic variants affecting the human plasma proteome achieved by combining label-free high-resolution LC-MS with genome-wide SNP data. We quantified 1,056 tryptic peptides representing 163 proteins in the plasma of 1,060 individuals from two population-based cohorts. The abundance level of one-fifth (19%) of the peptides was found to be heritable, with heritability 0.08-0.43. The levels of 60 peptides from 25 proteins were influenced by cis-acting SNPs. We identified and replicated individual cis-acting SNPs influencing 11 peptides from 5 individual proteins. These SNPs represent both regulatory SNPs and non-synonymous changes defining well-studied disease alleles such as the e4 allele of APOE, which has been shown to increase risk of Alzheimer's disease. In this study, all statistical analyses were performed on the peptide measurements rather than aggregate or derived protein abundances. The peptides directly represent the MS measurements and better capture the protein heterogeneity in the populations. The results show that label-free LC-MS is a viable alternative to SRM, especially for the few hundred most abundant proteins. The composition of the proteome play an important role in the etiology, diagnosis, and treatment of a number of diseases, and a better understanding of the genetic influences on the proteome is important for evaluating potential biomarkers and therapeutic agents for common diseases.
Keywords: population, plasma proteome, genome

POS-01-224 Quantitative Mass Spectrometry (SRM/MRM) to Amyloid Peptides, Tau Protein, and Apolipoprotein E in Human Cerebrospinal Fluid for Alzheimer Disease Diagnosis

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BACKGROUND: Recent improvements in mass spectrometry (MS) allow this technology to quantify with clinical grade analytical sensitivity and specificity, peptides and proteins in biological fluids. We believe that in some cases MS will represent a valuable alternative to immunodetection methods. We followed this path for biomarkers in Alzheimer disease (AD) which represents major cause of dementia. AD is associated with specific apolipoprotein E (ApoE) isoforms, and with alteration of cerebrospinal fluid (CSF) biomarkers. As a matter of fact, the decrease of amyloid peptides (Aß) and the increase of Tau proteins in CSF are currently use for AD diagnosis. Many isoforms of these molecules exist and MS represent an interesting tool to quantify their diversity, and therefore, to improve AD diagnosis and follow-up.
METHODS: For this purpose, quantitative targeted mass spectrometry (SRM/MRM) was developed using a triple quadripole. SPE, trypsic digestion and sample clean-up were realised using an automated liquid handling robot. Quantotypic peptides (Aß1-40, Aß1-42, tau, ApoE..) were synthetized in light and heavy (13C/15N) versions and used in calibration curve to evaluate LOD and LOQ. Experiments were run on series of human biological samples.
RESULTS: Optimal MRM methods for the different analytes were developed. Detection of specific Apo E peptides resulted in a rapid method for e2/e3/e4 phenotyping. Different isoforms of Aß and Tau proteins were detected with sensibility compatible with pathophysiological variations. Correlation with immunodetection methods and validation of the clinical relevance of the results are on-going.
CONCLUSIONS: The MS detection of several isoforms of Aß, Tau protein, and Apo E in CSF represents an important achievement that opens new avenue for quantitative Clinical Chemistry Proteomics (qCCP). The perspective is to exploit these results to improve phenotyping, diagnosis and follow-up of dementia.
Keywords: quantitative clinical mass spectrometry, neurodegenerative diseases, Alzheimer

POS-01-225 Quantitative Target Proteomics-Based Personalized Molecular Target Chemotherapy for Recurrent Brain Tumor

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Malignant brain tumors are aggressive and incurable, and tend to recur even after complete surgical resection and subsequent radiation therapy. Therefore chemotherapy is important for the treatment of malignant brain tumors. Molecular target drugs usually interact with membrane protein for providing their effects, suggesting protein, rather than gene, level of target molecule is suitable indicator for predicting their efficacy. The purpose of this study was to clarify the protein expression levels of membrane proteins, which are the target for anti-tumor drugs, in brain tumors by quantitative target proteomics (QTAP), and then personalized molecular target chemotherapy was applied for the malignant brain tumor according to the results of protein expression. The case presented here is anaplastic meningioma, which recurred repeatedly and disseminated in the short period. The residual survival time was anticipated to be within 1 month. QTAP analysis of surgical specimen was performed for 11 receptors and detected high expression of PDGFRβ. This finding was confirmed by immunohistochemistry with PDGFRβ antibody. According to the result, sunitinib, which inhibits multiple receptor tyrosine kinases including PDGFRβ, was administrated to the patient. MRI showed remarkable effect against the tumors for 10 weeks. After 2 cycles, treatment was terminated due to side effect, and patient died due to the tumor invasion into the midbrain. This result suggests that QTAP-based personalized molecular target chemotherapy is promising treatment for the malignant brain tumors.

Keywords: brain tumor, molecular target drug, quantitative proteomics

POS-01-226 Absolute Quantitative Analysis of Human Tear Fluid Proteome Using Wheat Germ Cell-Free Protein Synthesis System and Quantitative Mass Spectrometry

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Mass spectrometry (MS) based proteomic approach has been applied to discover protein biomarkers of eye diseases in human tear fluid and recent studies revealed the presence of over 1,000 tear proteins; however our understanding of the absolute protein expression profile in tear fluid is still premature. In this study, we have established an absolute quantitative platform of human tear fluid proteome using selected reaction monitoring (SRM) MS and wheat germ cell-free protein synthesis system. We initially selected 815 proteotypic peptides for 417 tear proteins and designed the sequences of 76 QConCAT genes, which are concatamers of selected proteotypic peptides. Stable isotope-labeled QConCAT proteins that can be used as an internal standard for absolute quantitative analysis using MS were synthesized by wheat germ cell-free system. Combining synthesized QConCAT proteins and SRM assay, we have conducted an absolute quantitative measurement of targeted proteins in normal tear fluid sample. After in-gel tryptic digestion of gel-separated tear proteins, extracted tryptic peptides were mixed with internal standard peptides derived from stable isotope-labeled QConCAT standards and subjected to LC-SRM analysis. Using established quantitative platform, we have so far estimated the absolute amount of the targeted 130 proteins from a small amount of tear fluid sample (5 μg total proteins) and successfully revealed the age-dependent alterations of their expression profiles.

Keywords: Tear fluid proteome, absolute protein quantification, selected reaction monitoring

POS-01-227 Development of an LC-MRM Based Assay of Prostate Specific Antigen (PSA) in Blood Samples

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Introduction Quantitative determination of Prostate Specific Antigen (PSA) in the human serum has been used for the early diagnostics and prognostics of prostate cancer. Most of the existing methods utilize enzyme-linked immunosorbent assay (ELISA) to measure the PSA in the serum. The ELISA method, while providing good sensitivity and high throughput for the PSA diagnostics, suffers from low specificity. There is a great deal of interest to find a more reliable diagnostic and prognostic method. In this paper we reported a method to quantify PSA in serum using a simple sample preparation followed by the LC-MS/MS analysis operated in multiple reaction monitoring (MRM) mode. The MRM method provides high sensitivity, high accuracy and good specificity. **Methods** PSA standard was trypsinized and analyzed for peptide identification on an ion trap mass spectrometer initially. The obtained MS/MS data was searched using Mascot software. Two signature peptides from PSA standard were selected for MRM analysis. The MRM method and ion source parameters were further optimized for sensitivity and interference, and linear responses. For serum samples, high abundant proteins such as albumin were removed by solid phase extraction (SPE) to reduce the matrix interference. After trypsin digestion, the PSA peptides were analyzed with the MRM method with external calibration. **Preliminary results** An initial MS/MS study on an ion trap mass spectrometer was carried out on the tryptic digested PSA standard. Based on the high relative abundance and spectra quality and other reported exclusion criteria, two peptides were chosen with their MRM transitions for further optimization. For PSA standard, the LOQ (S/N>10) of the developed MRM assay are at pg/mL level. For PSA in serum after the removal of high abundance protein, the LOQ is at high pg/mL level, sufficient for clinical diagnosis purpose.

Keywords: clinical diagnostics, MRM triple quadrupole, biomarker

POS-01-228 Proteome Analysis of Exhaled Breath Condensate for Medical Diagnostics

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Exhaled breath condensate (EBC) attracts significant attention as a potential object for non-invasive diagnostics of pulmonary diseases and the object of fundamental investigations of human physiology and pathology by modern analytical methods. The analysis of the protein composition of exhaled breath to diagnose diseases of the respiratory system raises a problem of differentiation proteins expressed in the tissues of the lungs and respiratory tract (endogenous) and proteins penetrated in the respiratory system from the ambient air in the process of respiration (exogenous). An analytical platform for EBC proteome analysis was developed. Different methods for EBC collection were compared. To estimate the contribution of exhaled exogenic proteins to the whole EBC proteome we analyzed protein composition of EBC from healthy donors subjected long term (six-month) isolation in the ground-based facility with cleaned air. We found that isolation conditions leads to removal the set of keratins from EBC that are considered therefore to be exogenic. Non-keratin proteins may also circulate between the ambient air and human respiratory ways, but their concentration appears to be significantly lower the keratin concentrations (especially epidermis keratin). Among non-keratins dermcidin seems to be the most significant exogenic protein of exhaled air. We demonstrate the possibility of analysis of EBC proteome and correlation between protein profile in EBC and different diseases and pathologies (pneumonia, lung chronic obstructive disease, lung cancer).

Keywords: exhaled breath condensate, pulmonary diseases, lungs and respiratory tract

POS-01-229 Plasma Protein Quantification from Dried Blood Spots Using Stable Isotope Labelled Protein Standards

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The quantification of blood proteins has long been established as a routine tool for *in vitro* clinical diagnostics. The analysis of plasma proteins from Dried Blood Spots (DBS) has attracted lots of interest, as such samples are relatively easy to acquire, needing only minimal training, and require only tiny sample volumes. Furthermore the DBS matrix affords stabilising effects for many of the analytes. The power of combining DBS sampling with the multiplexing capabilities of mass spectrometry based quantification has been demonstrated through the successful quantification of 37 plasma proteins using stable isotope labelled peptide standards following protein digestion (Chambers, A.G. *et. al*. (2012) *J. Mol.Cell.Prot., 12,781-791)*. Although a very powerful technique, potential quantification errors can arise through variations in peptide recovery due to incomplete sample digestion for example. We established a method for the high throughput generation and quantification of recombinant isotope labelled plasma protein standards which can be added to samples prior to digestion and workup. These standards were subsequently incorporated into an immunoaffinity mass spectrometry based multiplex assay using group specific anti-peptide TXP antibodies for the parallel determination of multiple protein biomarkers from Dried Blood Spots. The results from different Dried Blood Spot sample preparation techniques will be evaluated by comparison with solution based digestion techniques for biomarker quantification and discussed. The maturation of such techniques could eventually enable population wide personalized medicine as samples can be acquired by the patients within their own homes as their transport is considered exempt from dangerous goods regulations.

Keywords: dried blood spot, group specific immunoaffinity enrichment, heavy labelled protein standards

POS-01-230 Urine Profiling of Patients with Preeclampsia

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Preeclampsia is a common cause of fetal and maternal morbidity and mortality, complicating 3-6% of pregnancies in the Russian Federation. Preeclampsia, as a pregnancy-specific hypertensive disorder, is defined as new-onset elevated blood pressure accompanied by proteinuria after 20 weeks of gestation. Since delivery of the fetus remains the only proven way preventing negative outcomes, it is important to discriminate preeclampsia from other conditions characterized by hypertension and proteinuria, such as glomerulonephropathies and chronic hypertension. Thus, discovery of relevant biomarkers allowing accurate prediction and/or confirmation of this pathologic condition remains crucial. In this work, an attempt was made reliable reproducible method for extraction of urine peptide constitution of patients with preeclampsia. The most reproducible results were obtained using combination of strong anion-exchange sorbent beads (QAE-Sephadex A-25) with desalting on Strata C18E SPE tubes (Phenomenex). This procedure allows us to extract both soluble urine peptide fraction and peptides adsorbed on carrier proteins (in particular, albumin). Among the peptides detected in urine SERPINA-1 and albumin fragments were the major constituents. Further comparative studies for a statistically significant sample of donors (control group and patients with preeclampsia) are required to find the fragments (or misfolded forms) of SERPINA-1 in urine characteristic for preeclampsia and create a panel of biomarkers specific or sensitive enough to be used routinely in clinical practice.

Keywords: preeclampsia, urine profiling, peptide extraction

POS-01-231 An Integrated Proteomics for Extracting Molecular Target of Malignant Gliomas

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Comprehensive analyses for studying disease related cellular signals to be clinically targeted had been difficult due to limitations in analytical software, biological databases and validation standards. We have developed an integrated analysis system using iTRAQ and 2D-DIGE methods, as well as DNA array, and following a sequential proteomic strategy that includes a proteome tool called MANGO (*Mol Cell Proteomics* 2009, 8(10):2350-67, *ASBMB Today* Aug 2009) and iPEACH (integrated Protein Expression Analysis Chart; PTC/JP2011/58366, *Mol Cell Proteomics* 2013 in press) to integrate voluminous information from several types of analysis into a useful data file that provides comprehensive proteome data including post-translational modification, transcriptomic data, and functional annotations from several databases. Using iPEACH, patient sample data were integrated and stored in a database. In this study, we constructed an iPEACH database for chemotherapy resistant glioma including glioma stem cells, and used GO and knowledge-based network analyses by KeyMolnet targeting upregulated signals to extract a novel candidate signal network for chemotherapy resistance. An unique signal consisting of TRK-cdc42-PAK-CSS-Vim-TRK activation loop was extracted. Statistical analysis of the expression or modification of these proteins in patient tissues and glioma (stem) cells and subsequent biological validation *in vitro* as well as *in vivo* using animal models by sequential analyses using small interfering RNAs (siRNAs), inhibitors, and the anti-cancer drug successfully identified protein targets of the network most likely to be involved in tumor cell chemotherapy resistance. This is the first report of the identification and targeting of signal networks related to chemotherapy sensitivity/resistance in gliomas by our new integrated proteomics strategy. *Mol Cell Proteomics* 2013, *PLoS ONE* 2013 in press

Keywords: integrated proteomics, glioma, chemotherapy

POS-01-232 Comparative Study of Global Protein Turnover in Tissues and Cell Lines

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Proteome is extremely dynamic and exists in constant flux. Most of current proteomics studies present one particular state of proteome at given time and given conditions and do not describe the level of protein synthesis and degradation. Here we performed the comparative analysis of rates of protein turnover from these different approaches from different tissues and cell lines using ProteinCenter software.

We have performed comparative analysis of rates of protein turnover determined by different approaches and different tissues and cell lines followed by bioinformatics statistical analysis by means of Gene Ontology, participation in KEGG pathways and others.

We have noticed that proteins with similar turnover rates share similar Gene Ontology distribution. Interestingly, over-representative analysis was able to detect high specificity for gene ontology categories. For example proteins with low protein turnover rates in HeLa cell line were highly enriched for antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent and negative regulation of cyclin-dependent protein kinase activity. Such an ability to filter and cluster data allows highlighting an interesting group of proteins for further analysis. This study is validating the effectiveness of data analysis strategies in protein turnover determination. Comparison of rates of protein turnover between different tissues, cell lines as well as comparison with alternative non-mass spectrometric methods based on large-scale measurements of mRNA levels will be also presented.

Keywords: protein turn over

POS-01-233 Shotgun Proteomics Data Analysis Using Personalized Protein Sequence Database Built from RNA-seq Results from the Same Sample

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Results from data analysis of shotgun proteomics depend on the protein sequence database searched for LC-MS/MS spectra. With the availability of faster and cheaper next generation sequencing, a personalized protein database can be made readily available. We expect such a sample-specific protein sequence database to provide proteomic level evidence more specific to each individual, if the shotgun proteomics data obtained from the same individual is searched against the personalized database.
Given a paired data sets of RNA-seq and shotgun proteomics from the same matched samples of cancer and normal tissues, we first construct a personalized protein sequence database consisting of expressed proteins and variant peptides. Expressed proteins are derived from mapping RNA-seq reads from each sample to the reference genome and calculating expression levels of each transcript. Unexpressed or low-abundance transcripts are excluded from the expressed proteins, therefore, the size of personalized database is much smaller than a reference protein database such as UniProt.
Variant peptides are generated by considering all SNVs in coding regions. When an SNV is called for a stop codon, translation is extended beyond the original termination position, only until the next stop codon is found and while read counts at the translated positions are sufficiently high.
Blind modification search using MODa was conducted with both personalized databases and Uniprot. Our results show that the search against personalized database is more effective while it is much smaller than Uniprot. In addition, the personalized database search identified new post-translational modification sites, dentified only in tumor samples.
Keywords: modification, NGS, database

POS-01-234 Proteomic Quantitative Pathway Analysis Aided Potential Target Mining of Alkaloids from Herbal Medicine

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Alkaloids are chemical compounds that are naturally occurred in many plants, bacteria, fungi and animals. Some alkaloids and/or their derivatives had been thoroughly studied about their pharmaceutical properties and underlining mechanisms, were being used as FDA approved anti-cancer drug. For example, topotecan and irinotecan the two derivatives of camptothecin found in Camptotheca (Camptotheca acuminate) are being used for cancer chemotherapy. Some of the alkaloids found in traditional Chinese herbal medicine had drawn enough attention from researchers around the world, for example, berberine found in coptis rhizome (huang lian) while some of them are less thoroughly explored. Those less explored alkaloids can be potential anti-cancer drugs in the future if the drug efficacies and mechanisms of drug action are known. Herein, we demonstrated the ability to use mass spectrometry based proteomics expression data for quantitative pathway analysis to aid finding potential drug targets of alkaloids from herbal medicine. Three types of cancer cell lines (HepG2, SKOV-3, AGS) were treated with each of the three selected alkaloids (camptothecin, corydaline and capsaicin) for 1hour, 6 hours and 48 hours. Treated and control cells were lysed, digested and subjected to LC-MS/MS analysis using LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, USA). The subsequent proteomics data was processed and analyzed with MaxQuant (Max-Planck Institute of Biochemistry). The quantitative proteomics data was then uploaded to ExPlain™ 3.1 (BioBase) for signaling pathway analysis and searching of potential drug targets. The method was first validated using a known system, camptothecin treated system. The validated procedures were then applied to other alkaloids.
Keywords: proteomic quantitative pathway analysis, alkaloids

POS-01-235 Optimal FDR Threshold Surfaces for Combined Search Engine Identification Strategies

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In proteomics, control of false positives in peptide identification is indispensable. Predominantly, either decoy or parametric methods are used to estimate negative and positive search score distributions, rendering a false discovery rate (FDR) for the used identification procedure applied on a particular dataset and peptide search space. This estimate results in a score threshold value, below which peptide spectrum matches (PSMs) are rejected or, otherwise, accepted. A search engine score attributing higher scores specifically to correct PSMs, will improve protein identification.
A more specific search score can be constructed by combining several search engines. In this case, the score of a PSM changes from a scalar value to a multidimensional score space point. Analogously, a score threshold value dividing PSMs into accepted and rejected identifications at a corresponding FDR, for several search engines, becomes a surface dividing the space of PSM scores into accepted and rejected regions.
We argue that previous approaches use ad hoc threshold surfaces that are suboptimal, and do not take full advantage of multiple search engines. Once positive and negative score distributions have been estimated, either using database or parametric methods, optimal threshold surfaces can be derived directly from these distributions. These threshold surfaces are optimal in the sense that for each corresponding FDR, the largest possible number of PSMs are passed as correct, leading to a larger number of peptide identifications. The practical utility of this method will be explored using public and in-house datasets.
Keywords: peptide identification, multiple search engines

POS-01-236 A Novel Approach for Processing LC-Ion Mobility-MS Metabolomics Data

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MS interfaced with LC and ion mobility (IM) is routinely used to measure the level and variation of metabolites within biofluids as data generated through metabolomics studies may yield insight into disease onset and progression. LC-IM-MS based metabolomics generates large and complex data sets with analysis and interpretation of the results being the rate determining steps. This has led to a demand for improved data analysis, including processing and advanced multivariate approaches, which are described for the large scale analysis of metabolomics datasets.
Urine from a healthy individual was centrifuged and the supernatant diluted. The urine was divided into control, low dosed (LD) and high dosed (HD) groups. To create a sample set, 11 different drugs were differentially spiked into LD and HD urine, contrasted with blank urine. A reversed phase gradient was applied using a HSS T3, 2.1 x 100 mm, 1.8 μm ACQUITY column and MS data acquired on a SYNAPT G2 operated in positive mode using (HD)MS^E acquisition(s).
Distinguishing biological variation and metabolic change from analytical interference is key to data processing and analysis. Samples were randomized and measured six times, including QC runs, to ensure statistically valid analysis. LC-IM-MS data were retention time aligned and deconvoluted to produce a feature list. Identified features were compound searched and interrogated with multivariate statistics to provide marker ions of interest. Relative high abundance levels of the standards were reported for LD and HD compared to controls, confirmed by trend plots analysis showing an increase in LD and HD groups compared to control. The standards were identified with an average score of 91 and mass error of 1.2 ppm. Three sample clusters were produced with the standards being the most differentiating features (top 20 based on q value) between groups. Functionality of the software will be demonstrated using biological samples.
Keywords: metabolomics, informatics - data processing, ion mobility

POS-01-237 iPEACH: Integrated Protein/Gene Expression Analysis CHart

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The appearance of new "omics" enables comprehensive analysis of biological samples, however, there are many technological limitations. For example, in trranscriptomics, a gene which any probe is not assigned to can not be measured by DNA microarray. In LC-shotgun proteomics, the number of analyzable peptides in one experiment is restricted, and in 2D-gel based proteomics, the analyzable proteins are limited and the analysis throughput is very low, although detailed protein status such as splicing variants and modifications can be revealed. To understand the biological system as a whole, it is obvious that each individual limitation must be effectively complemented by combining different types of omics data. We recently developed the iPEACH (integrated Protein/gene Expression Analysis CHart) system, which provides an easy way to integrate the data from transcriptomics and proetomics. The system gets input in three types of files: CEL files from Affymetrix DNA microarray, Protein and Peptide Summary files from the ProteinPilot software for iTRAQ; and xml files from the Decyder software for 2D-DIGE, and outputs iPEACH_Index followed by the sorting of iPEACH_Index descending order. In addition, iPEACH adds annotations from UniProt to the identified gene/protein, which are useful for further functional analysis such as GO/network analysis. iPEACH is implemented by the Ruby programming language, and the R statistical package for DNA microarray analysis and the MySQL database for storage of annotations are called inside the system. iPEACH is an useful tool for the extraction of specific molecules to be focused and contribute to understand the biological systems [1].

[1] Hirayama *et al*., Integrated proteomics identified novel activation of dynein IC2_GR-COX-1 signaling in NF1 disease model cells. *Mol. Cell. Proteomics, in press,* doi:10.1074/mcp.M112.024802.

Keywords: iPEACH, data integration, data processing workflow

POS-01-238 Finding Representatives in and for Protein Classes

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When the Venter Institute organized the Sorcerer II Oceanic Metagenomics Expedition in 2003 this led to a data treasure of roughly 2 million hitherto unknown protein sequences of unknown functionality from which we hope to uncover proteins with possible benefits in medical/industrial research and production.

At this time we are aiming at two goals. First to find a sensible method of ordering the sequences into families of hopefully similar functionality. So far this is done using tools and methods like Blast or MCL. Second, given such families, we endeavor to find suitable representative proteins for actual synthesis and specific complementation studies and biochemical assays to assign functions. So far this is done using tools like Mafft. On our poster we present this and our progress made so far.

Keywords: classification, metagenomics, function

POS-01-239 CAPER 2.0: An Extensible, Configurable and Interactive Workflow-Based Platform to Mine the Knowledge from the Chromosome-Assembled Human Proteome

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Current proteomic research approaches have generated a great amount of heterogeneous datasets. Mining knowledge from these datasets brings significant challenges. To fit the requirement of data visualization, we have developed a chromosome-centric visualization strategy to effectively integrate, organize, and analyze proteomic datasets [J Proteome Res. 2013; 12:179]. This browser allows users to locate and visualize chromosome regions using intuitive graphical interfaces. However, more sophisticated analyses (e.g., "analyze the function of protein located in chromosome 1") still need their programming skills. To solve this problem we developed CAPER into the higher version-an online knowledge mining system. CAPER will present a powerful toolbox, together with a configurable workflow system to combine these software tools, supporting workflow running, editing, sharing and viewing. These features allow users to conduct independent queries on proteomic data from multiple sources and use software in the toolshed to combine, analyze and visualize them by simple interface. These updates will facilitate the analyses and annotations of proteomic data and also the generation of documentations for analytical report, sharing and publication.

Keywords: bioinformatics, Human Proteome Project, knowledge mining and visualization

POS-01-240 New Functionality for the Trans-Proteomic Pipeline: Tools for the Analysis of Proteomics Data

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High throughput LC-MS/MS is capable of simultaneously identifying and quantifying thousands of proteins in a complex sample; however, consistent and objective analysis of large datasets is challenging and time-consuming. Over the past ten years, we have continually developed and provided improvements to the Trans-Proteomic Pipeline (TPP), an open source suite of tools that facilitates and standardizes such analysis. The TPP includes software tools for MS data representation, MS data visualization, peptide identification and validation, protein identification, quantification, and annotation, data storage and mining, all with biological inference. We present an overview of the TPP and describe newly available functionality.

We recently introduced several new features in the TPP: Support for COMET and MSGF+ results, and enhanced support for OMSSA, Myrimatch, and InsPecT, including a utility that fixes the pepXML result files. We include support for exporting TPP results into the mzIdent PSI format. We have improved the various scripts that assist in launching the TPP on clusters and on the cloud. We added a new decoy database generating utility. Many updates to the user interfaces, including a 'dashboard' view of all models derived by the various TPP tools and an updated spectrum viewer, among others. Various security patches, bug fixes, and overall enhancements to the user interfaces.

All of the TPP software tools are available for download under an open source software license at tools.proteomecenter.org, and can be installed on including Microsoft Windows, UNIX/Linux, and MacOS X. Free email support for the installation and operation of these tools is also available through a popular, community supported listserver, as is a searchable knowledge base.

Keywords: PeptideProphet, ProteinProphet, iProphet

POS-01-241 Development of Web-Based Repository System for Mass Spectrometry Data and Equipment with Links to Chromosome-Centric Human Proteome Database

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Repositories for mass spectrometry data have been progressed for cooperative proteome research after mzML ver1.1.0 was released from HUPO PSI in 2009. We started developing our original repository system for mass spectrometry (MS) data in 2012 as an open source software system. The repository system has been developed as a PostgreSQL-PHP-Apache system for supporting web interfaces, and further equipped with links to chromosome-centric human proteome database for Human Proteome Project (HPP). We developed the system to store MS data files in mzXML or mzML format, which were exported from various mass spectrometers. It has been also able to register username, date of creation, date of registration, data ID, names of identified protein and notes as the supplementary information of mass spectrometry data. The mass spectrum stored in the system could be shown on the browser in arbitrary magnification. The m/z value, the difference of m/z values and the peak area pointed by mouse click on the mass spectrum could be calculated. The system also locally keeps the proteome database in xml format, which was downloaded from neXtProt release Jan 2013. An application to search the chromosomal locations by keywords as protein names etc. has been developed in the system. The mass spectrometry data in the repository has been linked to the related publications and the URL references to Uniprot, PeptideAtlas, 2-D gel databases etc. We have further improved the in-house repository system to register supplementary information released from HPP. We expect that our repository will be a useful system for proteomic research performed using mass spectrometers.

Keywords: mass spectrometry, repository, HPP

POS-01-242 PCDq and H-EPD: Human Protein Complex Database and Human Protein Sequence Database Developed to Research Uncharacterized Human Proteins

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H-Invitational Database (H-InvDB; http://hinv.jp/) is an integrated database of all human genes and transcripts based on comprehensive annotation of a largest collection of human full-length cDNAs and mRNA sequences in DDBJ/EMBL/GenBank. By comparing H-Inv proteins (release 8.0 extended) with UniProtKB/Swiss-Prot and RefSeq protein, a total of 14,323 proteins were common among the three databases, while 17,363 proteins were unique to H-InvDB. To discover the function of uncharacterized human proteins or identify novel human protein, we newly developed two proteomic databases based on H-Inv proteins. The first is a human protein complex database with a complex quality index (PCDq). We integrated six PPI data (BIND, DIP, MINT, HPRD, IntAct, and GNP_Y2H), and predicted human protein complexes by finding densely connected regions in the PPI networks. They were curated with the literature so that missing proteins were complemented and some complexes were merged, resulting in 1,264 complexes comprising 9,268 proteins with 32,198 PPIs. We identified 78 hypothetical proteins that were annotated as subunits of 82 complexes, which included known complexes. Of these hypothetical proteins, after our prediction had been made, four were reported to be actual subunits of the assigned protein complexes. PCDq is freely available at http://h-invitational.jp/hinv/pcdq/. The second is H-Inv Extended Protein Database (H-EPD), that provided cDNA based protein sequence data set of H-InvDB. H-EPD was designed as a reference database for human proteome research using mass-spectrometry. H-EPD is freely available at http://hinv.jp/hinv/h-epd/. Furthermore, we will push forward with the integration of genome, transcriptome, and proteome databases using a unique tool for connecting distributed databases and would like to develop a knowledge discovery system by incorporating data mining tools.

Keywords: protein-protein interaction, protein sequence database, proteome

POS-01-243 Integrating ENCODE in the Spanish Human Proteome Project: A Bioinformatics Approach

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We propose a bioinformatics workflow as one of the approaches for the integration of ENCODE (Encyclopedia of DNA Elements) in the Chromosome-centric Human Proteome Project (C-HPP) using the Spanish Chromosome 16 (Sp-HPP) as a case study. The workflow consists in three consecutive phases. First, we do a transcriptome analysis (RNA-seq) of those cell lines in ENCODE that were selected for shutgun proteomics in Sp-HPP. During this phase, a complete transcriptomic map across cell lines of the Chr-16 is created. Data from gene expression microarrays data either obtained in house or mined in GEO database will also be integrated at this point to complement and complete the analysis. In a second stage, we also identify novel RNA transcripts and isoforms that can translate into putative protein products. These proteins can potentially be sequenced and quantified by mass spectrometry and mapped back to their encoding transcripts. In the final stage of this proposal, we focus in understanding the regulatory elements of Chr-16 by combining Chip-seq and small RNA-seq information provided by ENCODE. Initial focus is on Chr-16 but networks will be extended genome-wide. Preliminary results center their attention on those coding proteins that have not been detected by any experimental procedure according to the criteria established by the HPP consortium (missing proteins).

Keywords: bioinformatics, ENCODE, Transcriptomics

POS-01-244 An Automated Workflow for Enterprise-Level High-Throughput Proteomics Analysis

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Large scale LC-MS/MS proteomic experiments requires a significant informatics ability with the capability to organize, process and track multiple large datasets to enable many users to interact and summarize their data. Large scale proteomics analysis systems entailed a complex symphony of data organization and execution of multiple programs operating from the command line. The traditional approach for both simplifying and automating this process and has been to develop workflow systems based on graphical "pipelines" such as Taverna, TOPPAS, and Galaxy. These software systems use an abstract representation of an analysis in the form of a directed graph and provide a visual front end that users use to build a workflow by connecting a series of programs (tasks) which serve to direct data flow and dependencies. The workflows are then run in the provided execution engine.

While such workflow systems employed by users that have little or no programming expertise, these workflow pipelines have had little adoption by experienced bioinformaticians who still prefer the humble command line. To unify the two approaches, we present an alternate computing pipeline system for proteomics analysis that is based on "Make", the ubiquitous software utility that has been widely used for over 25 years to automatically build programs and libraries from source code. Utilizing its target-dependency rule syntax with associated commands, any analysis can be easily defined in a single file that is simply invoked. Thus it provides a straightforward approach that is robust, cross-platform, parallelizable, and reproducible.

This comprehensive workflow utility is made available as open source and released as part of the Trans-Proteomic Pipeline. It provides a simple, robust, and reproducible approach to managing the analysis of multiple proteomic data sets.

Keywords: bioinformatics, proteomics, scientific workflow

POS-01-245 Development of "Proteome Tools" - A Web Application Suite for Proteomics Experiments

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Data processing is an essential stage for proteomic analyses, though many fields in proteomics are still underdeveloped in software development. However, typical analysis program operation procedures are often too complex for users, requiring cumbersome operation steps to exchange data with spreadsheet software such as Excel, which experimental scientists are most familiar with.
We therefore began to develop a web application suite: "Proteome Tools." While several excellent web-based applications for proteomics such as ProteinProspector are already available, our intended purpose is "experiment support," as our tools are designed for users to easily and practically confirm their experimental information. Our tools to date include:

- Obtaining the Gene Symbol and Gene Ontology (GO) terms by querying the sequence database ID
- Obtaining the sequence ID of a protein containing the query sequence using short amino acid sequences as the query
- Computing the m/z value of the derived peptide by inputting the amino acid sequence and the digestion enzyme used

Inputting data into these tools require a simple "copy & paste" by users from multiple cells in an Excel sheet column. Results are outputted as CSV (comma-separated values) file, which can be opened directly in Excel.
We are currently developing new tools for proteomics-related biochemical experiments. The first tool is a "time-series timer," which can be used for a series of routine works; e.g. adding a reagent into multiple series of samples at set time intervals series. This tool runs on web environment in PC and iOS, and includes a countdown function with pre-recorded voice.
Keywords: web application, experiment support, utility

POS-01-246 Identification and Label-Free Quantitation of Mass Spectrometric Data Via Freely Available Plug-In Software, Mass++

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Mass++ is a plug-in style visualization and multi-purposed analytical tool for mass spectrometry. Mass++ has core libraries for accessing raw data and program components called plug-in. This structure allows us to customize and develop new applications. Mass++ has already contained several plug-ins, which support various data formats and have multiple functions such as viewers and algorithms. Since Mass++ can be used not only as a visualization/analytical tool but also a plug-in execution platform, users who have programming skills can add new functions by implementing plug-ins, writing scripts or utilizing other functions, but without editing the source code of Mass++.
We have improved identification and quantitation tools. A conventional method begins with extracting peaks, saves them in a text file and then posts it to a search engine; hence it is quite time-consuming. On the other hand, Mass++ can directly post peak lists and parameters to a certain search engine, such as Mascot, X! Tandem or MassBank, which are linked with Mass++, and searched results are stored in Mass++ internal database and can be displayed in the viewer of Mass++.
In addition, Mass++ provides quantitation data of peaks and can also manage quantitation results using a "peak matrix"; where its row represents each peak and its column represents each sample. The quantitation results are also stored in the Mass++ internal database and linked to corresponding identification results. Hence, peaks related to target substances can be easily found in original mass spectrometric data. Mass++ can be freely downloaded from the following website: http://www.first-ms3d.jp/english/achievement/software/mass2.
Keywords: identification, quantitation, label free

POS-01-247 PRIDE-Q: Providing a Condensed and Quality-Scored View of Public MS Proteomics Data in the PRIDE Database

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The PRIDE (PRoteomics IDEntifications) database (http://www.ebi.ac.uk/pride) at the European Bioinformatics Institute (Cambridge, UK) is one of the main public repositories for MS-based proteomics data. In PRIDE, the amount of MS-based proteomics data is constantly growing. Aside from the data management challenge, it is also very challenging to decide which of the peptide and protein identifications are reliable.
PRIDE-Q is a new resource providing a condensed and quality scored view of PRIDE data. Data quality assessment is performed at different levels: experiment (based on metadata annotation), protein, peptide and peptide-spectrum match (PSM). At the PSM level, two quality metrics are used at present: 'PRIDE-Cluster'(using a spectral clustering approach [1]) and the 'peptide score' (a score independent from the search engine used [2]). This information is then propagated to the peptide and protein level by using a set of rules. All the peptides are mapped to a recent release of the UniProtKB/Swiss-Prot database. The first beta version of PRIDE-Q is now available for four species *via* a rich and interactive web application (http://wwwdev.ebi.ac.uk/pride/prideq). Future updates will periodically and iteratively update the data content and quality defining metrics of PRIDE-Q.
[1]. J. Griss *et al.*, *Nat Methods*, 10(2):95-6, 2013.
[2]. S.A. Beausoleil *et al.*, *Nat Biotechnol.* 10:1285-92, 2006.
Keywords: MS proteomics, databases, quality assessment

POS-01-248 Unrestricted Modification Search in Formalin-Fixed Paraffin-Embedded Samples Using MS/MS Spectrum Library

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FFPE tissue is being considered as an alternative to frozen samples due to its stability and comparable protein information storage to frozen tissue. However, the details of the possible protein cross-links induced by formalin fixation and the chemical modifications still remains unclear which would hinder extracting protein/peptide information thoroughly from FFPE sample search. Here, we sought to characterize the chemical modifications occurred in formalin fixation by unrestricted spectrum comparison between FFPE and frozen samples. Firstly, trypsin-digested FFPE and frozen samples were prepared using laser microdissection (LMD) and on-site direct digestion (OSDD) before LC-MS/MS analysis. Secondly, spectrum libraries were created after peptide identification by meta-search engine PepArML. Thirdly, FFPE spectra were compared with frozen spectrum library (-260 to +260 Da for mass shift window) using an open modification search (OMS) tool, QuickMod, to investigate the possible modifications in formalin-fixed samples. Peptide identification indicated that around 80 % of peptides (FDR < 0.01) from both samples were overlapped which is in consistence with the clustering analysis suggesting high similarity of spectral data between both samples. So far, QuickMod results implicate that FFPE spectral data obtained by OSDD method might be quite close to those from frozen samples though a few of mass shifts on amino acids were significantly detected including L (14.04, -61.94, 122.02), V (14.02, 217.12), A (15.99), G (15.99), Y (16.00, 90.09), D (-25.97, -61.94), Q (-25.97), S (-25.97). Surprisingly, well-reported cross-link reactions derived from Schiff base (-CH_2, mass shift 14) on primary amines of amino acid residues were not significantly detected here. Our study suggested that comparable spectral data and protein/peptide identifications could be possibly obtained from FFPE and frozen tissues.
Keywords: modifications in FFPE tissue, open modification search, spectrum library

POS-01-249 Cross-Organism Comparison of the Uniqueness in Protein Terminal Region Sequences

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Post-translational modification is deeply related to protein function. Drastic modifications such as signal peptide and propeptide removals are well known to occur in protein terminal regions; hence gathering precise information on terminal region sequences is of great importance in biological research. We compared the uniqueness of protein terminal regions in the whole proteomes of human, mouse, rat, fly (*Drosophila melanogaster*), worm (*Caenorhabditis elegans*), weed (*Arabidopsis thaliana*), yeast (*Saccharomyces cerevisiae*), and *Escherichia coli*.

We extracted three amino acid length sequences from both N- and C-terminals ("terminal tags") of all proteins of the eight organisms stored in the UniProt release 2013_3 reference proteome. All sequences of *E.coli*, >99% of yeast, and >40% of human and mouse proteomes are stored in Swiss-Prot (all human-curated sequences) while >70% of rat and fly and >80% of worm proteomes are stored in TrEMBL (not human-curated). More sequences in TrEMBL are expected to share the same terminal tags because signal/propeptide sequences are not deleted. Similar amounts of unique terminal tags were found in human (69%), mouse (68%), and rat (66%) proteomes. Numbers were greater for fly (80%) and worm (83%) proteomes, and greatest for yeast (93%) and *E.coli* (97%) proteomes.

Results show that differences between organisms do not reflect curation dependency, but proteomic differences that may be attributed to certain genomic aspects; splicing variants that share the same terminal tags may reflect the number of alternatively spliced genes in non-terminal regions. Data will be web-published as a database in the near future.

Keywords: PTM (post-translational modification), database, comparative proteomics

POS-01-250 iProX: Integrated Proteome Resources

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Since Human Liver Proteome Project (HLPP) launched in China in 2002, several proteome profile datasets of human liver have been published, including the human adult liver proteome dataset, the fetal liver proteome dataset, the human liver phosphoproteome dataset and so on. For the data sharing purpose, these datasets were submitted to prominent data repositories, including PRIDE, Human Proteinpedia and NIST spectral library. Recently, CNHPP was initiated in China to perform the proteome research including B/D/C-HPP. CNHPP will produce large-scale datasets for several human tissues. In order to support the project and facilitate the data sharing, the integrated Proteome resources (iProX) were built. Currently, iProX is composed of an experiment data submission system and a proteome database (http://www.iprox.org). iProX submission system is a public platform for collecting and sharing proteomics experiment raw data and standardized meta-data, using controlled vocabularies to describe the Minimum Information About a Proteomics Experiment (MIAPE). This submission system is set up following the data-sharing policy of ProteomeXchange consortium. Registered users can submit their proteomics datasets to iProX by the public or private modes. The public datasets are openly accessible and private datasets could only be accessed by the authorized users. The iProX proteomics database is developed as a structured storage platform for data deposited in this system. All datasets submitted to iProX can be automatically imported into the database; comprehensive information of data resources as well as detailed identifications can be viewed in different visualized interfaces of iProX. Nowadays, huge number of proteome data generated in CNHLPP and in the first phase of CNHPP has been submitted to iProX. Definitely, iProX will facilitate the data analysis and sharing process of proteomics experiments.

Keywords: data sharing, proteome database, submission system

POS-01-251 "Antibody Ranker" to Find Antibodies Used in Recent Publication

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The antibody is one of two important tools in proteomics, and is also one of the most pivotal reagents in biomedical researches. Currently, so many antibodies against one molecule or protein are produced by different commercial companies or researchers. However, this variety makes researchers troublesome to select a suitable antibody for their studies. Researchers consume a lot of time to find and select antibodies for their purposes by referring articles. Therefore, we aimed to develop a tool for researchers to select antibodies suitable for their purposes."Antibody Ranker" is a searching engine to look for antibodies for all human proteins in recent open access articles viewed in the Pubmed Central and to collect information. The antibodies used in the articles were arranged in the rank order of the number of articles. To obtain antibody information, full-text search was performed in the section of the materials and methods in each particle to find antibody information. Variety of names for one protein was used by getting from Uniprot Database. In this study, we looked for antibodies for human proteins, but the system will be extended to search antibodies to proteins of other spices or to other key words. In our database, 363 major antibody-producing or selling companies were listed. All the data will be opened in the Aitibody Ranker website by updating periodically. The web system displays a list of human protein names, company names in rank order of numbers of articles, in which antibodies to each protein, article source information and figures with links to original article in the Pubmed Central.

In conclusion, this web system will provide useful information of which antibodies are most used and how they are employed in the articles published in the PubMed Central for researchers to select antibodies.

Keywords: antibody, database

POS-01-252 Identification of Short Terminal Motifs Enriched by Antibodies Using Peptide Mass Fingerprinting

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Reducing the complexity of digests by immunoaffinity enrichment leads to a substantial increase in throughput and sensitivity in proteomic mass spectrometry. The limitation of using such techniques is the availability of appropriate peptide specific capture antibodies. Novel methods use antibodies directed against short terminal epitopes, promise a significant gain in efficiency. It has been shown that subsets of peptides with short identical terminal sequences can be successfully enriched. After purification the epitope of the novel binders has to be elucidated. This is a substantial effort in the lab. Custom peptide libraries and numerous mass spectrometry experiments are needed to identify the epitope using a classical approach. We propose a peptide mass fingerprinting algorithm, which determines which epitopes are most significantly enriched in a complex sample. Each potential terminal sequence of a predefined length is associated with a p-value. The score is estimated by sampling random spectra. Another algorithm combines the predicted sequences to binding motifs. The result of the algorithm is a list of motifs, which balance model complexity and peak coverage. We have compared library screenings for three antibodies to our approach using three different complex samples obtained from common cell lines. The predictions made by the method give reliable and reproducible indications about the motif of the antibody. A web-based user interface allows the prediction for a given mass spectrum and renders a graphical representation of the search result. The novel approach reduces cost and time for such experiments significantly.

Keywords: bioinformatics, epitope, motif

POS-01-253 Data Processing Within Multi '-OMICS' Projects

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Background:

Within a cell, the most important bio-molecules (i.e. DNA, proteins, mRNA, metabolites) are highly interrelated. These relationships influence the regulation of virtually all cellular processes like gene expression, cell cycle etc. to a great extent. Hence, data from different multi-Omics platforms ("cross-platform analyses") should be considered holistically in order to acquire new insights into the highly complex cellular mechanisms. From a medical point of view such an integrated multi - OMICS approach is most likely indispensable to accelerate progress in biomarker discovery and may lead to an -in silicio driven - development of new therapeutic approaches.

However, processing and interpretation of multi-OMICS data is challenging and requires a highly standardized and reproducible data analysis pipeline.

Results:

Here, we present such a data analysis scheme for the processing of Proteomics, Epigenomics (DNA methylation) and Transcriptomics (mRNA and miRNA) data. This workflow comprises several steps of data conversion, data comparison, text mining and statistical analyses. Additionally, a software named CrossPlatformCommander is sketched, which facilitates several steps of the proposed workflow in a semi-automatic manner. The performance of major workflow steps is shown using a hepatocellular carcinoma data set, obtained from a multi-OMICS project named PROFILE (http://www.profile-project.de/). Utilization of this approach was shown for the detection of novel biomarkers. The final result is a list of biomarker candidates that can be further validated using an independent data set or an independent method.

Conclusion:

A workflow / software solution is proposed that handles and integrates data obtained from several -OMICS platforms.

Keywords: multi-OMICS, data processing workflow, biomarker

POS-01-254 New 2-dimensional Image-Converted Analysis of Liquid Chromatography and Mass Spectrometry (2DICAL) Optimized for High-Performance Mass Spectrometry

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Mass spectrometry is essential for proteomic and metabolomic research. Improvements in measuring speed have enabled the acquisition of vast amounts of tandem mass spectra for determining peptide sequences and dramatically increased peptide identification. However, these improvements have resulted in an abundance of data, which require an effective analyzing system. We developed a new quantitative method that uses a bottom-up approach that is based on our label-free analyzing system 2-dimensional image-converted analysis of liquid chromatography and mass spectrometry (2DICAL). With this approach, all annotated materials, including metabolic substances, can be quantified. This new system is applicable for biomarker detection in a large number of clinical samples and can be provided as a Web application with an easy interface for researchers.

The system can deal with multiformatted liquid chromotography and tandem mass spectrometry (LC-MS-MS) data with file-converting software, such as ProteoWizard or SpiceCmd, in the background. Mascot or LipidSearch is used for the identification of tandem mass spectra. Dynamic programming is adopted for retention time adjustment utilizing the similarity index of the mass spectrum pattern. The peak quantity is calculated by the peak area of the ion chromatogram at the parent mass that is positioned at the same substance in different LC-MS-MS runs. When the same substance is not contained in an LC-MS-MS run, the peak quantity is calculated at the position of the parent mass that has been estimated from other LC-MS-MS runs. Because of this approach, the quantity of the same substance in every LC-MS-MS run can be calculated, and a comprehensive peptide list is obtained, including peptides at small amounts that have been neglected by conventional quantification software. The quantified peptide number has increased 2-3 times compared to the former 2DICAL version.

<B>Keywords:</B> Proteomics, Biomarker, Informatics

POS-01-255 Development of a Tool for Label-Free Quantitation of Proteins Identified by Mass Spectrometry and Verification of the Results

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Quantitation of proteins in samples is primarily significant for obtaining the biological conditions. Especially, label-free determination of quantity is very useful for the purpose since it is simple and cost-free. In addition, statistical analysis of the data is becoming not easy because of the large protein information data obtained by mass-spectrometry. In this study, we aimed to develop a tool by using an EXCEL spreadsheet for quantitative comparison of protein data obtained by label-free LC-MS/MS analysis.

Then, the tool automatically calculates ratio of intensity of each protein in the total intensity and ratio of intensity/protein length. High ratio-proteins of the glomerulus, proximal tubule, distal tubule and collecting tubule were selected by using the tool and those proteins localization in kidney was verified by immunohistochemistry images provided by the Human Protein Atlas.

Results

1. LC-MS/MS and MASCOT analysis identified 277 proteins in glomeruli, 852 proteins in proximal tubule, 550 proteins in distal tubule and 518 proteins in collecting tubule.
2. By the tool, 36, 218, 6 and 4 proteins were uniquely localized in glomeruli, proximal tubule, distal tubule and collecting tubule, respectively.
3. The Human Protein Atlas revealed the localization of those proteins in each nephron segment. Conclusion

This Excel-base tool enabled to provide quantitative information from label-free MS analysis data easily and to compare the quantitative data among several samples to select unique or common proteins in each sample.

Keywords: LC-MS/MS, label-free, EXCEL

POS-01-256 A Comparison of Two Different Library Construction Processes

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With the development of mass spectrometers, large scales spectra were achieved. However, the identification rate of spectra was still unsatisfactory. Since libraries with equal ion intensity were widely used at present, it would be possible to increase identification efficiency by building new libraries, of which the different intensities could represent reliable fragments. Here we would like conclude the present methods and offer a comparison. The first process with MassAnalyzer prediction (1), which was generated using a kinetic model, offered a relative theoretical library. The other process was based on a series of data analysis: alignment with SpecAlign (2), representation of similar spectra by a single consensus spectrum with Spectral archives (3) and output of the library with SpectraST (4), all of which together built an experimental library. In the following data analysis, we tried to measure the difference and similarity between the new libraries. And we decided to calculate the normalize dot-product with the similarity score. In the side of spectra identification, the experiment-based library was supposed to be more reliable. Yet, the MassAnalyzer prediction could afford more theoretical spectra within short time.

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Keywords: spectra library

POS-01-257 'exKRT': Automated Extraction Analysis of Contamination in Proteomic

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Background: For understanding human diseases mechanism by proteomics research, exact peptide information of clinical human samples is necessary for us. Unfortunately there is no any method could avoid contamination completely during the sample preparation.
Method: In this study, we provide a VBA-based tool named as ʻexKRTʼ. By using this tool, exported identification results from few kinds of searching engines can be analyzed automatically. From the information of peptide samples a few kind of proteins including keratins were considered as point of contamination. Quality and quantification of keratins of the exported results will be calculated individually. As well known, the main reason of contamination of proteomics sample is coming from dandruff and epithelium. We first confirmed the types of few keratins coming from dandruff by western blotting, and then compare them with identified results of our experimental peptide samples. We focused the ratio of several keratins to other proteins in dandruff samples.
Results: We developed this tool to exclude the effect of contamination on peptide identification results so that we can understand the clinical samples status more clearly. It also supports to distinguish the quantity of other proteins in the peptide samples by extracting their fragments intensity of MS analysis results. We hope the ʻexKRTʼ tool can help more researchers getting their real ʻtrueʼ data from proteomics.
Keywords: exKRT, keratin, contamination

POS-01-258 Estimation of Protein Species Number for Mammalian, Bacteria, Insecta and Yeast

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The sequencing of the human genome was completed over 10 years ago during the Human Genome Project. As a logical continuation of this project, Human Proteome Project was launched in 2010. At the same time, the target size of the human proteome is still obscure: from 10 000 (Adkins et al., 2002) to 1 billion (Kelleher, 2012) of different protein species assumed. The diversity of protein species arises from the appearance of single amino-acid polymorphisms (nsSNP, SAP), alternative splicing variants (AS) and post-translational modifications (PTM). During last 3 years the numbers of entries in UniprotKB concerning SAP, AS and PTM stable for Drosophila melanogaster and Saccharomyces cerevisiae S288c, while for human there is a slight increasing SAP-related entries, and number of PTM-related entries are increased for Rattus norvegicus and *Escherichia coli*. Multiplying the average number of variations per gene, we could estimate the number of protein species coded by one gene; applying this calculation to all genes, we could expect the number of protein species. For human, rat and E-coli the minimal number of protein species estimated as 1,9 mln., 390 000 and 45 000 respectively. We obtain approximately 307 000 protein species for Drosophila melanogaster and 290 000 for Saccharomyces cerevisiae S288c (UniprotKB, v.03_2013). As the number of possible protein variants will definitely increase due to growth of the database, here we use the term «minimal number of protein species» for mammalian and *E-coli*. The number of protein species is necessary for understanding the target size of the proteome of each organism. Moreover, such kind of data is necessary for determination the each protein species abundance. Dependence of the number of detected proteins and the analytical sensitivity could be based on a comparison of the theoretical calculations with experimental results of number of protein species detected using 2DE with various dyes types.
Keywords: data analysis, number of protein species, Human Proteome Project

POS-01-259 How to Submit MIAPE Compliant Data to ProteomeXchange Consortium

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ProteomeXchange consortium was launched with the aim of providing scientific community a unique entry point for sharing proteomics data, developing the appropriate tools based on HUPO-PSI standards.
It is expected the amount of submitted data will continually grow as Human Proteome Project (HPP) begins to produce data from the characterization of human protein-coding genes products. Metadata about sample processing and experimental procedures becomes crucial for HPP project where information about how each proteoform has been experimentally detected from a certain sample/tissue should be part of the aimed human proteome map.
ProteomeXchange provides the appropriate tools for preparing proteomics data for its deposition. However, the metadata about sample and experimental equipment and protocols that is currently required seems to be insufficient for the HPP submissions.
Here we present an alternative tool, the ProteoRed MIAPE Extractor, which prepares data for a ProteomeXchange submission and additionally provides the way for assuring MIAPE compliance of the submitted data.
In 5 steps, the tool is able to firstly extract MIAPE data from PSI standard data files. Secondly, it provides a way for completing the required metadata to reach the MIAPE compliance. Thirdly, it compiles and integrates data coming from several experiments, allowing its filtering, keeping just the reliable data. Fourthly, it provides a great number of views for comparing and inspecting compiled data. Finally the MIAPE Extractor prepares all data for a ProteomeXchange submission by compiling all required files in a single folder and creating a PRIDE XML file from each processed experiment data, also including human readable MIAPE compliant reports. All these files can be later been used in an automatic submission using the ProteomeXchange submission tool.
This tool is being used by the Spanish HPP consortium and its open for its use from any other initiative.
Keywords: ProteomeXchange, MIAPE, sharing

POS-01-260 Update on the Transcriptomic and Proteomic Expression Data for the Placenta within the GenomewidePDB

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As a member of the international consortium working on the Chromosome-centric Human Proteome Project (C-HPP), we developed a gene-centric proteomic database called GenomewidePDB, which integrates proteomic data for proteins encoded by human chromosomes along with transcriptomic data and other information from the public databases. This GenomewidePDB contains not only the experimentally identified proteins that are present in normal human placenta tissue as well as preeclampsia patients but also the genomic profiling data (e.g., cDNA arrays). Integrated were also publicly available transcriptomic expression data from NCBI UniGene EST profile and proteomic expression data from Human Protein Atlas. Thus, this database will provide an ideal interface between multi-omics fields in the course of comprehensive genome-wide proteomic initiatives. GenomewidePDB is available publicly at http://genomewidepdb.proteomix.org/. (*This study was supported by a grant from MediStar (A112047 to SKJ) and the National Project for Personalized Genomic Medicine (A111218-11 to YKP).*
Keywords: Chromosome-centric Human Proteome Project, database, placenta

POS-01-261 Novel Data Analysis Pipeline Based on Mass Spectrum Sequential Subtraction for High-Throughput Proteogenomics

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Since tandem mass spectrometry-based shotgun proteomics generates huge numbers of MSMS spectra of tryptic peptides with high efficiency, it is of importance to establish the highly efficient pipeline for data analysis to identify as many peptides as possible. Therefore, various kinds of peak picking algorithms, database search engines and databases containing amino acid sequences or nucleotide sequences have been employed in an integrated manner to maximize the identification efficiency. However, the entire execution of these steps in series is just time-consuming, especially database searching of huge numbers of spectra against large size nucleotide databases. Recently we reported an approach to subtract spectrum data identified by the search engine for the next round search against different databases, named mass spectrum sequential subtraction (MSSS). We applied the MSSS approach to HeLa
phosphoproteome data against two protein databases (IPI and Swiss-Prot), cDNA (RASV), mRNA (RASV), genome (HG19) and cervix-EST (UniGene) database, as well as three cancer-driven databases such as Hela-EST database (UniGene), cervical-cancer EST database (UniGene) and COSMIC database. As a result, 29 phosphopeptides were identified from these cancer databases, meaning that these peptides possibly included cancer-related somatic mutations. In addition, whole proteome data of HeLa cells were analyzed by MSSS with a series of databases. Consequently, additional 462 peptides were found. In this presentation, we will also show the MSSS-based strategy for different database search engines.

Keywords: proteogenomics, LC-MS/MS, cancer

POS-01-262 Benefits and Challenges of Combining Search Results from Multiple Algorithms

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Presented here are results of applying iProphet to different combinations of search engine searches. The iProphet tool is part of the Trans-Proteomic Pipeline (TPP) that allows for computing accurate probabilities of identifications for distinct peptide sequences. It improves classification of spectrum search results on single search engine results and can be used to generate combined results from multiple search engines.
In this work we examine the performance of iProphet on different combinations of search engine results currently supported by the TPP and compare to other search engine result combiners. All of the iProphet results for this work can be generated using the Amazon Elastic Compute Cloud (EC2). Using EC2 it is possible to buy time on Amazon cloud computers to perform searches and TPP analyses.
A detailed cost benefit analysis will be demonstrating the additional peptide identifications that can be gained at a controlled false discovery rate using multiple searches. Additional costs in terms of storage, compute time and/or cloud costs will be analysed and compared for different combinations.

Keywords: bioinformatics, machine-learning, cloud-computing

POS-01-263 Solving the Bioinformatics Bottlenecks of Massive Storage & Data Distribution, Huge Computational Needs, and Flexible and Fast Reporting

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Introduction
With today's modern mass spectrometers easily producing one to two gigabytes of data per hour, it was a critical requirement to have an informatics service that could process the MS data at least 10x faster than the MS could generate. Further, increasing the number of identified spectra and proteins was desired. Finally, the lab needed a mechanism for the MDs and biologists to interrogate their data in a supremely flexible manner such that user queries produced reports within seconds. Finally, the lab sought a system that would be accessible from any geographic location.
Methods
Working with Integrated Analysis (since 2008) and Shimadzu Scientific (since 2013), the JHU NHLBI lab provided requirements and extensive feedback for a subscription-based integrated informatics service that would be accessible from anywhere in the world, scaled to terabytes worth of data, supported multiple search engines running in parallel, and allowed high flexibility for querying and reporting of data.
Preliminary Data
The lab achieved a 100x fold increase in search speeds. Approximately 30% more proteins were identified at low (~1%) FDRs by combining multiple search engines. Reports that previously crashed when run on the lab's high-end desktops now ran in 1 to 30 seconds even when accessed from low-end laptops. Terabytes of data were stored securely and automatically and robustly uploaded from the MS instruments, all with built-in logging. Most notably, the lab's collaborators have identified putative biomarkers (some of which were validated by ELISA and are now being commercially validated) using the informatics service's query and reporting abilities.

Keywords: protein identification, workflow, data management

POS-01-264 A New Algorithm for Peptide *De Novo* Sequencing with Multiple Complementary Spectra

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Different fragmentation methods in MS/MS produce complementary spectra of the same peptide. Combining complementary spectra lead to more accurate *de novo* sequencing. In all of the few existing algorithms for multi-spectra *de novo* sequencing, score functions are designed from scratch, without taking advantage of the accumulated development in existing single-spectrum *de novo* sequencing software. As a result, an inadequate multi-spectra *de novo* sequencing tool may not necessarily compete with the best single-spectrum *de novo* sequencing tool.
We propose a new algorithm that reconstructs a more accurate sequence from the top 10 PEAKS *de novo* sequencing results of each spectrum. This algorithm calculates a new score for each residue with Bayesian rule based on the residue's local confidence, and selects confident sequence tags for reconstruction. The goal of the reconstruction is to maximize the total residue scores in the reconstructed sequence. A dynamic programming algorithm is designed to conduct the reconstruction efficiently.
Comparative study is performed using a dataset of 4641 ETD/HCD spectral pairs generated from a trypsin-digested proteomic mixture. By database search, 387 out the 4641 pairs had both the ETD and HCD spectra identified as the same sequence. These 387 sequences, consisting of 5531 residues, were used to validate *de novo* sequencing results. The new algorithm was compared with a recent multi-spectra *de novo* sequencing software, UniNovo, and PEAKS single-spectrum *de novo* sequencing, using either the ETD or the HCD spectra. The four methods correctly computed 2585 (new algorithm), 2082 (UniNovo), 2063 (PEAKS HCD), 1792 (PEAKS ETD) residues.
The result demonstrates that the new algorithm is significantly better than the other approaches. This is attributed to both the reconstruction from HCD and ETD spectra, and the utilization of the excellent PEAKS HCD and PEAKS ETD single-spectrum *de novo* sequencing results.

Keywords: de novo sequencing, complementary fragmentations, tandem mass spectrometry

POS-01-265 Peptide *De Novo* Sequencing Result Validation

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De novo sequencing is essential for a complete proteomics analysis. As a supplement to protein database search, *de novo* sequencing interprets the large number of high quality spectra that do not match any database peptides, and helps characterize amino acid mutations and PTMs. Meanwhile, the lack of an automated method for result validation is currently preventing the adoption of *de novo* sequencing in high-throughput proteomics analysis. In this research, we present a novel method to automatically estimate a "residue-level" FDR (false discovery rate) to validate *de novo* sequencing results, making it possible to use *de novo* sequencing in every proteomics experiment.
An algorithm is proposed to calculate a local confidence score for each residue in *de novo* sequence result. The score indicates the probability that the residue is correctly computed, and can be used directly to filter the *de novo* results in the case when no database is available. Experiments show that local confidence score accurately reflects the correctness probability, and well separates the correct and incorrect residues.
In proteomics analysis, after protein database search is performed, *de novo* sequencing result is validated on those spectra with confident database peptide assignments. By plotting the score distributions for *de novo* residues that agree/disagree with database peptide, a score threshold can be determined to give a desired residue-level FDR. The threshold is then applied to filter the *de novo* results on the spectra with no database peptide assignments.
In experiments, we demonstrate that this method is capable of highlighting confident residues in *de novo* sequencings result of unassigned spectra, and essentially enables residue-level FDR quality control for "*de novo* only" result in high-throughput proteomics analysis.

Keywords: *de novo* sequencing, proteomic analysis, LC/MS-MS

POS-01-266 PAA - A New R Package for Autoimmune Biomarker Discovery with Protein Microarrays

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Background: Protein microarrays like the ProtoArray (Life Technologies, Carlsbad, CA, USA) are used for autoimmune antibody screening studies to discover biomarker panels. For ProtoArray data analysis the software Prospector (Life Technologies) is often used because it provides an advantageous feature ranking approach ("M score"). Unfortunately, Prospector provides no capabilities regarding multivariate feature selection, classification, batch effect adjustment and computational biomarker candidate validation.
Results: Therefore, we have adopted Prospector's M score approach and implemented a new R package called Protein Array Analyzer (PAA) that provides these features and a complete data analysis pipeline for ProtoArray and other single color microarray data that come in gpr file format. After optional data pre-processing and M score-based feature pre-selection a multivariate feature selection is performed. For this purpose, a backwards elimination (wrapper) approach ("gene shaving" with random forest) has been implemented. For the selection and validation of stable panels a frequency-based approach has been adopted. Furthermore, different plots and results files can be obtained to outline the analysis results.
Conclusions: We propose the new R package PAA for protein microarray data analysis. PAA has been used to successfully analyse several different ProtoArray data sets (e.g. "Parkinson", "Alzheimer", "Amyotrophic Lateral Sclerosis"). Thereby, its suitability for biomarker discovery with protein microarrays has been shown.

Keywords: bioinformatics, biological markers, protein array analysis

POS-01-267 Computational Studies of Post-Translational Modifications

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Post-translational modifications (PTMs) greatly expand the proteome diversity and play critical roles in regulating the biological processes. Identification of site-specific substrates and the regulatory enzymes is fundamental for understanding the molecular mechanisms and functions of PTMs, which is still a great challenge under current technique limitations. Development of computational approaches, which could rapidly generate useful information for further experimental investigations, has promoted studies of PTMs. My recent progresses in this area are focused on two aspects as follows:
1) Improving the GPS algorithm and employed it to implement a series of softwares including GPS-CCD, GPS-PUP, GPS-YNO2 and GPS-ARM to predict PTMs sites including calpain cleavage, pupylation, tyrosine nitration and APC/C E3 recognition motifs (D-box and KEN-box) respectively; Extending the GPS algorithm to develop predictors of GPS-MBA for prediction of MHC Class II Epitopes. With these predictors, a number of large-scale computational studies were carried out to dissect function of PTMs.
2) Systematical analyses of PTM proteome and PTM-related enzymes: Integrating the experimental protein lysine acetylation information into a comprehensive database of CPLA, followed by construction and analysis of human lysine acetylation regulatory network; Comprehensive analysis of the Plk-mediated phosphoregulation in eukaryotes followed by experimental assay which verified the predictions and analyses; Genome-wide prediction of enzymes for ubiquitin and ubiquitin-like conjugation among 70 species while the results were integrated into a comprehensive database of UUCD.
Token together, we believe that computational analysis backed up with subsequent experimental identification can propel systematic studies of PTMs into a new and highly productive phase.

Keywords: post-translational modifications (PTMs), predictor, network

POS-01-268 GlycoPepDecipher: Automated Identification of Intact *N*-Linked Glycopeptides

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Protein glycosylation is one of the important post-translational modifications and plays crucial roles in diverse biological functions. However, annotation of high-throughput mass spectrometric data, in terms of identifying site-specific glycan structure and the peptide sequence of the carrier protein, still remains a great challenge in glycoproteomics field. There is a pressing need to develop an automated tool for confident glycopeptide identification from fragmentation pattern in MS/MS spectra. Therefore, we present a bioinformatics tool to identify intact *N*-linked glycopeptides, specifically, identification of Y1 ion, i.e. peptide core carrying one HexNAc, and monosaccharide residues. After filtering out low-quality spectra, we first examine mass differences in each spectrum to determine the Y1 ion and then adopt a clustering strategy based on elution time and *m/z* information to adjust the precursor shift due to an adduct or a modification. Finally, neutral losses of monosaccharide residues were annotated to determine glycan composition and/or structure in each spectrum. On the preliminary study on horseradish peroxidase (HRP), 8 out of 9 previously reported glycosylation sites and their corresponding attached glycans were confidently identified by our tool. Out of 312 candidate spectra, 118 spectra were found to correspond to the reported glycopeptides and were also confirmed manually. The result demonstrates that our algorithms are effective and robust for identifying glycopeptides from tandem mass spectra. Validation of the tool on other standard glycoproteins or mixture of standard glycoproteins is ongoing. We expect that our tool may facilitate the identification of the under-represented *N*-linked glycoproteome.

Keywords: bioinformatics, N-linked glycopeptide, mass spectrometry

POS-01-269 Bioinformatics Study for Elucidation of Lectin Recognition Patterns

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Proteins called lectins bind to glycans and play important roles in biological processes. There are many lectin binding and steric mechanisms involving glycan structures in the control of protein-protein interactions, but it is often difficult to analyze. In general, lectins bind to the terminal monosaccharides of glycans on glycoconjugates. However, it is suggested that some lectins recognize not only terminal monosaccharides, but also internal monosaccharides, possibly influencing the binding affinity. Thus, we have developed a tool that can aid in the analysis of glycan patterns recognized by glycan-binding proteins, based on our new multiple glycan alignment algorithm called MCAW (Multiple Carbohydrate Alignment with Weights). The MCAW algorithm is based on the KCaM algorithm, which aligns pairs of carbohydrate structures, and ClustalW, which is a popular multiple amino acid sequence alignment algorithm. The MCAW tool has now been implemented on the web to output a multiple glycan alignment of an input dataset of glycans structures. Lectin recognition patterns can be analyzed using data sets of glycan structures from binding affinity data which can be obtained from glycan array data such as that from the Consortium for Functional Glycomics. Additionally, it is possible to configure parameters in the calculation of the alignment such that monosaccharides and bonds are aligned appropriately for the data set. Presently in the MCAW tool, we have confirmed the possibility of aligning up to 88 glycans at once, and we have been able to extract interesting glycan patterns from array data.

Keywords: glycan, lectin, bioinformatics

POS-01-270 Firmiana: An Integrated Platform for Mass Spectrometry-Based Proteomics Studies Based on Galaxy Framework

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Data analysis is essential and critical for proteomics studies. However, increasingly large amouns of high through-put data produced from mass spectrometry experiments pose significant challenges for computational analysis. Although lots of bioinformatics tools have been available, they are difficult to use, especially for experimental biologists with no programming experiences. Meanwhile some computational analyses are complex and integrative. Thus, user-friendly data analysis platforms integrating a broad range of tools are quite necessary.

Here we present a web-based platform called Firmiana for mass spectrometry-based proteomics study, built upon open source Galaxy framework which was originally designed for genomics research but allows easy integration of tools used in proteomics study as well as rapid configuration of proteomics data analysis workflows. This platform has integrated a collection of current and newly developed representative tools involved in most steps of mass spectrometry proteomics data analysis, from preliminary experiment management, file format conversion, spectrum identification with database searching, statistical validation of peptide and protein identification results, peptide and protein quantification, to downstream analysis such as significance test and Gene Ontology analysis. In addition, more new functions and tools can be included in this platform.

The user-friendliness of Firmiana platform will greatly facilitate mass spectrometry-based proteomic studies. The intuitive graphical user interface of tools liberates researchers from complex software operations, and the flexible construction of workflows simplifies complex data analyses. Besides, benefit from the scalability of Galaxy framework, tools can be configured to run on clusters or cloud to utilize more powerful computational resources.

Keywords: proteomics data management & analysis system, galaxy, mass spectrometry experiments

POS-01-271 A Simple and Fast Label-Free Quantitation Algorithm for LC-MS

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Liquid chromatography-mass spectrometry (LC-MS) is becoming an increasingly important technology, especially in differential proteomic analysis for biomarker discovery. However, LC-MS generates huge amounts of analytical data, which is often impossible to interpret manually. We developed an algorithm called AB3D, a label free and unbiased peak detection and quantitative algorithm using MS spectral data. AB3D utilizes XIC (extracted ion chromatogram) to calculate the quantitative value such as peak intensity or peak area for a given retention time (RT) and m/z with tolerances. Some MS vendors provide mass chromatogram extraction features, but an automated approach is necessary for large scale samples analysis. In AB3D, at first the XICs are generated automatically according to extraction features provided by MS instrument vendors. The XICs are then further analyzed by applying our XIC peak detection algorithm that uses a combination of local minimum and weighted average peak detection for quantitation.The practical application of AB3D for LC-MS data was evaluated using BSA peptides and the peptide mixture from BSA and HeLa cells with different concentrations. A comparison was then carried out between widely used software tools such as XCMS, MZMine and our algorithm AB3D from the generated LC-MS data. All quantitative results were confirmed manually, and we found that AB3D could properly identify and quantify known BSA peptides with less false positives and false negatives compare to XCMS and MZMine. Our AB3D algorithm will be integrated as one of the plug-ins for Mass++ and will be freely available on the Mass++ distribution site:http://www.first-ms3d.jp/english/achievement/software

Keywords: label-free, quantitation, algorithm

POS-01-272 Isobar: Making Sense Out of Protein and Modified Peptide iTRAQ/TMT Quantitative Data

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While current mass spectrometry is able to measure thousands of non-modified and posttranslationally modified peptides, the identification of significantly regulated molecules remains challenging. We have introduced a new software package - isobar - to address this problem both at the protein and the modified peptide levels for iTRAQ and TMT data.

The isobar platform relies on carefully tested statistical models (Breitwieser et al., J Proteome Res, 2011) that separate technical variability, originating from the instrumentation, and biological variability. Significantly regulated proteins can be naturally selected by requiring a clear measure and biological significance. Isobar can also take advantage of replicates present in a single iTRAQ or TMT experiment.

We have extended isobar to analyze the regulation of PTMs (Breitwieser & Colinge, J Proteomics, 2013) by assessing the statistical methods in this special condition and by introducing necessary new features. In particular, isobar provides a generic mechanism of validating the localization of PTMs by means of the Mascot Delta Score approach or specialized external tools such as PhosphoRS. The navigation of peptide regulation results is facilitated by a sophisticated hyperlinked spreadsheet user report which integrates references to known PTMs from neXtProt and PhophoSitePlus.

Isobar can be run without programming skills and it is released as a Bioconductor R package thus allowing more advanced users and bioinformaticians to fully exploit its rich repertoire of functions. It can parse the most common file formats (Mascot, Phenyx, MSGF+, MGF, Rockerbox, MzIdentML). Isobar also supports label free quantitation by computing emPAI and dNSAF protein abundance indexes.

Web site: http://www.ms-isobar.org

This work is supported by the Austrian Science Fund (FWF) grant P 24321-B21.

Keywords: quantitative proteomics, bioinformatics, PTM

POS-01-273 "FindPairs" - the Protein Quantification Module of the PeakQuant Software Suite

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Accurate protein quantification is a major task in Proteomics. A wide range of stable isotope labeling techniques allow simultaneous quantification of thousands of proteins by using mass spectrometry. Here, the FindPairs module of the PeakQuant software suite is presented. It automatically determines peptide and protein abundance ratios based on the automated detection of isotopic peak patterns in stable isotope-coded mass spectrometric data. Hence it also works with SILAC and iTRAQ, the practicability of FindPairs is shown on the quantitative analysis of proteome data acquired in $^{14}N/^{15}N$ metabolic labeling experiments. This works on the one hand "database-driven", when sequences are known. On the other hand, a special feature of FindPairs is the application of an average mass shift factor to identify isotope patterns of $^{14}N/^{15}N$ peptide pairs, even if no sequence information is known. This is interesting especially for Quantitative Proteomics in unknown species, i.e. "Meta-Proteomics". Furthermore we give an overview of the features of FindPairs and compare these with already existing quantification packages. The software is publicly available at http://www.medizinisches-proteom-center.de/software and free for academic use.

Keywords: bioinformatics, quantitative proteomics, metabolic labeling, 14N/15N labeling

POS-01-274 Approximative Statistical Approach for Absolute Quantification of the Human Chromosome 18 Proteins

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Absolute protein quantification is a crucial parameter for many biomedical researches, particularly biomarker research. One of the most sensitive, selective, reproducible methods of absolute protein concentrations estimation is Selected Reaction Monitoring (SRM).
We used statistical model [Ludwig et al, 2012] for analyzing of SRM-data obtained during Russian part of the Human Proteome Project. This model is based on the hypothesis that for absolute protein identification the specific MS signal intensity of the most intense tryptic peptides per protein is approximately constant throughout a whole proteome.
Basing on the experiment results obtained during chromosome 18 proteins detection, we selected 15 "best-flyer" peptides as anchor points for the statistical model and quantified their copy numbers per HepG2 cell. Quantification was performed with Mass Hunter Quantitative analysis Software, version B5.0. Using the model we estimated the number of copies for all the proteins of chromosome 18.
We plan to perform quantitative analysis for all the 277 proteins of chromosome 18 for approving of the estimated values and to determine the variability of the model's sensitivity depending on the number of anchor points.

Keywords: selected reaction monitoring, protein quantification, statistical modelling

POS-01-275 Condenser: A Statistical Aggregation Tool for Multi-Sample Quantitative Proteomic Data

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We present Condenser, a freely available, comprehensive open-source tool for aggregating and merging quantitative proteomics data from the Matrixscience Distiller work package into a common format ready for subsequent bioinformatic analysis.
A number of different relative quantitation technologies, such as 15N and amino acid stable isotope incorporation, label-free and chemical-label quantitation are supported. The program features multiple options for curative filtering of the quantified peptides, allowing the user to choose data quality thresholds appropriate for the current dataset, and ensure the quality of the calculated relative protein abundances. Condenser also features optional global normalization, peptide outlier removal and calculation of t-test statistics for highlighting and evaluating proteins with significantly altered relative protein abundances. Condenser provides an attractive addition to the gold-standard quantitative workflow of Mascot Distiller, allowing easy handling of larger multi-dimensional experiments using the supplied statistical tools. Source code, binaries, and documentation are available at http://condenser.googlecode.com/

Keywords: bioinformatics

POS-01-276 Bioinformatic Workflow for Chr16 Characterization Using Proteomic Shotgun and Transcriptomic RNA-seq Experiments

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Spanish team of the Human Proteome Project (Sp-HPP) marked the annotation and data analysis of Chr16 genes as one of the priorities for its bioinformatics group. Three cell lines (CCD18, MCF7 and Jurkat) were initially selected to cover the chromosome 16 proteome, and shotgun proteomic and microarray transcriptomic experiments were performed to characterize their molecular profiles. The information available in ENSEMBL, UniprotKB and GPMDB databases was used for the analysis of the generated data. In order to improve the identification of coding genes and properly define the so-called "missing" proteins (coding proteins that have not been detected by any experimental procedure according to the criteria established by the HPP consortium) we propose the incorporation of public RNA-seq experiments provided by the ENCODE project and the Illumina Human Body Map database for the bioinformatic analysis pipeline of Chr16. Following the guidelines presented in the iPRG-2013 ABRF study we have derived proteomic databases from RNA-Seq data for peptide identification. We have focused our preliminary study in shotgun experiments from Jurkat cell line. Using MASCOT and X!TANDEM search engines and MAYU algorithm for peptide and protein FDR calculations, a new annotation map of the Chr16 was developed based on gene and protein databases in addition to the information obtained from RNA-seq data analysis. Finally, a comparison study between public proteomic databases and RNA-seq shotgun analysis methods for chromosome 16 annotation is presented.

Keywords: Human Proteome Project, RNA-seq, Chromosome 16 annotation

POS-01-277 ProteomeXchange: Globally Coordinating Proteomics Data Dissemination

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The ProteomeXchange consortium (PX) provides a globally coordinated infrastructure for the deposition and public dissemination of MS-based proteomics data. For each dataset, PX aims to capture raw data, metadata, and processed results, to allow different views of the submitted data, from the original author analysis and interpretation in e.g. PRIDE to re-processed views in e.g. PeptideAtlas to higher abstraction levels in molecular biology resources like UniProtKB.
For all types of PX datasets, all the data remains private by default and each submission becomes publicly available only on author instruction or publication of the manuscript supported by the dataset. When this happens, a short summary announcement is released though a public announcement system, as a RSS feed. All the PX announcement messages are stored and searchable in ProteomeCentral. This resource generates a unique identifier and constitutes a registry for each PX data dataset. In addition, it provides the users with an efficient way to identify datasets of interest.
ProteomeXchange has been in production mode since summer 2012. As of March 2013, 160 submissions with a total volume of 7.7 TB from 28 countries have been received, ranging from a few hundred spectra to 1.5 TB in a single submission. A significant fraction of depositions stems from HPP participants. ProteomeXchange submission and dissemination sites are accessible from http://www.proteomexchange.org/

Keywords: database, HPP, data integration

POS-01-278 Development of a Genome Annotation Pipeline of Protein-Coding Genes of the Liverwort, Marchantia Paleacea Var. Diptera for *De Novo* RNA-Seq

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Marchantia paleaceae var. diptera is the key liverwort for study of mycorrhizal symbiosis. We performed *de novo* RNA-Seq of Marchantia paleaceae along with its genomic sequencing. To define the annotation of Marchantia paleaceae for *de novo* RNA-Seq, next generation sequencing reads from RNA-Seq were mapped on its partial gemome sequences and a genome annotation pipeline based on transcriptome was developed. Genome sequences of chromosomes (135,212 contigs, N50 4,507bp, mean length 1,169b (by Velvet-Oases); 103,118 contigs, N50 3,235 bp, mean length 1,012 bp (by Trinity)) were obtained. Complete sequences of the mitochondrial (mt) genome (total length 186,637bp) and the chloroplast genome (total length 121,027bp) were also determined. Sequence reads of RNA-Seq from Illumina GAIIx (148,600,679 reads) and Roche 454 (1,096,602 reads) were mapped with TopHat on the genome sequences. Sequences mapped with RNA-Seq reads were extracted, searched and assigned suitable protein names based on rice and Arabidopsis genome databases (IRGP and TAIR, respectively) by BLAST. Several gene prediction and gene finding software, such as GeneMark and GlimmerHMM, were also used for constructing a GFF/GTF file, which is a file format used for describing genes and other features of DNA, RNA and protein sequences. The genomic expression profiles were analyzed by the combination of Trinity and TopHat/Cufflinks with a fresh prepared GFF/GTF file based on RNA-Seq reads. This transcriptomics pipeline for *de novo* RNA-Seq could provide a quite sensitive, comprehensive and thorough quantitative method for RNA-Seq using non-model organisms.

Keywords: bioinformatics, annotation, non-model organisms

POS-01-279 Improving the Efficiency in Obtaining True Protein IDs with Biological Significance in Protein Biomarker Discovery Platforms

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There have been enormous improvements in mass spectrometry driven "proteomics" applications since the term was first coined nearly 20 years ago. However, there remain challenges, including the efficient determination of high confidence "true" protein identifications that are both statistically significant, and biologically relevant. To gain a better understanding of the problem, we have taken a step back to offer what we think is a simple solution to an otherwise very complex issue. In this study, human plasma was analyzed using a multiplex tandem mass tag (TMT) technology in combination with a 14-fraction-MudPIT, carried out on a high resolution Orbitrap Velos Pro instrument. Multiple data sets were generated on the same sample set to serve as a model system to compare three main areas: 1) perturbation of matching algorithms by way of instrument settings alone, 2) poor scoring and protein grouping due to a high level of database redundancies, which include human parasites, and virus's, and 3) GO mapping, required for adequate systems/ pathway analysis, and yet often decreased due to newly added and poorly annotated NGS data. The results led us to improve the number of significant protein ID's with biological significance by over 200%, with conclusions driven by pathway analysis that made clinical sense. This was achieved by improving the instrument settings with a focus on increased efficiency in peptide mapping from 10% to ~50%, decreasing the redundancy of current databases (in some cases by as much as 10-fold), and finally by focusing on the protein sequences that map to GO terms.

Keywords: MudPIT, GO mapping, systems biology

POS-01-280 Automatic Extraction of Correlation of Annotation Terms in Databases to Find Similar Concepts, Synonyms, and Multifunction

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Characterization of a given gene set, such as "gene set enrichment analysis (GSEA)", has become an important task in omics era. In such analyses, annotations are used as if they were independent, despite that some annotations are correlated each other. To interpret complex multiple annotations, we comprehensively examined correlation among each annotation for human genes. We selected ten gene annotation (gene family, Gene Ontology, InterPro, KEGG pathway, protein-protein interaction, SCOP, SOSUI membrane protein prediction, OMIM, tissue specificity of gene expression, and subcellular localization) from the integrated human gene database, H-InvDB. For all pairs of terms, the correlations were evaluated using Fisher's exact (two-side) test with Bonferroni correction. As a result, we found 21,047 and 793 pairs with positive and negative correlation. Many of the positive relationships were synonyms, such as "SCOP g.44.1.1 (RING finger)" and "IPR001841 (Zinc finger, RING-type)". We found other pairs with relevant but not apparent relationships, such as "GO:0006470, protein dephosphroylation" and "hsa04940: Type I diabetes mellitus". We also obtained negative relationships. Many of them seemed unlikely to co-exist in a gene and related to subcellular localization, such as membrane protein and soluble protein. Those information will help to refine predictive annotation, or perhaps include multifunctions of the genes. By integrating these annotation relationships together with other integrated databases, such as UniProt, we can re-evaluate complex annotations of GSEA results and produce a new summary report of the gene set.

Keywords: Gene Set Enrichment Analysis, database

POS-01-281 Proteome Analysis of Fenitrothion Exposed Adrenal and Pituitary Gland

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A sensitivity of children to organic phosphorous compounds seems to be higher than adults, and it is feared that the compounds irreversibly influence for reproduction and endocrine system of children. Therefore, we studied about the effect of the fenitrothion to the endocrine system. The fenitrothion was administered to male rat of the 3 weeks-old at ratios of 0.1, 1, 10 and 30mg/kg/day. Each concentration in the serum Testosterone, FSH, Progesterone and ACTH was measured, after the administrations for 2 months. Sera testosterone in the fenitrothion treated groups of 1, 10 and 30mg/kg/day were significantly higher than a control group. Sera FSH in the treated groups of 1 and 10mg/kg/day were significantly higher. Sera progesterone in the treated groups of 1 and 10 mg/kg/day were significantly lower. Sera ACTH of 1, 10 and 30mg/kg/day treated groups were significantly higher. Then, proteomic analyses of adrenal and pituitary was carried out. Nucleoside diphosphate kinase A, Ubiquitin conjugating enzyme E2-N, Peroxiredoxin-6 and Cytochrome B were detected as interest proteins in the pituitary gland. The present time, a relationship between expression level changes of these proteins and hormone concentration fluctuations of the pituitary gland is being examined. HSP-90 (decreased) and apolipoprotein A-1, prohibitin, proteasome activator complex subunit-1, 6-phosphogluconolactase were detected as interest proteins in the adrenal gland. The change of the expression level of these proteins seemed to reflect load of a stress of the fenitrothion to the adrenal and the progesterone secretion reduction from the adrenal cortex.

Keywords: fenitrothion, adrenal gland, pituitary gland

POS-01-283 Global Protomap Profiling for Biomarker Discovery of Human Hepatocellular Carcinoma

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This study reports the global protein expression profiling of hepatocellular carcinoma (HCC) tissues surgically resected from the patients with or without recurrence within 2 years after the curative surgery (termed "early-recurrent" or "late-recurrent" HCC tissues respectively), as well as of non-tumor tissue adjacent to the tumor and of normal liver tissue. We employed the "PROTOMAP" strategy for comparative profiling, which integrates SDS-PAGE migratory rates and high-resolution mass spectrometry-based identification of in-gel digested tryptic peptides with semi-quantitative emPAI parameters. The PROTOMAP strategy thereby provides a dataset that could reveal global changes in the molecular size, topography and abundance of proteins in the complex tissue samples. This approach allowed the identification of ~8,500 unique proteins with ~46,000 non-redundant peptides from the tissue samples described above, and generated a proteome-wide map displaying the changes in expression and proteolytic events potentially induced by the intrinsic apoptotic or necrotic pathway. In the "early-recurrent" HCC tissue, we found 87 proteins expressed differentially (≥ 20-fold in emPAI) from other tissues, of which 41 were upregulated or/and their fragments increased by the potential proteolytic events and 46 were downregulated. The protein dataset consisted of proteins with various functional categories; notably a protein subset involved in the catalytic pathway responsible for major liver function such as urea cycle and detoxication metabolism. Finally, we present a number of candidates that could serve as prognostic HCC markers and potential therapeutic targets to prevent the recurrence of HCC.

Keywords: PROTOMAP, hepatocellular carcinoma, biomarker

POS-01-282 Proteomic Analysis of C14-Sphingosine-Triggered Germination of the Entomopathogenic Fungus, Nomuraea Rileyi

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The conidium of the entomopathogenic fungus *Nomuraea rileyi* has been found to germinate rapidly in the presence of host insect-derived extract. The factor contribute to this germination has been identified as D-erythro-C14-Sphingosine. The activity of D-erythro-C14-sphingosine is superior to that of sphingosines with shorter and longer carbon chains. Therefore, the molecular species with 14 carbons chain of a sphingosine is important for host recognition. To elucidate the mechanism of germination process in the presence of D-erythro-C14-Sphingosine, the 2D-PAGE analysis followed by mass analysis has been done for each stage of fungus. In this study, the conidium of *Nomuraea rileyi* was extracted by phenol method. The protein extract was then subjected to 2D-PAGE, pH3-10, 13cm. To detect the specifically developed proteins by sphingosine induced germination, the protein spots of germination samples with and without sphingosine were compared by differential display. Specific protein spots (4 spots) and increase spot (17 spots) were detected in sphingosine induced germination. These spots were analyzed by mass spectrometry after tryptic in-gel digestion. TOF/MS ions were analyzed by PMF analysis and followed by MS/MS *de novo* sequences using BLAST search. The results showed that the specifically developed functional proteins were permease, contributes secondary active transport to carry nitrogen, and increased were peptidyl-proryl cis-trans isomerase, cytosolic folding cataryst in periplasm, Sec14 cytosolic factor, transporter of secretory proteins, and translationally controlled tumor protein, contributes microtubule stabilization. Therfore, these proteins might be responsible for sphingosine mediated germination.

Keywords: sphingosine, Nomuraea riley, entomophathogenic fungi

POS-01-284 Comparative Proteomic Analysis of Virulence Variations in *Xanthomonas campestris* pv. campestris Strain 17, 11A and P20H

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Xanthomonas campestris pv. *campestris* (*Xcc*) is a Gramnegative plant pathogenic bacterium causing black rot in crucifers, resulting in tremendous loss in agriculture. The ability of *Xcc* to infect plants successfully depends on certain factors including extracellular enzymes, exopolysaccharides and biofilm production. A newly isolated pathogenic XC17 from an infected cabbage leaf for some phenotypic characteristics have previously been implicated as factors contributing to pathogenicity. A wild-type strain of *X. campestris*, XC11, was found to have lost its pathogenicity spontaneously after frequent subculturing for years. This non-pathogenic derivative, designated XC11A. *X. campestris* P20H was a non-mucoid mutant previously isolated from XC11A by mutagenesis with nitrous acid. Recently the proteomics have been developed more widespread, the techniques can offer insights into the quantity and quality of the final gene products. In this study, we aim to elucidate the physiological and molecular mechanism response to virulence variations in *XCC* by using proteomics method. Total proteins of intracellular and extracellular were extracted for comparative proteomics analysis via SDS-PAGE, 2D-PAGE and LC-MS/MS analysis. The result shows several differentially expressed proteins spots of bacterial proteome in XC17, 11A and P20H. These identified proteins may be helpful in elucidating the molecular basis of virulence variations in *Xcc.*

Keywords: Xanthomonas campestris pv. campestris, 2D-PAGE, LC-MS/MS

POS-01-285 Proteins Expression Clustering of Normal and Alzheimeric Rat Hippocampus Treated with Lavandula Angustifolia

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Anti-inflammatory drugs have been approved as drugs for Alzheimer postpone but many side effects and limitations of these drugs, the discovery of effective medications to treat or improve symptoms of Alzheimer plant origin have been more interesting. In this study are applied proteomics, bioinformatics and biostatistics to protein identification of drug targets for Alzheimer's disease. Hippocampus proteome of normal and Alzheimer's rats treated with aqueous extract of lavender (Lavandula angustifolia) evaluated with protein expression clustering. Protein samples extracted from normal and Alzheimer's rats that were treated with extracts (CE and AE) and without extract (C and A). Proteins separate by using two-dimensional electrophoresis and gels stain by silver stained methods. Bioinformatics and biostatistics analysis of proteins of four groups to be studied by bioinformatics software. 990 protein spots were detected in four groups. Proteome comparison of A and AE was found 49 proteins in A that AE was inhibited their expression, while there are 26 new spots in AE because of present of extract. Proteome comparison between groups C and CE showed the expression of the 80 new proteins and inhibition of 104 proteins. After removal of proteins between the two groups that were affected by the extract found proteins that are Alzheimer's drug candidate. Changes at the molecular level with multivariate statistical analyzes such as principal component analysis, correlation analysis and Clustering has been revealed and introduced proteins with changed expressed level in three main cluster. Finally, it can be concluded that lavender extract caused significant expression changes in the proteome and possibly activates specific biological processes in the rat's hippocampus that associated with enhancement of learning and memory in normal and Alzheimer's rats.

Keywords: Alzheimer disease, lavender, proteome

POS-01-286 Proteomic Analysis of the Cellular Signal Regulation Mechanism by Cholesterol Sulfation

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Background: The membrane microdomains which enriched cholesterol were existed on the cell surface membrane. These microdomains called lipid rafts, concentrate signaling molecules to amplify cellular signal. However, the dynamic regulatory mechanisms of raft microdomains are still remained an open question. Here, we investigate the possible regulatory mechanisms by cholesterol sulfation. The sulfation was catalyzed by SULT2B1b, which principally catalyzes the transfer of the sulfonate group from PAPS (3'-phosphoadenosine 5'-phosphosulfate) to the hydroxyl group of cholesterol.

Method: The SULT2B1b cDNA was subcloned into pcDNA3 vector plasmid, and transfected to Jurkat cell line. After transfection, stably expressing cells were selected. SULT2B1b expressing cells were activated with the use of concanavalin A (ConA) treatment. Detergent insoluble glycolipid-enriched microdomains (DIG) are isolated as lipid rafts fractions by sucrose density gradient centrifugal method. The proteins contained in DIG are separated and analyzed by two dimensional difference gel electrophoresis (2D-DIGE). The differentially expressed protein spots were identified by peptide mass fingerprinting method using MALDI-TOF/MS.

Results and Discussion: Differentially expressed proteins were detected in DIG fractions from SULT2B1b stably expressing cells and Jurkat cells. Up-regulated proteins were primary localized in mitochondrial, and were involved in cholesterol biosynthesis. Down-regulated proteins have been known to be primary localized in lipid rafts, and were involved in signal transduction. These results may suggest that possible involvement of cholesterol sulfation is one of raft regulatory mechanism.

Keywords: sulfotransferase, lipid rafts, sulfation

POS-01-287 Immunoproteomics Analysis of Antibody Response to Proteins of *Candida tropicalis*

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Candida spp. is major human fungal pathogen and the causative agent of candidiasis that affects mainly immunocompromised patients. Importantly, the incidence of candidiasis has continued to increase over the past few decades. Systemic candidiasis is of particular importance as it is associated with high mortality and morbidity rate. The difficulty in establishing definitive diagnosis and effective treatment for candidasis has prompted the search for specific and sensitive biomarkers for the disease. This study was conducted to shed light on *Candida tropicalis* as an increasingly important pathogen that was not widely studied before. An immunoproteomics approach combining two-dimensional gel electrophoresis, Western blotting and mass spectrometry was used in an attempt to discover immunogenic proteins of *C. tropicalis* as potential biomarkers. Antibody response against the 2-DE separated cell wall proteins was investigated. Protein spots that were recognized by IgG antibodies in the immune sera were subjected to mass spectrometry analysis. This led to identification of 12 distinct proteins. Ten of the proteins have been previously reported as antigens of *Candida albicans*. Additionally, species-specific and new antigens were also found in this study. Kgd2p and Idh2p were first time described as immunogenic proteins for *Candida* spp. The immunogenic proteins provide new insight into host-pathogen interaction during infection and could be potential biomarker candidates for candidiasis. Further work is required to evaluate the applicability of the proteins as biomarkers.

Keywords: immunoproteomics, candida tropicalis, antigens

POS-01-288 Mechanistic Analysis on Potent Intermolecular Isomarase Activity of Protein Disulfide Isomerase-P5 with Down-Regulation in Sperm Maturation Highlighted by Electrophoresis-Based Proteomics

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In mammalian spermiogenesis, sperm mature during epididymal transit to get fertility. We examined the expression profiles of proteins from boar epididymal caput, corpus, and cauda sperm by SDS-PAGE and LC-MS/MS. We identified 880 proteins with a number of unannotated ones. Using emPAI method, each about 300 proteins were up or down-regulated during the transit. Sperm proteins have been thought to be fully transcribed and translated in the testis, however, our search using KEGG pathway indicated that some are newly transcribed and translated during the transit. Disulfide isomerase-P5 (PDI-P5, P5) was also down-regulated. P5 is reported to be involved in asymmetric organogenesis in zebra fish and in promoting the ability of human tumors to evade the immune system and we have reported its potent intermolecular isomerase activity to inhibit oxidative refolding of reduced and denatured lysozyme, although most PDI family promotes oxidative refolding but the details have not been elucidated. We investigated the properties of domain-deleted or cysteine variants of P5 having active *a* and *a'* domain, and an inactive *b* domain with each active domain containing CGHC motif. The results showed that the activity of boar P5 was enhanced when the *a* and *a'b* domains were covalently linked, suggesting the cooperative potent isomerization by two active domains that possibly reflects the ancestral-type structure of P5 among PDI family. Proteomic approach enabled us to focus on analysis of P5 structure-function, and our finding on its isomerization mechanism provides insight into molecular basis of sperm maturation, asymmetric organogenesis and tumor immune evasion.

Keywords: KEGG pathway, protein disulfide isomerase-P5, potent isomerase activity

POS-01-289 Proteomic Analysis of Cell Populations in Artificial and Clinical Peritoneal Dialysis Effluents

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Depressed levels of heat shock proteins (HSP) upon exposure of cells to peritoneal dialysis fluids (PDF) represent a novel mode of cytotoxic action. Due to high abundant serum proteins in PD-effluents, analysis of expression profiles of cellular proteins is hampered. This study aims to reveal the cellular protein pattern after depletion of plasma proteins to elucidate molecular stress mechanisms. To investigate the dynamic complexity of the PD-effluent proteome, artificial PD-effluents and clinical samples the PD-protec phase I trial (ClinicalTrials.gov; NCT01353638) were analysed by gel-based fluorescent detection of protein abundance and MALDI-MS protein identification before and after depletion. The methodology was translated into patient material, following PD with and without therapeutic interventions with cytoprotective agents such as alanyl-glutamine (Ala-Gln). Without the serum background, 2D-gels allow the discrimination between protein patterns of cells under control and heat stress conditions. After depletion, the cellular protein fraction reaches the detection limit and expression profiles can be examined. Accordingly, heat shock proteins, which were differentially abundant in the cell extracts and serve as the gold standard of the cellular stress response, could be identified by MS and Western blotting. Recently our group has described the inadequate cellular stress response of MC following PDF exposure as a novel pathomechanism in PD. Early data from analysing effluents of the PD-protec phase I trial also suggest that addition Ala-Gln may induce cytoprotective cellular responses during clinical PD. The implication of the addition of this cytoprotective agent on outcome in the course of clinical PD is currently under investigation.

Keywords: end stage renal disease, high abundance protein depletion, difference gel electrophoresis

POS-01-290 Basic Nuclear Proteins Alteration in Neurodegenerative Disorder

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Neurodegenerative proteinopathy in advancing age, the Alzheimer's Disease is associated with extracellular senile plaques contributed by abnormally cleaved β amyloid peptide and neurofibrillary tangles formed by paired helical filaments of phosphorylated tau and synaptic deterioration which are the hall marks of the disease. Although being one of the major health concerns, the complete etiology of the disease is still obscure. Learning and memory processes in the brain are intricately associated with long lasting expression of genes. These processes are dependent on post translational modification signatures on the expressed products. Nuclear proteins are thought to undergo combinatorial posttranslational modifications specific to different genes the mechanism for which is still vague and needs to be explored. DNA associated basic proteins regulate the chromatin remodeling and gene expression by undergoing an array of modifications after their synthesis. Slight variation in these epigenetic marks alter the entire function of these proteins culminating in the form of neurodegenerative disorder such as Alzheimer's disease Expression profiling of the human brain DNA associated proteins was carried out through acid urea gel electrophoresis followed by by silver staining. Significant number of proteins was expressed and interesting observations were made. The expression profiles of DNA binding proteins will be helpful in the identification of the diagnostic and/or prognostic biomarkers at proteome level. The present research will greatly facilitate the identification of cellular alterations associated with AD resulting in the identification of novel diagnostic as well as drug and therapeutic targets.

Keywords: neurodegenerative, DNA associated proteins, combinatorial PTM

POS-02-001 Bridging the Gap between Imaging Mass Spectrometry and LC-MS/MS Identification

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Identification of mapped analytes is a crucial step in MALDI imaging mass spectrometry (MALDI-IMS) workflows on formalin-fixed tissues as it provides tissue marker candidates which can subsequently be validated downstream (e.g immunohistochemistry, IHC). Highly abundant tryptic peptides can be identified directly from formalin-fixed tissue by *in situ* MS/MS {Groseclose, 2008}. However, *in situ* fragmentation regularly fails to provide sufficient daughter ions for many tryptic peptides detected at the MS level {Gustafsson, 2010}. Here we present the combined results of two recently published manuscripts. The first illustrated the generation of an in-house, tissue specific, tryptic peptide LC-MS/MS reference library for formalin-fixed ovarian cancer {Meding, 2013}. The second demonstrated that re-calibrating MALDI-IMS peptide peaks with a set of internal calibrants leads to much higher confidence (< 20 ppm error) in matching of peptides to tissue specific LC-MS/MS identifications {Gustafsson, 2012;Schober, 2011}. These results support the use of dedicated data bases of LC-MS/MS identified peptides for matching to high mass accuracy tryptic peptide MALDI-IMS data. Confirmation of these matches has been achieved with a combination of IHC and *in situ* MS/MS in late stage ovarian cancer samples {Gustafsson, 2012}. Future application of these methods will thus provide an avenue for identifying tissue markers in clinical samples.

Keywords: MALDI imaging, LC-MS/MS

POS-02-002 Detection of Individual Cells in Tissue Using MALDI-TOF Imaging at 10 μm Pixel Size

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As MALDI Imaging instrumental parameters approach the low μm range, sample preparation becomes the resolution limiting experimental factor. Here, we combine sample preparation by matrix sublimation with optimized instrument settings to explore the limits of MALDI Image resolution. We have used biological model systems that have features on the 10 μm scale to evaluate these limits (rat cerebellum and porcine retina). Ten μm sections of fresh-frozen tissue were mounted on conductive glass slides. After tissue desiccation, DHB matrix was sublimated using a home-built device following P. Chaurands protocol. MALDI images were acquired on a MALDI-TOF MS in reflector mode, at a laser focus diameter of 5 μm. At 1 kHz laser frequency 100 shots were accumulated per pixel with a 10 μm raster width. Post-acquisition H&E staining followed standard protocols; the resulting microscopic images were co-registered with the MALDI images. In rat cerebellum are several prominent anatomical features such as the granular layer and molecular layer. Located between the granular and molecular layer are the bodies of purkinje neurons, these appear as individual cell bodies with a ~ 30 μm diameter. These cells were clearly identified in our datasets, e.g., by a lipid peak at m/z 878.9. Individual cells with 30 μm diameter were represented by 3x3 pixels, indicating that the true pixel size was indeed 10 μm. Porcine retina displays several highly organized layers of cells at ~ 10 μm thickness. The absence of unique m/z signals prevented obtaining high resolution images based on single peaks. However, multivariate statistical analysis such as Hierarchical Clustering allowed resolving the layered structure of the retina based on peak populations rather than individual peaks.

Keywords: imaging

POS-02-003 MALDI-FTICR Tissue Image Analysis of Rat Testis at 10 μm Pixel Size and 200 k Mass Resolution - High Mass Resolution x High Spatial Resolution

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Spatial and mass resolutions are both important parameters in MALDI imaging. We have used the sublimation method to obtain 10 μm spatial resolution from tat testis sections providing a rich tissue morphology at mass resolution >200k using MALDI-FTICR.Frozen rat testis sections were cut at 10 μm thickness using a cryo-microtome, transferred to ITO-coated glass slides and dried under vacuum. DHB matrix was sublimated in a home built device as described by Chaurand et al. 2011 MCP. MALDI images were acquired on a 7T-FTICR instrument with a 1 kHz smartbeam II laser and 10 μm flat top focus. The analyzed mass range: m/z 500-900 comprised lipids, which were identified based on accurate mass by matching against Lipidmaps.org database.Even anatomic features such as sub-structures inside the seminiferous tubules as small as 10 μm were clearly resolved in rat testis sections. Notably, ion images at m/z 758.545 and m/z 788.607 show clear differentiation of the interstitial space from the smooth muscle layer lining the basal lamina of the tubules. The muscle layer (<10 μm) and the interstitial space (<40 μm) were clearly resolved, indicating that a real image resolution of 10 μm pixel size was achieved, without oversampling conditions. The mass resolution in the lipid mass range was 200k, providing for the unambiguous assignment, e.g., for a pair of ions with a mass distance of only 3mDa. The lipid at m/z 808.5819 was found in the interstitial space, m/z 808.5849 in the lumen of the seminiferous tubules. The high mass accuracy allowed a tentative assignment of the molecular formulas of the two substances and their identities as $[C_{44}H_{84}NNaO_8P]^+$ as phospholipid PC36:2 and $[C_{46}H_{83}NO_8P]^+$ PC38:5.

Keywords: imaging

POS-02-004 Spatial Correlation Combined with Hierarchal Clustering Analysis for Reducing Complex Multi-Dimensional MALDI Imaging Dataset

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Mass spectrometry imaging allows for the correlation of spatial localization and chemical information from biological surfaces. It affords simultaneous visualization of thousands of lipid species in complete tissue sections, generating complex and high dimensional data from a single experiment, especially if ion mobility separation is used to enhance measurement specificity. This resulted in the growing need for automated image processing. A novel MALDI imaging workflow is presented including date acquisition incorporating ion mobility, processing using High Definition Imaging (HDI) software and a spatial correlation using Pearson product-moment correlation algorithms. Data were acquired using a MALDI SYNAPT G2-S mass spectrometer in IM-MS mode. The acquisition mass range was 100-1000 Da. The data were processed and visualized using HDI 1.2 MALDI software. Experiments were conducted using parts of a thin section of a rat whole body section, produced using a cryotome and deposited on tape and mounted on a standard target. Matrix was applied evenly to the sample in several coats.

The first step to reduce data set size was by peak picking the data in the m/z and drift time dimensions. The second step comprised the generation of ion distributions containing coordinate information. Thirdly, all processed distributions were correlated using a Pearson product-moment algorithm, generating a correlation matrix. The degree of correlation is expressed by a correlation coefficient that represents the linear dependence between two variables. Negative numbers are a negative correlation and positive a positive one. Hierarchal clustering analysis (HCA) was performed on the correlation matrix with K-means top down divisive clustering, allowing for useful visualization of the correlation matrix. This, subsequently, enables easy determination of groups of peaks with increasingly correlated distributions, within the large complex dataset.

Keywords: MALDI imaging

POS-02-005 Computational and Experimental Pipeline for MRI-Compatible Three-Dimensional MALDI Imaging Mass Spectrometry

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MALDI-imaging has emerged as a spatially-resolved label-free bioanalytical technique for direct analysis of biological samples and was recently introduced for analysis of 3D tissue specimens. In this work a new experimental and computational pipeline for 3D MALDI-imaging was established using PAXgene fixation, which was compatible with magnetic resonance imaging and evaluated this pipeline by analyzing a mouse kidney. Constructing 3D MALDI-images from 122 consecutive 2D MALDI-imaging data sets, required realigning the optical images of the serial sections and reconstructing their original spatial relationship. The computational analysis of this large data set was conducted by spatial segmentation, a multivariate analysis previously established for 2D MALDI-TOF imaging data, which permits determining regions of distinct molecular profiles in an automated fashion. In addition, correlation analysis was used to find distinct peptide and protein m/z-values co-localized with these 3D regions. The massive data set comprised 122 individual sections with 2,171,451 spectra with 7,680 m/z-values each; its total size was 200 GB, and it was processed using the SCiLS Lab 3D software, allowing the visualization and analysis of the entire data set. Mouse kidney was used as a model system to screen for functionally interesting features, such as molecular co-localization in tissue and the 3D analysis of human biopsy material. Our experimental and computational approach shows the feasibility of combining 3D MALDI-imaging with *in-vitro* MRI and H&E-staining to obtain a 3D spatially resolved proteomic snapshot of a specimen.

Keywords: MALDI imaging

POS-02-006 Investigation of Different Hierarchal Clustering Approaches for Protein Identification Directly from Tissue Section in a MALDI Imaging Experiment

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Mass spectrometry imaging (MSI) is an emerging tool for biomarker discovery. Identifying proteins directly from tissue has pushed the development of on-tissue enzymatic digestion, combined with the co-detection of lipids, leading to the generation of complex, high dimensional data from a single experiment, especially if ion mobility separation (IMS) is used to improve specificity. This has resulted in the growing need for automated data processing. Two types of hierarchal clustering methods (HCA) following spatial Pearson product-moment correlation are presented, divisive top down and agglomerative bottom up.

Tissue sections (rat brain and mouse fibrosarcoma model) were washed and on-tissue digested. After overnight incubation, matrix was applied evenly to the samples in several coats. Data were acquired using a MALDI SYNAPT G2 operated in IMS-MS mode. The acquisition mass range was 700-3,000 Da. Data were processed using High Definition Imaging 1.2 MALDI software.

Data reduction is achieved by peak picking the image data in m/z and drift time dimensions. Next, distributions comprising ion image coordinates are generated before being analyzed with a Pearson product-moment algorithm to generate a correlation matrix. The degree of data correlation is expressed by coefficient R, which measures the linear dependence between two variables, ranging from -1 (negative correlation) to +1 (positive correlation). To recognize the correlation results, matrix HCA was performed. For divisive clustering, K-means and K-medoids options were explored. For agglomerative clustering, the use of different distance metrics and linkage criteria were investigated. The different HCA methods were assessed in terms of their ability to cluster peaks from digest image data sets into groups of related peptides identified through PMF analysis. Evaluation of the time required to complete the clustering analysis and number of hierarchal levels created was also investigated.

Keywords: MALDI imaging

POS-02-007 ImageID: A New Spatially Resolved Proteomics Approach Providing Access to Disease Biomarker Proteoforms

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There is great interest in identification of biomarkers for clinical conditions resulting from MALDI imaging studies of clinical cohorts. We developed a new workflow that combined spatial information from MS Imaging (MSI) with LC-MS/MS identification of proteins. Protein digests were generated by trypsinization of two subsequent tissue sections maintaining the spatial distribution of the peptides. One of the trypsinized sections was analyzed by MSI. Peptides from the other section were identified by LC-MS/MS. Fresh frozen rat brain, testis samples and human breast cancer biopsies were sectioned to 10 μm slices. They were all MSI analyzed without and with tryptic digestion. In rat, more than 80 % of all peptides present in the MALDI images were identified by the parallel LC-MS/MS analysis and approximately 120 proteins were localized. Cystein-rich intestinal protein 1 (CRIP1) - a novel marker for cancer survival was previously identified by top-down analysis of HER2+/CRIP1+ specified human breast cancer biopsies. Such breast cancer biopsies were further analyzed using the ImageID workflow. CRIP1 was identified again as one in 150 proteins and 3 CRIP1 proteoforms were identified in addition. Arg-68 was present predominantly in methylated forms and, to a lower extent, in the non-modified form. The Arg-methylation heterogeneity described for CRIP1 was in agreement with the unexplained top-down imaging peak pattern in breast cancer biopsies providing additional structural details about the CRIP1 proteoforms in cancer. The new "ImageID workflow" allows to routinely identify more than 100 proteins from tissue sections and to simultaneously determine their spatial distribution at the 50 μm level. ImageID is also suitable for protein imaging and identification from FFPE tissue, which is largely accessible from tissue banks but in which currently employed top-down analysis is made impossible by protein crosslinks.

Keywords: MALDI imaging, proteoforms, cancer biomarker characterization

POS-02-009 Peptide MALDI Imaging - How to Get Most Out of Your Sample?

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Introduction: Matrix-assisted laser desorption ionization (MALDI)-Imaging mass spectrometry (IMS) has become a powerful and successful tool for biomarker detection especially peptide IMS. The overall goal of this study was to test and compare various protocols of tissue digest in order to improve the quality of IMS data.
Material and Methods: In the presented work the MALDI-ImagePrep™ device from Bruker Daltonics was used for automated spraying of trypsin and matrix. Ten μm thin rat brain tissue sections served as samples. Subsequent measurements were carried out with an UltrafleXtreme instrument (Bruker Daltonics). The experimental set up consisted of different experiments varying the trypsin incubation time, the matrix, the protease and the raster width of the laser. Every obtained image was analyzed using the flexImaging™ and the SCiLSLab software (Steinbeis Innovation Center SCiLS, Bremen).
Results: A total of 73 slides were processed and measured. It has been noted that the type of matrix has a significant impact on the number and quality of resolved structures. Similar observations were made for the experiments with trypsin overnight digestion. However it also became clear that the vast amount of external factors can have a large impact on the overall performance and outcome of an on-tissue digestion protocol. The SCiLS Lab software facilitated the comparison of sample sets due to the analysis of more than one sample simultaneously.
Conclusion: A crucial aspect is without doubt the quality of the sample. Analyzing such a comprehensive dataset is time consuming and difficult due to the variability of the parameters and more importantly the lack of tools for objective post-processing. Help may be provided by the SCiLS Lab software allowing the analysis of many samples in parallel.

Keywords: MALDI imaging, method improvement, rat brain

POS-02-008 A Study of Drug Distribution in Malignant Melanoma Tissue by MALDI Mass Spectrometry Imaging for Evaluation of Drug Efficacy

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Malignant melanoma (MM) is a disease with ever-increasing detection rates in the industrialized countries. However, the elevated incidence frequency has not been paralleled by the development of novel therapeutic agents with a significant impact on survival. Currently, limited therapeutic options exist for patients with metastatic melanomas, and all standard combinations used in metastasis therapy have low efficacy. Therefore, to develop the novel therapeutic agents is required for improving outcome of MM patients. In the process of drug development for therapy purposes, one of the key objectives is to optimize the efficacy and safety. Mass spectrometry imaging (MSI) is a powerful tool for pharmacokinetics/pharmacodynamics. MSI will provide a way forward in characterizing drugs and their spatial localization in tissue sections. The aim of this study was to examine the drug distribution within melanoma tissue sections using a pharmacokinetic model that allows quantitative analysis by MALDI MSI. MM tissues were prepared in sections of 10 μm in thickness prior to be exposed to drug by our in-house designed microdispenser platform. Samples coated with a matrix (α-cyano-4-hydroxycinnamic acid) were analyzed by a MALDI LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany). The drug fragment ions were visualized by the ImageQuest software (Thermo Scientific) and quantified by QUANTINETIX (Imabiotech, Loos, France). We examined the tissue localization of several drugs, which have previously reported in MM studies, and detected the parent and fragment ion mass signals with high sensitivity. These compounds were evaluated for further investigations to measure the drug efficacy in clinical settings.

Keywords: imaging, mass spectrometry, malignant melanoma

POS-02-010 Comparative Analyses of Peptidome and Proteome of CSF Samples from Patients with Guillain-Barre Syndrome and with Non-Neurological Diseases

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Guillain-Barre syndrome (GBS) is a very rare but highly dangerous immune-mediated inflammatory polyneuropathy disease with the most accepted mechanism involving autoantibody reaction against Schwann's sheath of the peripheral nerves leading to demyelinisation and damaging nerve signal transmission, in some cases involving respiratory muscles and autonomous nervous system. It was shown that autoantibody reaction might evolve as a side reaction of immune system response to bacteria or virus infection, when pathogen antigens mimic host Schwann cell membrane components, however in many cases exciting cause remains unknown. There are still a lot of questions concerning molecular mechanism of this disease and further studies are needed. Although GBS involves peripheral nerves only it is also known to be accompanied by increase in protein concentration in CSF. To understand the composition change of peptidome and proteome we compared CSF samples from GBS patients and the control group of patients with non-neurological diseases. Endogenous peptides as well as tryptic protein fragments were analyzed by LC-MS/MS. Totally in all CSF samples 1653 unique endogenous peptides (1% global FDR) - fragments of 200 proteins were identified. Among analyzed patient groups those identifications were distributed as follows: GBS patients - 1475 peptide fragments of 181 proteins; patients with non-neurological diseases - 641 peptide fragments of 84 proteins. 1012 of all identified peptides were specific for GBS samples and 178 peptides were specific for patients with non-neurological diseases. Proteome studies resulted in identification of 946 unique proteins (1% global FDR), 306 of them were specific for GBS samples and 65 were found only in CSF samples of control group. Obtained results will be discussed in the poster presentation.

POS-02-011 Protein Profiles of Peripheral Blood Mononuclear Cells as a Biomarker for Behcet's Disease

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Objective. To investigate the pathophysiology of Behcet's disease (BD) and find biomarker candidates for the disease, we analyzed protein profiles of peripheral blood mononuclear cells (PBMCs).
Methods. Proteins, extracted from PBMCs, were comprehensively analyzed in 16 patients with BD, 16 patients with rheumatoid arthritis (RA), and 16 healthy control subjects (HC) by 2-dimensional differential gel electrophoresis (2D-DIGE). Differently expressed proteins were identified by mass spectrometry.
Results. In total, 563 protein spots were detected. Intensity of 25 and 115 spots showed at least 1.2-fold intensity difference between the BD and HC groups and between the BD and RA groups, respectively ($p<0.05$). We completely discriminated between the BD and HC groups and between the BD and RA groups by multivariate analysis of intensity of 23 and 35 spots, respectively. The protein spots with significantly different intensity and also selected by the multivariate analysis included proteins functionally related to cytoskeleton, transcription/translation, T cell activation, bone turnover, regulating apoptosis, and microbial infection. Interestingly, intensity of only 3 protein spots provided area under the receiver operating characteristic curves (AUROC) of 0.922 for discrimination between the BD and HC groups. Similarly, intensity of 2 protein spots provided AUROC of 0.883 for discrimination between the BD and RA groups.
Conclusion. PBMC protein profiles, especially those of the 3 and 2 proteins, would be candidate biomarkers for BD. The identified PBMC proteins may play important roles in the pathophysiology of BD.
Keywords: Behcet's disease, peripheral blood mononuclear cells, biomarkers

POS-02-013 Immuno-Proteomics of *Helicobacter pylori* Antigens for IgG Antibodies in *H. pylori*-Positive Japanese Child Sera

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Helicobacter pylori infects human stomach in childhood and persists for lifelong time in case without eradication therapy. Serum antibodies against *H. pylori* are used commonly for diagnosis of *H. pylori* infection. Antigen proteins of anti-*H. pylori* antibodies in adult patients have been reported, however, these in child sera were not studied well. Here, we analyzed antigen proteins by immune-proteomics, reacted with IgG in sera from 14 asymptomatic and 8 symptomatic *H. pylori*-positive Japanese children whose ages were 2 to 18. Total 41 protein spots of a Japanese *H. pylori* strain were reacted with the sera by two dimensional electrophoresis (2D)-immunoblot analysis, and 24 proteins including previously not identified 10 proteins were identified as candidate antigen proteins by mass spectrometry. Nine antigen proteins were not detected by asymptomatic child sera, but by symptomatic child sera. All the *H. pylori*-positive sera were reacted with a major virulence factor, cytotoxin-associated gene A (CagA) protein and its fragments. Antigenic reactivity against CagA was confirmed using the *cagA* deletion strains compering with wild type strain. CagA may be one of the earliest antigen proteins recognized by the early host immune system.
Keywords: Helicobacter, antibody, CagA

POS-02-012 Identification of Naturally Processed MHC Class I-Restricted HIV Epitopes Presented by Human HIV-Infected Cells

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HIV-specific cytotoxic T-lymphocytes play a critical role in containing HIV infection. Yet correlates of immune protection against HIV are not defined. Identification of peptides endogenously processed and uniquely presented by HIV-infected cells remains essential to identify potent immune responses against HIV and for vaccine design. Human 293T cells expressing HLA-A02/B07 were transfected with, HIV-derived lentiviral vector producing GFP and pseudotyped with VSV-G envelope: LV-GFP-VSVg (293T-HIV-transfected). This LV-GFP-VSVg was used to infect HLA-A03/A11/B35/B51 EBV-transformed human B cell line (B-HIV-infected). Peptides displayed by 293T-HIV-transfected, B-HIV-infected cells and control-uninfected cells were eluted by mild acid treatment. Eluted peptide pools were enriched by size fractionation and analyzed by LC-MS/MS. We identified 43 HIV-derived peptides eluted from 293T-HIV-transfected and 16 from B-HIV-infected cells. All HIV peptides identified so far were derived from the most abundantly expressed Gag protein. 80% of peptides were in epitope-containing areas of p24. 80% were 8-12aa long peptides suitable for MHC-I loading and 20% were >12aa, indicating fairly frequent presentation of longer HIV peptides by MHC-I. 35% (293T-HIV-transfected) and 56% (B-HIV-infected) of 8-12aa long peptides contained anchor residues for HLA expressed by the cells. 52% (293T-HIV-transfected) and 44% (B-HIV-infected) represented potential extended HLA-restricted binders. 13 (293T-HIV-transfected)% were non-HLA-restricted. 10% of 293T-HIV-transfected peptides corresponded to optimal HIV epitopes reported to elicit immune responses in HIV-infected individuals. This first report of peptides displayed by HIV-infected cells provides fundamental insight into antigen presentation during HIV infection and may lead to unbiased identification of novel immune responses important for control of HIV infection and for immunogen design. Supported by NIAID-A1084106.
Keywords: HIV infection, MHC class I-HIV Epitopes, Vaccine immunogens

POS-02-015 Proteomic Analysis of HLA Class I Binding Peptides from Prostate Cancer Cell Lines to Seek for Novel Cancer Vaccine and Cancer Biomarker

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Background/Aim: Since antigenic peptides of the cancer-associated antigens presented on human leukocyte antigen (HLA) molecules are recognized by specific cytotoxic T-lymphocytes, they have the potential to become effective peptide vaccines for cancer immunotherapy. Antigenic peptides from human prostate cancer cells were analyzed for identification of candidates for novel cancer vaccine or cancer biomarker by mass spectrometry (MS).
Materials and Methods: Peptides binding to HLA-A*0201 and HLA-A*2402 obtained from human prostate cancer cells by acid-elution were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The source proteins of the peptides were determined based on the HLA-binding capacity listed on the Syfpeithi. mRNA expression of candidate source protein was analyzed by quantitative RT-PCR in cancer cell lines and normal tissue.
Results: We identified five peptides from human prostate cancer cell lines. TKLSA possibly derived from absent in melanoma 1-like protein, and RLRYT from trans-membrane protein 191C or c20orf201. mRNA encoding these proteins were expressed in various cancer cell types but none or very few in noncancerous tissues except for testis, cerebellum and ovary.
Conclusion: HLA class I-binding peptides of unique cancer-associated proteins, which could become novel cancer vaccine, could be identified by MS analysis. MS analysis might become a promising tool for the searching of novel cancer vaccines or cancer biomarkers.
Keywords: cancer vaccine

POS-02-016 Immune Complexome Analysis for Identifying the Immune Complex Antigens

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Immune complexes (ICs) produced during an immune response may reflect some aspects of an ongoing immune response. Therefore, the identity of antigens incorporated into ICs provides the information that in the future may aid in the development of diagnosis and treatment strategies for autoimmune diseases, infection and cancer. Here, we propose a novel proteomic strategy (immune complexome analysis) consisting of immune complexes (or antigen-antibody complex) isolation from serum and direct tryptic digestion followed by nano-liquid chromatography-tandem mass spectrometry for the identification and profiling of antigens in circulating immune complexes (CICs). At first, the effects of gradient elution program and column types for nano-LC (C18-packed, C8-packed, silica monolith, and packed spray capillaly columns) on the numbers of identified peptides and proteins were investigated. The longer gradient elution time (25-600min) provided higher identification capability and the packed spray capillaly column allowed the identification of largest number of peptides and proteins. We applied immune complexome analysis to the analysis of CICs in the patients with rheumatoid arthritis (RA). Sera samples from healthy donors and osteoarthritis patients were used as controls. CIC-associated thrombospondin-1 (TSP-1) was found in 81%, and CIC-associated platelet factor 4 (PF4) was found in 52% of established RA patients, but neither protein was found in CICs from any of the controls. Furthermore, CIC-associated TSP-1 was found only in early RA patients and was found in neither other disease controls (Sjögren's syndrome and systemic lupus erythematosus patients) nor healthy donors.

Keywords: immune complexes, rheumatoid arthritis, optimization

POS-02-017 Shotgun Proteomic Analysis of Human Multiple Sclerosis Lesions

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Multiple Sclerosis (MSc) is a chronic inflammatory disorder of the CNS. Oligodendrocytes and their myelin sheaths are lost in focal white matter lesions, compromising the capacity of axons to conduct electrical impulses and it can lead to axonal/neuronal loss. Remyelination is an endogenous repair mechanism where oligodendrocyte progenitor cells are recruited from the surrounding tissue and regenerate the myelin, a process which fails with increasing disease duration. The molecular pathways that underpin these processes are poorly understood. In this study we examine the proteome of characterised MSc lesions and their corresponding perilesional regions using a quantitative shotgun proteomics approach.

Ten micrometer formalin-fixed sections containing MSc lesions from 3 secondary progressive MSc patients were characterised using oil red O (ORO) and luxol fast blue. For proteomics, fifteen 50 μm ORO-stained sections containing chronic, late remyelinated and recently active lesions and their corresponding periplaque regions were collected via laser guided manual dissection. Proteins were extracted, digested, labelled with iTRAQ tags and analysed by 2-D LC-MS/MS. Differentially abundant proteins were determined using ProteinPilot.

A two-sample unequal variance T-test performed on tissue areas that were sampled resulted in 15 pair-wise comparisons identified over 200 differentially abundant proteins. Analysis using Ingenuity Pathway Analysis (IPA) revealed the majority of the proteins were involved in these networks: cellular assembly and organisation, nervous system development and function, cellular movement and cellular function and maintenance. A number of proteins, such as Stathmin I, had biological functions that may be important in the remyelination process and were investigated further using immunohistochemistry.

Keywords: multiple sclerosis, remyelination, iTRAQ

POS-02-018 Identification of Novel ACPA Targets in Rheumatoid Arthritis Synovial Tissues Using 2D Gel Electrophoresis and Mass Spectrometry

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Background: Rheumatoid arthritis (RA) is an inflammatory autoimmune disease characterised by synovial joint inflammation that leads to degradation of cartilage and the underlying bone. Presence of anti-citrullinated protein/peptide antibodies (ACPA) in 60-70% of patients with RA is one of the major characteristics of the disease and associates with a more aggressive disease course, suggesting a direct pathogenic involvement of ACPA in disease initiation and progression. In this study, we aim for the identification of novel ACPA targets in synovial tissues of patients with RA. **Material and methods:** RA synovial tissues were obtained from patients undergoing joint replacement surgery for rheumatoid arthritis. Proteins, extracted from pulverised frozen synovial tissues and solubilized in lysis buffer, were resolved in 2D PAGE. Separated proteins were directly transferred to a nitrocellulose membrane and probed with human ACPA pool obtained using CCP2 affinity columns (EuroDiagnostica, Sweden) as described previously[1]. Human IgG and CCP2 flow-through fraction were used as control antibodies. Silver stained gel spots, corresponding to WB signals, were extracted from 2D gels, in-gel digested, and resulting peptides were identified using mass spectrometry. **Results:** By combining 2D gel electrophoresis with mass spectrometry, we have identified several novel potential ACPA targets as well as already characterized proteins. It remains to demonstrate if these proteins are citrullinated. **Conclusions:** Here we demonstrate an extensive ACPA reactivity against novel proteins in RA synovial membranes. The results encourage further exploration of the role of these proteins/peptides in rheumatoid arthritis both as additional biomarkers as well as their potential roles in the pathogenesis of RA.

Keywords: ACPA, rheumatoid arthritis, 2D-PAGE

POS-02-019 Identification of Minor Antigens by Quantitative Proteomics and Its Impact on Next Generation Leukemia Immunotherapy

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Allogeneic hematopoietic cell transplantation (AHTC) via graft vs leukemia effect (GVL) is an effective approach to eradicate leukemic cells. The curative effects of AHCT is mainly associated to immune system cells that recognize or target tumor minor histocompatibility antigens (MiHAs). These antigens are peptides presented by MHC I molecules and are derived from polymorphic genomic regions that give rise to amino acid substitutions. Although approximately 200,000 subjects diagnosed with hematological cancers (HC) have been cured with AHCT, the use of adoptive immunotherapy is relatively rudimentary and is hampered by the variable anti-HC activity of AHCT, and the risk of a devastating autoimmune response (or graft vs. host disease, GVHD). Currently, the unpredictability of these two factors seriously limits the success rate of immunotherapy. The enhancement of anti-HC activity for immunotherapy relies on the identification of patient-specific MiHAs that can be targeted by primed T cells.

Here, we present a novel approach that combines genomics, transcriptomic and MS-based proteomics data to profile and identify human MiHAs. HLA-matched B-lymphocytes from different individuals were used to generate personalized protein databases using *in silico* translation of genome/transcriptome sequencing data. MHC-I peptides isolated from B-cells by mild acid elution were analyzed by LC-MS/MS on a LTQ-Orbitrap Elite and searched against individual and human reference databases using Mascot. Our high throughput quantitative proteomics platform identified more than 5000 MHC-I peptides from B cells of HLA-identical siblings, of which typically 0.3-2 % were identified as MiHAs. This study represents the first large-scale genomic-proteomic analyses of MiHAs and opens up new avenues for leukemia immunotherapy.

Keywords: immunology, cancer immunotherapy, cancer antigens

POS-02-020 Extensive Phenotypically Characterization of Exosomes Derived from Activated, Non-Activated APCs and T-Cells Using an Extracellular Vesicle (EV) Array

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It has been known for several decades that major histocompatibility complex II (MHCII) can be found on human CD4+ T cells after activation and the presence of this molecule on the T cells might be facilitated by transfer from antigen presenting cells (APCs), such as dendritic cells (DCs).
In this study it was investigated whether MHCII is transferred via exosomes from human APCs to CD4+ T cells during co-cultivation. MHCII was detected on the T cells after co-culturing, indicative of a molecular transfer.
The co-stimulatory protein CD86 could also be detected on the T cells after the co-cultivation. The combination of these two molecules is normally only found on APCs and the thus obtained results could suggest an unknown role of CD4+ T cells as APCs.
Blood samples were obtained from healthy donors with known MHCII types. Blood mononuclear cells (MNCs) were isolated and CD4+ T cells were positively selected and B cells were negatively selected. Immature and mature monocyte-derived dendritic cells (MDDCs) were generated from human MNCs. The cells were then cultured separately or co-cultured in various ratios (APC:T cell). Exosomes produced during the maturation, activation and cultivation were phenotyped for the presence of 21 different antigens using an Extracellular Vesicle (EV) Array. The EV Array is based on the antibody capture of exosomes by the 21 individual surface exposed proteins and subsequent detection of the captured microvesicles by biotin labeled anti-tetraspanin antibodies (CD9, CD63 and CD81).
The quantification of exosomal presence of CD3, CD4, CD45, CD80, CD83, CD86, MHC I and MHC II were performed together with known exosomal markers as Alix, CD9, CD63 and CD81. The amount of exosomes produced and their phenotypes showed variance in relation to different activation states and co-culturing circumstances.

Keywords: Antigen Presenting Cells (APC), Exosomes, Extracellular Vesicle (EV) Array

POS-02-021 Systems-Level Analysis of Proteolytic Events and Post-Translational N-Acetylation *In Vivo* in Increased Vascular Permeability and Complement Activation in Skin Inflammation Revealed by N-Terminomics Analyses

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In inflammation, local tissue responses are augmented by complement and acute phase proteins that exude into the tissue because of increased blood vessel permeability mediated by bradykinin, which is proteolytically released from kininogen. We quantified changes in the proteome and the nature of protein N-termini (the N-terminome) and the altered abundance of murine proteases and inhibitors during skin inflammation. Through analysis of the N-terminome by iTRAQ-terminal amine isotopic labeling of substrates (TAILS), we identified cotranslational and posttranslational αN-acetylation motifs *in vivo*, quantitative increases in protein abundance, and qualitative changes in the proteolytic signature during inflammation. Of the proteins identified in normal skin, 50% were cleaved, which increased to 60% during inflammation caused by phorbol esters, including chemokines and complement in which we identified previously uncharacterized cleavage sites. In mice deficient in matrix metalloproteinase 2 (MMP2), exudation of serum proteins was diminished compared to that in wild-type mice, and their proteolytic networks differed. Quantitative analysis of the neo-N terminal peptides revealed a novel MMP2 cleavage site in complement 1 (C1) inhibitor that was detected *in vivo*. Cleavage and inactivation of the C1 inhibitor by MMP2 increased complement activation and bradykinin generation by plasma kallikrein in wild-type mice, leading to increased vessel permeability during inflammation. In the absence of MMP2, the intact C1 inhibitor levels rose and exerted negative regulatory effects on complement activation and generation of bradykinin by reducing plasma kallikrein activity and kininogen cleavage. Our degradomics analysis dissecting proteolysis in skin inflammation demonstrated perturbance of the proteolytic signaling network and its functional consequences arising from lack of a single protease.

Keywords: terminomics, alpha amine acetylation, degradomics

POS-02-022 Comprehensive Analysis of Aberrantly Glycosylated Proteins in Rheumatoid Arthritis

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Objective.
To identify aberrantly glycosylated proteins and investigate their immunological and pathological functions in rheumatoid arthritis (RA).
Methods.
Proteins extracted from peripheral blood mononuclear cells (PBMCs) of patients with RA and of healthy (HL) donors were separated by 2-dimentional electrophoresis (2DE). Then, the whole proteins and glycoproteins were detected by SYPRO Ruby® and Concanavarin A (ConA) recongnizing Man (a-1,3) [Man(a-1,6)] Man of *N*-glycans, respectively. Mannosylation levels of each detected glycoprotein were compared between the RA and HL groups. Proteins differently glycosylated between the two groups were identified by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS) and MALDI-TOFTOF/MS. Further, the antigenicity and enzymatic activity of superoxide dismutase 2 (SOD2), one of the identified proteins, were examined.
Results.
465 glycoprotein spots were detected by 2DE and ConA-blotting, 11 out of which showed different glycosylation between the RA and HL groups ($P < 0.05$). Seven out of the 11 protein spots were identified. One of the identified proteins, SOD2, which showed very low glycosylation in the RA group, was further investigated from the immunological and functional aspects. As a result, autoantibodies to the amannosyl-SOD2 were detected in 8 (40%) out of tested 20 patients with RA. Functionally, the antioxidant activity of SOD2 was decreased by deglycosylation.
Conclusion.
Seven proteins including SOD2 were found to show altered mannosylation in RA. The amannosyl-SOD2 may play pathogenic roles in RA through the autoantigenicity and the decreased enzymatic activity.

Keywords: autoantibody, peripheral blood mononuclear cells, rheumatoid arthritis

POS-02-023 Proteomic Analysis of Effects of Antirheumatic Drugs on Exosomes Derived from Synovial Sarcoma Cells

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Background. Exosomes are nanometer-sized vesicles that have been reported to have immune-modulatory functions recently. At present, effects of disease-modifying antirheumatic drugs (DMARDs) on exosomal protein profiles remain to be elucidated. **Objective.** To assess effects of DMARDs on synovial cell line-derived exosomes. **Methods.** We used human synovial sarcoma cell line of SW982 as a model for rheumatoid arthritis-derived synovial cells. SW982 were cultured in media containing IL-1β and/or DMARDs (methotrexate (MTX) and Salazosulfapyridine (SASP)). Exosomes were isolated from the conditioned culture media using Exoquick-TC. Then, exosomal proteins were comprehensively analyzed by 2-dimensional differential image gel electrophoresis (2D-DIGE). Protein spots intensity of which was significantly altered by the treatment with DMARDs were identified by mass spectrometic analysis. **Results.** 294 protein spots were detected in the exosome preparations. Among them, 8, 10, and 21 spots showed more than $\pm$2.0-fold expression with statistical significance in SASP-, MTX-, and SASP+MTX-treated groups respectively, as compared with the group without DMRAD. Similarly, 62 spots showed significantly different expression between the control and IL-1β groups. The IL-1β-induced change of 22 spots were suppressed by the simultaneous addition of DMARDs. **Conclusion.** Exosomal proteins derived from synovial sarcoma cells are affected by DMARDs and IL-1β. A part of the effects of IL-1β on the exosomal proteome were suppressed by DMARDs.

Keywords: exosome, rheumatoid arthritis, disease-modifying antirheumatic drugs

POS-02-024 Ultra High-Density Peptide Microarrays Reveal a Receptor Domain as Novel Autoimmunity Target in Multiple Sclerosis

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Broad scaled screening and profiling of autoantibody repertoires for the discovery of new autoimmune targets requires large antigen collections representing a significant part of the human protein coding genes. We have here utilized both planar and bead based arrays containing protein fragments from the Human Protein Atlas as well as ultra-high-density peptide microarrays for the profiling of plasma and CSF samples within multiple sclerosis. More than 11.000 protein fragments, representing more than a third of the human protein coding genes, on planar microarrays were initially screened with 90 plasma samples and followed up by a verification phase with 380 samples on 380 targets on suspension bead arrays. The outcome was 51 verified protein fragments as potential autoimmunity targets differentiating between subgroups of multiple sclerosis. An ultra-high density peptide array platform was also utilized for screening and discovery of novel autoimmunity targets. The peptide arrays were designed with either 2 million peptides with whole proteome coverage with 6 out of the 12 amino acids overlap or 150.000 peptides representing a selected set of targets and then also enabling a higher resolution with 11 amino acids in overlap. The whole proteome arrays revealed a frequently repeated and specific part of a receptor domain as a potential novel autoimmunity target in multiple sclerosis. A subsequent high resolution design of peptide arrays was utilized for further exploration with larger sets of samples and increased understanding of the autoimmunity targets identified through both screening of protein fragment as well the high density peptide microarrays.

Keywords: peptide microarrays, autoimmunity, multiple sclerosis

POS-02-026 Global Quantitative Analysis of IL-2 and IL-15 Signaling Pathways

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Interleukin-2 (IL-2) and IL-15 are the only members of the common cytokine receptor gamma chain family sharing also the IL2Rβ subunit. Not surprisingly, both cytokines have overlapping functions. However, they also have distinct and often contrasting roles, as evident from the different phenotype showed by IL-2$^{-/-}$ and IL-15$^{-/-}$ mice. So far, the paradox of how IL-2 and IL-15 produce divergent functions despite signaling through the same receptor subunits remains to be elucidated. Attempts to identify any difference in the signaling profiles of IL-2 and IL-15 using traditional and targeted approaches have failed so far. Recent comparison of the IL-2 and IL-15 quaternary complexes also did not reveal significant differences that may account for the functional discrepancies between the two interleukins. Nevertheless, a global quantitative analysis of the signaling pathways initiated by both cytokines may result in the identification of key factors that would provide a better understanding of the opposing roles of IL-2 and IL-15. In order to compare the signaling events triggered by the two interleukins, we combined SILAC with enrichment of tyrosine-phosphorylated proteins followed by mass spectrometry analysis. Our results demonstrate that even if the signaling cascades triggered by IL-2 and IL-15 are markedly similar, a discrete number of differences do exist. Further characterization of those discrepancies will shed light on the functional dichotomy of both interleukins and may provide new insights for the development of specific immunotherapies based on IL-2 or IL-15.

Keywords: interleukins, quantitative phosphoproteomics, signaling pathway

POS-02-025 Differential Proteomic Analysis of Human Hippocampal Regions of Interest (CA1, CA2, CA3, fascia dentata) - Relevance for Alzheimer's Disease

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In an aging world Alzheimer Disease (AD) is the most common form of dementia. Regarding the last 20 years of scientific research we conclude that the reason for AD might be more complex then known so far. Engelhardt and colleges (Engelhardt et al., 2000) demonstrated that the injection of human IgGs from AD patients into the basal forebrain of living rats results in a loss of cholinergic neurons. Giving special tribute to this work we postulate that AD is a disorder with an autoimmune character which starts in the early lifespan of human beings. Furthermore Braak et al. (2000) observed that neuronal damage of the different regions of the human hippocampus (CA1, CA2, CA3, fascia dentata) occurs in a time dependent matter, a process which might be also forced by an autoimmune reaction. The CA1 region is affected early, while the damage of the CA2, CA3 region and the fascia dentata is observed in a later state (Braak et al., 1991). Taken all this together we decided to analyze the content of these hippocampal regions of interest. We use a broad spectrum of classical biochemical methods and modern technologies like laser microdissection to isolate the hippocampal brain regions of interest from post mortem AD and control samples. To obtain valid data concerning the difference between these regions we perform a differential proteomic study by a label free LC-MS/MS approach combined with a couple of functional analyses.

Keywords: Alzheimer disease, autoimmune

POS-02-027 Improving Chr-16 Proteins Coverage with High Confidence and MIAPE Compliant MS Data

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A pilot shotgun study was conducted among 8 laboratories of the Chr-16 (Spanish) HPP consortium. The main goals of this experiment are: 1) to provide "in house", high confidence experimental MS data of the Chr16 proteins that could be used to develop SRM/MRM assays, 2) to define the standard operation procedures for further large scale experiments, 3) to submit generated data on the ProteomeXchange repository according to the HUPO PSI's guidelines and 4) to get information about the total proteome coverage of high confidence by integrating different mass spectrometry analysis platforms.

Three human cell lines were selected, MCF7 breast cancer epithelial cells, CCD18 colon fibroblasts, and Jurkat T lymphocytes since a combination of fibroblasts, lymphoid and epithelial cells could provide theoretically up to 71% coverage of chromosome 16 proteins. This coverage was confirmed by transcriptomic experiments (J Proteome Res. 2013;12:112-22).

A robust pipeline to submit full MIAPE compliant data to PRIDE/ProteomeXchange repository were developed and 24 large scale shotgun 2D-LC-MS/MS experiments were performed in different MS platforms, giving a 45% Chr-16 protein coverage. However, data analysis did not show a clear-cut protein number as several factors may influence decisively on the final number of identified proteins or protein groups. The protein grouping issue and the level at which FDR is applied (peptide or protein) might generate variability in the number of proteins. The total coverage were improved by the combination of cell lines, extraction/fractionation procedures and analytical platforms more than accumulation of replicas from the same laboratory, underlying the importance of a multi-centric approach for this kind of projects. Properties and features of the detected and undetected peptides have been evaluated in order to identify experimental conditions that could improve the proteome coverage.

Keywords: HPP, networking, data standarization

POS-02-028 Dissecting Subcellular Compartments for Deep Proteome Coverage of Chromosome 16 in T Cells

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As part of the Chr-16 (Spanish) Consortium, our group set out to dissect the human T lymphoblast cell line (Jurkat cells) for deep proteome profiling. Current shotgun analyses reach on an average 50% coverage of the human proteome. To further complete this data, we focused on certain protein subsets including plasma membrane and secretoma, since these subproteomes are down-represented in crude cell lysate analyses.

We combined different protein extraction methods with gel electrophoresis/in-gel digestion and in-solution digestion/off-line RP-HPLC at basic pH in order to gain coverage of all subsets of proteins as well as to establish optimal SOPs. Sequential digestion with Lys-C and trypsin was also performed for better protein digestion efficiency. We have faced protein grouping inference following the PAnalyzer algorithm, identifying 8,912 different protein groups with 93,310 unique peptides, covering 44% of the human genome and near 50% of the Chr16 protein-coding genes expressed in Jurkat cells (FDR below 1% protein level).

We then analyzed particular cell compartments using cell fractionation methods to enrich low abundance proteins. Overall, according to GO terms, we identified about 30% of the membrane proteins coded by Chr16 genes. We also analyzed the secretome of Jurkat cells under different stimuli, identifying about 10% of Chr16 proteins annotated as secreted but not observed in previous experiments. Our overall analyses enabled the identification of more than 9,200 protein groups. This information constitutes the data source to set up targeted analyses (SRM and pseudo-SRM) for a particular subset of the observed Chr16 proteins. These methods will be employed for routine measurement of Chr16 proteins in different cell lines or tissues and, importantly, will be translated to disease-related studies.

Keywords: Chr16 proteome coverage, MRM, HPP

POS-02-029 First Draft of the Human Proteome

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Although the human genome sequence has been established more than a decade ago, it is still unclear how many of the roughly 20,000 human genes exist as proteins. Here, we have undertaken to identify the complete protein complement of the human genome using experimental evidence provided by mass spectrometry based proteomics. To build the draft of the human proteome, we analyzed >100 human cancer cell lines, >50 human tissues and body fluids as well as hundreds of affinity purifications. A large number of experiments were downloaded from repositories or contributed by other labs. All data were processed via two parallel pipelines using Mascot and MaxQuant/Andromeda and results were loaded into ProteomicsDB which is based on the SAP HANA in-memory computing technology for subsequent data mining. At the time of writing, the assembled proteomic data consisted of >500,000 distinct fully tryptic peptides at 1% FDR. These map to >18,000 human genes representing >90% coverage of the human proteome. Analysis of cell lines contributed ~12,000 proteins, human tissues extended this to ~16,000 while body fluids and affinity purifications contributed the remainder. Interestingly, all but the Y-chromosome are evenly represented. ProteomicsDB not only provides new protein evidence for thousands of genes, it also highlights an astounding diversity of protein expression in different tissues. Many proteins were detected exclusively (but at high levels) from rather special sources (e.g. earwax) while others showed stable expression across many cells. The current evidence suggests that proteome coverage will not be significantly increased by adding more high-throughput data. Instead, more focused experiments will likely fill the gaps. By engaging the scientific community via ProteomcisDB, we believe that we can provide protein evidence for every human gene by the end of 2013 before taking the next steps to tackle protein isoforms and modifications.

Keywords: human proteome

POS-02-030 Identification and Characterization of Cow's Milk Proteins from the Rat Intestinal Lymph Using a Proteomic Strategy

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Traditionally, food proteins were considered to be absorbed into the body through the small intestine after being digested by enzymes into amino acids, dipeptides and tripeptides. However, there were studies indicated that some proteins can pass through the intestinal epithelium under normal physiological conditions, perhaps not in sufficient quantities to be of nutritional importance, but in quantities that may be antigenically or biologically active. It has been reported that presentation of food antigens to T cells is more efficient and long-lasting in the mesenteric lymph nodes than in the peripheral lymphoid organs. In the present study, rat intestinal lymph samples were collected using a modified lymph fistula rat model in fasted and cow's milk postprandial states. Low molecular weight proteins were enriched by ultrafiltration and differential solubilization, separated by 1D-SDS PAGE, digested in-gel based on molecular weight and identified using nanoLC-MS/MS. In the postprandial rat intestinal lymph, 9 bovine-specific proteins were identified in different molecular weight regions, which implied that they might enter the lymph in diverse forms. Most proteins identified in the lymph were highly abundant proteins in the milk, such as β-lactoglobulin and caseins. The physicochemical properties, structure and post-translational modifications of these proteins were also analyzed. Seven of the 9 identified bovine-specific proteins are major allergens in milk. This strategy can be used to search for proteins that can enter the intestinal lymph and analyze their common features. Understanding the common features of these proteins might help to develop protein drugs taken orally.

Keywords: lymph fistula rat model, bovine-specific proteins, proteomics

POS-02-031 LC Retention Behavior of Tryptic Peptides in Proteomics LC-MS

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In LCMS-based shotgun proteomics, tryptic peptides are separated by means of reversed-phase LC as the front end of MS to reduce the sample complexity prior to MS and MSMS analyses. Therefore, maximizing the separation capability in LC is a key step to maximize the proteome coverage in shotgun proteomic analysis. In this study, we aimed to optimize the LC conditions to increase the number of identified peptides in LC-MS by elucidation of the retention behavior of tryptic peptides. At first, we examined the retention behaviors of alkylbenzenes and peptides using particle-packed columns and a monolithic silica column under the isocratic conditions. Retention factors of peptides were highly sensitive to the content of organic solvent, compared with those of alkylbenzenes, independent of the column length, the particle pore size and the packing material. Under the gradient conditions with constant t_G/t_0 (t_G ; gradient time), optimum t_G was obtained to provide maximum peak capacity (*Pc*) for alkylbenzenes, whereas *Pc* increased linearly and larger t_G/t_0 gave larger *Pc* as t_G increased for peptides. These results suggest that the use of longer column, lower flow rate and shallower gradient would give larger *Pc* in peptide separation. To validate this assumption, we employed two different columns such as 17 cm long C18-silica particle-packed column and 100 cm long C18-silica monolithic column under the various conditions for analyzing trypsin digest of HeLa cells. At the same flow rate, the number of the identified peptides increased as t_G was longer, while lower flow rate gave more identified peptides. We will apply this optimized LC-MS system with the meter-long C18 monolithic silica column to uncover the entire human proteome analysis.

Keywords: HPLC, monolithic silica column

POS-02-032 Maximizing Spectrum Identification Rate in Shotgun Proteomics on the Q Exactive Mass Spectrometer

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Due to its high sensitivity and fast scan speed, Q Exactive mass spectrometer has been widely applied in shotgun proteomics field. A typical shotgun proteomics experiment is data-dependent-TopN experiment, in which tens of thousands of MS/MS spectra are acquired. The quality of a MS/MS spectrum, in terms of signal-to-noise ratio, mass accuracy and numbers of fragments, determines whether a MS/MS spectrum identify a peptide via database search. The number of ions analyzed (AGC target) has an impact on the above parameters, while unnecessary high exposure of ions to the instrument could result in shortened cleaning frequencies. In this application, different AGC values (2e4, 5e4, 1e5, 5e5, 1e6, and 3e6) and data-dependent parameters are investigated to optimize the shotgun analysis on the Q Exactive.

1 µg of *Hela,* tryptic whole cell lysate digest, separated over a 70 min gradient via a reversed-phase chromatography. MS data was acquired using a data-dependent top15 method. With a total of 95mins MS acquisition time, > 40,000 MS/MS spectra were acquired per run with all AGC target values. The number of identified proteins and peptides per single run increases while the AGC target value increases until 1e5, where the maximal number of identified proteins and identified unique peptides are reached. An average of ion injection time for MS/MS scans is around 30ms for the AGC target value 1e5. With a ten time higher target values for MS/MS, 1e6, the maximal injection time of 60ms is always reached, which slightly increases the duty cycle and results in less MS/MS spectra in total per run. Good quality of MS2 spectra can already be achieved with 5e4, with a comparable peptide identification score to 1e6. 1e6 target ions for MS/MS do not increase the number of identified proteins while unnecessarily exposing the ion optics to ions.

Keywords: Q Exactive, protein identification number, AGC target value

POS-02-033 Proteomic Analysis of the *abi3* Deletion in *Physcomitrella patens*

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The plant specific transcription factor ABSCISIC ACID INSENSITIVE3 (ABI3) functions in ABA signaling during seed maturation and germination. We previously demonstrated that ABI3 is evolutionarily conserved in the moss *Physcomitrella patens*. Furthermore, both ABA and ABI3 are required for *P. patens* vegetative tissue to survive desiccation, similar to seeds of angiosperms. To reveal the regulatory network controlled by ABA and ABI3 during the desiccation-rehydration process, we performed quantitative proteomics using iTRAQ (Isobaric Tags for Relative and Absolute Quantification). A total of 3582 proteins were identified with a false discovery rate of less than 1%. A subset of 94 proteins from this group were increased more than 2.5-fold with ABA treatment. Interestingly, most of these proteins were also increased in ABA-treated *abi3* mutant plants. Only four proteins required both ABI3 and ABA, suggesting that the gene regulatory pathway that requires both ABA and ABI3 control a relatively few proteins, and that these proteins are likely to be essential for desiccation tolerance in vegetative tissues of bryophytes. We note that genes encoding three out of the four proteins are also conserved in Arabidopsis. Expression of these Arabidopsis genes is also under the control of ABI3 and are specifically expressed in late stage of seed maturation, when seeds acquire desiccation tolerance. It might be suggested that this ABA- and ABI3-mediated regulatory network was established in the ancestral land plants to regulate a set of genes, which confers cellular desiccation tolerance of land plants.

Keywords: transcription factor, phytohormone abscisic acid, moss *Physcomitrella patens*

POS-02-034 Proteomics Analysis of USP20 for Identifying Its Functions Through the Characterization of Putative Substrates

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USP20, one of deubiquitinating enzymes (DUBs) belonging to the subfamily of ubiquitin-specific protease (USP), regulates ubiquitin-mediated protein degradation. In order to investigate the biological functions of USP20, we searched for its substrates through two-dimensional electrophoresis (2-DE) and MALDI-TOF/MS analysis. We found several putative substrates, and some of them are related to cancer metabolism or neural disorders. In addition, we generated a polyclonal and two monoclonal antibodies against USP20 and confirmed through immnunoblotting, immunoprecipitation, and immunofluorescence analyses. Since USP20 is known to bind a protein associated with von Hippel-Lindau (VHL) disease tumor suppressor (pVHL) stability, it may be useful for investigating mechanisms of pVHL-derived carcinogenesis with regard to USP20 and its substrates. VHL disease generatesbenign and malignant tumor in the kidney or the central nervous system and pVHL suppresses that. Although several studies revealed the roles of various DUBs, the functions of USP20 in cellular mechanisms are poorly understood. Thus, our data will help us to elucidate the biological functions of USP20 in cellular and disease-related pathways.

Keywords: deubiquitinating enzyme

POS-02-035 The Effects of 5-Fluorouracil on the Proteome of Colon Cancer Cells

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The pyrimidine analogue 5-fluorouracil (5FU) is widely used as a treatment for solid tumours but its mechanism of action is not fully understood. We have used mass spectrometry to comprehend the overall mechanism of action of 5FU and for that we have measured the effects of this drug on the composition and on the turnover of the proteome of RKO colon cancer cells. We have shown that 5FU has a massive effect on the proteins involved in RNA metabolism. After only one hour of treatment, 5FU causes a reduction in the abundance of components of the translation machinery (mostly ribosomal proteins), and this reduction is accompanied by a down-regulation of the translational capacity of the cells. Neither rapamycin nor raltitrexed, two drugs that also block cell proliferation, reduce the abundances of ribosomal proteins as 5FU does, which suggests that the down-regulation of ribosomal proteins is coupled to the mechanism of action of 5FU. This reduction is also seen when analyzing two other types of colon cancer cells. These results show that the effect is at the protein level, and they conflict with previous reports based on RNA quantification. This shows how important it is to complement RNA profiling studies with analyses of drug toxicity in a proteomic scale. Our study has identified many novel proteins that are affected by 5FU after very short exposure times. The usefulness of these newly identified molecules as therapeutic targets can now be investigated, and we hope that the findings in our work will lead to improvements in the efficacy of 5FU-based therapies.

Keywords: mass spectrometry, cancer, ribosomal proteins

POS-02-036 Targeting Cancer Metastasis Using Global, Quantitative and Integrative Network Biology

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Cell behavior and phenotype are driven by the complex integration of cellular and environmental cues. Phosphorylation networks play a major role in these processes and are often involved in disease progression. By conducting an integrative and quantitative network analysis using data from (phospho) proteomics, genomics and RNAi screening, we set out to comprehensively explore the phosphorylation networks underlying metastasis in colon cancer, in an attempt to identify potential network drug targets and novel therapeutic strategies.

We characterized global quantitative phosphorylation dynamics from metastatic and non-metastatic SILAC-labeled cell lines, and identified >15,000 confidently localised phosphorylation events that may be important for the metastatic phenotype. Through exome sequencing of the cell lines, we identified 8,375 point mutations in 5,040 genes, many of which may give rise to the differential signaling network dynamics associated with enhanced metastasis. Additionally, to characterize the role of the observed phosphorylation dynamics, we modeled their regulating kinases using the quantitative MS data combined with our novel KinomeXplorer algorithm. To experimentally validate the resulting network models, we deployed a kinome-wide siRNA screen to investigate which kinase perturbations affect the proliferation of metastatic cells *in vitro*. Finally, in order to assess the relevance of our cell line based network models for a representative patient population, we analyzed 16 patient tumors originating from 4 different colorectal cancer stages using global quantitative MS and deep sequencing.

Data from these experiments were computationally integrated in order to establish an integrative network model. From this model, we are currently testing combination therapies in *in vivo* mouse models. Ultimately, these results can potentially aid the development of a network-based therapeutic strategy for treatment of metastatic colon cancer.

Keywords: quantitative mass spectrometry, network medicine, cancer metastasis

POS-02-037 Profiling and Annotation of Human Kidney Glomerulus Proteome

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The comprehensive analysis of human kidney glomerulus, we previously performed, provided a dataset of 6,686 unique proteins representing 2,966 distinct genes. Since this dataset contained a considerable redundancy, we reanalyzed the raw data files using the Mascot search engine under highly stringent criteria, which generated a high-confidence, non-redundant dataset of 1,817 proteins representing 1,478 genes and enabled us to perform extensive profiling, annotation, and comparison with other proteome datasets. These proteins were represented by 2-D protein array specifying observed MW and pI range of identified proteins, which indicated obvious differences in the observed and calculated physicochemical properties. Characteristics of glomerular proteome could be illustrated by GO analysis and protein classification. The depth of proteome analysis was well documented via comparison of the dynamic ranges of identified proteins with other proteomic analyses of human glomerulus, as well as a high coverage of biologically important pathways. Comparison of glomerulus proteome with human plasma and urine proteomes suggested the extent and characteristics of proteins contaminated from plasma and excreted into urine, respectively. Among the latter proteins, several were demonstrated to be highly or specifically localized in the glomerulus by cross-reference analysis with the Human Protein Atlas immunohistochemistry database, and could be biomarker candidates for glomerular injury. Furthermore, comparison of ortholog proteins identified in human and mouse glomeruli suggest some biologically significant differences in glomerulus proteomes between the two species. In conclusion, the high-confidence, non-redundant dataset of glomerular proteins could provide a more extensive understanding of human glomerulus proteome and could be useful as a resource for the discovery of biomarkers and disease-relevant proteins.

Keywords: human glomerulus proteome, human plasma proteome, human urine proteome

POS-02-038 Unraveling the Role of Progesterone in Cerebral Aneurysm Among Postmenopausal Women: Utilizing HBMECs

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The molecular pathogenesis of diseases has become a crucial step in the development of new treatment strategies. Although the pathogenesis of cerebral aneurysms (CAs) has been studied intensively, it is poorly understood. Hemodynamic stress in the form of hypertension, arteriosclerosis and secondary inflammatory reactions are thought to be elementary preconditions. The incidence of CA disease in postmenopausal women is more than in men of similar age, and the menopause-associated increase in cardiovascular disease in women, has led to speculation that sex hormones have a key role in the development and evolution of CAs among this group. The increased rate of cerebral aneurysms among postmenopausal women has been documented; however, the molecular mechanism for this high rate is largely unknown. In order to understand the molecular pathogenesis of intracranial aneurysm, we utilized human brain microvascular endothelial cells (HBMEC) subjected to 17β-estradiol, progesterone, Fulvestrant, mifepristone and vehicle treated cells. This cell line was subjected to different forms of oxidative stress and the protective effects of E2 and PG4 were also studied. HBMEC were further fractionated into three subcellular components for detailed analysis. Different proteins and genes induced by these exogenous factors were characterized by Isobaric tags for relative and absolute quantitation (iTRAQ) after 72 hours of PG treatment. Bioinformatic analysis lends insight into the molecular mechanism of estrogen and progesterone in CAs. PG may be a major player in vascular wall protection, arrest of apoptotic markers. Progesterone Receptors in PGR- HBMEC may be a potential target for treatment of CAs in postmenopausal women.

Keywords: Cerebral aneurysm, Brain endothelial cells, Progesterone

POS-02-039 Protein Profile of HaCaT Cells Under UV Radiation and Lipoic Acid Treatment

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Oxidative stress (OS) has been recognized as a main pathologic pathway in aging and in age-associated diseases including diabetes, inflammatory, cardiovascular, and neurodegenerative diseases as well as cancer. Reactive oxygen species (ROS) are central role in oxidative stress and they are by-products which are made from cellular metabolism in the mitochondria primarily. Lipoic acid (LA) is an essential cofactor for mitochondrial enzymes and ideal antioxidant present in prokaryotic and eukaryotic cells. LA acts as strong antioxidant through several mechanisms, involving scavenging of ROS, chelation of metal ions, and regeneration of endogenous and exogenous antioxidants, such as glutathione, ascorbic acid and so on. Recently, LA was used as the potential antioxidant in cosmetic products to against the formation of wrinkle and melanin. We successfully applied two methods to detect LA in cosmetics. UV radiation is an important fact to cause cell damage in human skin. To mimic the antioxidative effect of LA in cosmetics, human keratinocytes (HaCaT) were utilized to investigate the protein profile with and without ultraviolet radiation (UVA and UVB). The cellular proteins were identified by nano liquid chromatography coupled with tandem mass spectrometry (nanoLC-MS/MS) after enzyme digestion. Protein identification was executed by peptide sequencing and MASCOT searching simultaneously. There are several apoptosis related proteins that are expressed or changed in the research. The results could help us to elucidate the antioxidative mechniam of LA in human skin.

Keywords: lipoic acid, HaCaT keratinocytes

POS-02-040 Protein Profile of Human Plasma After Acrylamide Exposure

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Acrylamide is a probable food or environmental carcinogen for humans and it is associated with several kinds of cancers. Its biological metabolite, glycidamide, is also harmful to human health. Acrylamide is found everywhere in our living environment (including biomedical laboratory and polymer industry) and this toxic chemical usually becomes an important issue for public health. Acrylamide and glycidamide could react with proteins to form new covalent bonds with target amino acids in metabolic processes. In this study, we used simple proteomic strategy to identify the acrylamide and glycidamide bound in abundant plasma proteins. After simple sample preparation, new protein binding sites by acrylamide and glycidamide were identified by using nano liquid chromatography coupled with tandem mass spectrometry. Only 10 μL of human plasma sample is required for protein binding sites survey by this micro-scale method. We also hope this strategy may help us to understand the metabolic pathways of acrylamide and glycidamide.

Keywords: acrylamide, glycidamide, binding site

POS-02-041 Comprehensive Peptide Searching Workflow to Maximize Protein Identifications

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Protein identification and characterization through mass spectrometry has become established method in recent years. However the number of identified proteins remains less than the actual number of proteins supposed to be present in complex biological samples. Undesirably, only a fraction of spectra generated have confident peptide matches for any complex biological sample. There are several factors that are being overlooked by many users in data searching strategy (e.g. appropriate combination of post translational modifications (PTMs), coding SNP, partially cleaved peptides etc.) that can possibly help identify these unmatched spectra. We herein develop a comprehensive MS/MS searching Proteome Discoverer workflow using combinations of multiple search engines (e.g., SEQUEST, SEQUEST HT, MS Amenda and Mascot) in an iterative fashion to maximise number of protein/ peptide identification. In Effect of various factors on peptide identification were explored and implemented in the process that includes protein isoforms, missed cleavage sites, semi tryptic digestion and most importantly appropriate combination of PTMs in each search node. The workflows were tested on a plasma digest sample acquired on a hybrid Orbitrap mass spectrometer. We compare the results of our comprehensive searching workflow with a standard SEQUEST and Mascot workflows. We found that on average, the number of high confidence peptides (FDR≤0.01) increased by approximately 45% in our workflow. The increase in the number of high confident grouped proteins was found to be 44%. Further analysis of the peptides that were exclusively identified with high confidence in the comprehensive workflow reveals that large fraction of them have multiple PTMs.

Keywords: protein identification

POS-02-042 Comparative Proteogenomic Analysis of *Streptococcus suis* by High Resolution Mass Spectrometry

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Streptococcus suis (*S.suis*) serotype 2 is an important human pathogen, causing more than 200 cases of severe human infection across the world. One of its virulent isolates in China, 05ZYH33 strain, caused the large-scale human streptococcal toxic shock syndrome outbreak in 2005. The whole genome sequencing of 05ZYH33 strain was finished in 2007. However, up to now, comprehensive proteome sequencing of the strain was not reported and accurate annotation of its genome remains challenging. In this study, in-depth proteome sequencing of *S.suis* 05ZYH33 was first implemented using high resolution mass spectrometry. Then comparative proteogenomic analysis of 05ZYH33 strain was carried out by database search of an *S.suis* complex database generated by integration of 05ZYH33 predicted protein sequences, six-frame translated sequences, and predicted protein sequences of other 16 *S.suis* strains. In all, 1,644 proteins from *S.suis* 05ZYH33 were identified representing 75.2% of its predicted genes. Besides, 157 novel peptides that only match to 05ZYH33 genome and other strains' protein sequences were identified and validated by spectra similarity comparation of the corresponding synthetic peptides. Based on these novel peptides, we discovered 27 novel protein-encoding genes in 05ZYH33 genome (5 of which are strain-specific novel genes), 41 new translation start sites, 11 frameshift mutations and 78 potential genome sequencing errors or SAPs. Finally, by the combination of bioinformatics analysis and experimental validation, a considerable proportion of new genes, new translation start sites and frameshift mutations were validated, which largely improved the genome annotation of *S.suis*.

Keywords: proteogenomics, pathogenic bacteria, genome annotation

POS-02-043 Analysis of the First Draft of the Human Proteome with ProteomicsDB

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ProteomicsDB is a new public protein centric database and data repository for mass spectrometry based proteomics facilitating the analysis of the human proteome. An easy to use and fast web interface allows users to browse the human proteome in a protein, chromosome or project centric fashion as well as upload/download data to/from the repository. ProteomicsDB also holds all the primary LC-MS/MS information and allows inspection of the tandem mass spectra underlying peptide identification. ProteomicsDB holds >10,000 raw files and >700 experiments from the analysis of over 100 human cell lines, 50 human tissues and body fluids as well as hundreds of affinity purifications. So far, we identified >18,000 human proteins representing >90% of the protein complement of the human genome with equal coverage of all (except the Y-) chromosomes. Analysis of protein expression across cell lines, tissues and body fluids highlights the astounding diversity and dynamic range of protein expression. Despite a high correlation of individual cell line proteomes, proteins may differ by orders of magnitude in expression between cell lines. These are particularly interesting since they may determine the functional make up of cell lines. Some examples are the stem cell transcription factors SOX-2, SALL4 and STAT3 or the immune cell specific tyrosine protein kinase BTK. ProteomicsDB has been implemented using SAP's HANA in-memory computing technology and is currently is backed with 50 TB of storage, 2 TB RAM and 160 processing units. A direct interface to the programming languages L, C++ and R allows calculations not possible with standard SQL. The webinterface is built upon a JavaScript framework for HTML5. At this stage, ProteomicsDB is confined to the human proteome, but extension to other organisms is envisaged.

Keywords: human proteome

POS-02-044 Molecular Signatures of Long-Lived Proteins: Autolytic Cleavage Adjacent to Serine Residues and Peptide Laddering

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Long-lived proteins are widespread within the human body. Over decades at body temperature, persistent proteins undergo structural damages and modifications that affect their functions. This phenomenon has been associated with age-related disorders such as cataract. However, its process is poorly understood. The center of the human lens is composed of proteins that were synthesized prior to birth. The lack of turnover, combined with an absence of proteolytic activity, allows this tissue to be used to evaluate the long-term stability of proteins. Utilizing nanoLC-ESI-MS/MS, we characterized 328 novel breakdown products (peptides) of adult human lens proteins. Prominently were peptides featuring N-terminal Ser and "peptide laddering" - the sequential loss of terminal residues with age. These cleavage characteristics could be reproduced by incubating synthetic peptides at elevated temperatures, suggesting that Ser residues may represent susceptible sites for non-enzymatic cleavage in polypeptides that are exposed to physiological conditions over long periods. MALDI-imaging and selected reaction monitoring analyses confirmed that these endogenous peptides emerged initially in the center of the lens at around middle-age, and increased significantly in abundance in later years. Larger protein complexes derived from the crosslinking of truncated lens proteins were also characterized using 2-D gel electrophoresis and LC-MS/MS. These findings suggest that Ser cleavages and "peptide laddering" may play a significant role in the degradation of long-lived proteins in the human lens. Such cleavages can severely compromise lens protein structures and lead to the accumulation of structurally-damaged proteins in the lens, leading to diseases such as cataract. More importantly, given the widespread distribution of long-lived proteins, the non-enzymatic cleavage mechanisms characterized here may have important implications for other age-related diseases.

Keywords: nanoLC-ESI-MS/MS, proteolysis, MALDI-imaging

POS-02-045 Proteomics Study of Serum from Patients with Acute Coronary Syndrome

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Acute coronary syndromes (ACS) including unstable angina pectoris (UAP) and acute myocardial infarction (AMI) is the major cause of sudden death in the world. It is an important cause of decrease in quality of life, disability, and contributes substantially to the escalating costs of health care. At present, the scientists summarized and given six notable signs of heart disease. However, this is just too late signs of the disease and it is easy to mistake them for other diseases. To have an accurate diagnostic for the best treatment from those laboratory tests, deeply understanding about the protein expression profiles and characterization of ACS patient's proteome is necessary. In this study, albumin-depleted sera from ACS patients (10 patients with AMI and 10 patients with UAP) and 10 normal volunteers, have been used for the proteome separation by SDS-PAGE/2-DE. After staining, gels with protein bands were cut and digested by trypsin. The peptide mixtures extracted from each gel slice were analyzed by Nano-liquid chromatography-electrospray ionization mass spectrometry (NanoLC-ESI-MS/MS). The proteins were identified, characterized by Mascot v1.8 software and validated by MSQuant v1.5 software. As the result, a proteome database including 1357 proteins in AMI group, 1431 proteins in UAP group, and 1365 proteins, was established. These proteins were classified into different functional categories, such as cellular component, biological process, molecular function and post translational modifications, based on their role according to Gene Ontology using bioinformatics tools. In addition, more informations of these proteins such as pI, mass, score, peptide matches were also defined. A list of proteins that might be associated with ACS has been proposed. Further research might be focused on these specific proteins for the potential biomarker discovery.

Keywords: acute coronary syndromes, LC-MS/MS, human serum proteome

POS-02-046 Preparation of Meter-Scale Monolithic Silica Capillary Column Modified with Urea Functional Group for Hydrophilic Interaction Chromatography and Its Application to One-Shot Proteomics Approach

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A meter-scale monolithic silica capillary column (2 - 4 m length and 100 μm internal diameter) modified with an urea-functional group for hydrophilic interaction chromatography (HILIC) was developed for the highly efficient separation of the biological components. It was possible to prepare the column with a minimum plate height of 12 μm for nucleosides and a permeability of 2.1 $\times$ 10^{-13} m^2, whose efficiency is likely catching up with that of existing long monolithic silica-C_{18} capillary columns and over 300,000 theoretical plates could be generated with a meter-scale column format (4 m in length). The further utility of the long ureidopropylsilylated monolithic silica capillary column was demonstrated by shotgun proteomics approach, especially as a support of "one-shot proteomics" which involves one-dimensional gradient HPLC with a long monolithic silica column, connected to an electrospray ionization (ESI)-mass spectrometry (MS). The recent approach of "one-shot proteomics" utilizes a long monolithic silica-C_{18} capillary column on reversed phase separation mode and provides the considerably high efficiency for the identification of peptides, however the complexity in biological systems continues to require more powerful separation systems to obtain the reasonable resolution and detection of components. Here, the proteomics strategy using a meter-scale ureidopropylsilylated monolithic silica capillary column as a HILIC mode offered the possibility of the further enrichment for peptides identification upon the existing "one-shot proteomics" strategy due to the orthogonal separation characteristics.

Keywords: monolith, HILIC, meter-scale

POS-02-047 Precise Column Temperature Control Enables Improved Protein Identifications in Proteomics Shot-Gun Sequencing Applications

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Temperature control is routinely used in laboratories to control peptide elution order and increase separation reproducibility. Here we use the ability to precisely control the temperature in a microcolumn format to deliberately change the elution time of the peptides and therefore improve number of identifications in shotgun proteomics experiments.

A tryptic digest of Hela were analyzed using top 15 HCD data dependent tandem MS method. The source temperature was set to be room temperature, 30degC, 35degC, 40degC, 45degC and 50degC. Under single temperature, heating control increased the reproducibility and reliability, by generating reduced retention time shift and similar total numbers of proteins and peptides in replicates. A temperature of 40degC was determined to be a good start for single proteomics analysis.

However, when replicated experiments were performed, same temperature resulted in 90% overlap between any two attemps, translating into a small increase contribution of each additional replicate experiment to the total number of proteins. A change in peptide elution order at different column temperatures were exploited to decrease the overlap between experiments significantly, thereby increasing the total number of protein/peptide identifications. The number of proteins/peptides detected by combining the results from three separate experiments performed at 40degC, 45degC and 50degC provided an additional 11%/25% increase compared to the triplicate runs at 40degC. This application of precise column temperature control is an inexpensive and effortless solution for proteomics users to achieve deep mapping of proteome without any hardware changes to the separation system or optimization efforts in the mass spectrometry method.

Keywords: proteomics, temperature, shot-gun

POS-02-048 Metaproteomic Analysis of Methanogens in the Thermophilic Reactor Treating Terephthalate at Different Loading

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Shotgun proteomic approach was used to study in situ activities of aceticlastic Methanosaeta and hydrogenotrophic Methanolinea in the terephthalate-degrading methanogenic reactor at the conditions of different loadings (3 vs 0.25 kg/m3-day). Totally, the 2-D nano HPLC combined with Obitrap mass spectrometry detected expression of 667, 453, and 323 coding genes of Methanosaeta thermophila, Methanosaeta concilii, and Methanolinea tarda, corresponding to a detected gene expression rate of 48%, 29%, and 26%, respectively. The KO-based annotation shows that about 50-60% of the detected proteins involved in the metabolism, where 50-60% of proteins are related to energy metabolism, followed by carbohydrate metabolism (10-20%) and amino acid metabolism (5-10%). The proteins related to genetic information processing, and environmental information processing and cellular processes accounted for 5-15% and 2-5% of total identified proteins, respectively, while the remaining 25-35% could not be assigned to known functions. In comparison, M. concilii and M. tarda had apparently differential expression on metabolism, particularly those associated with energy conservation and metabolism of cofactors and vitamins. Although protein expressions of M. thermophila were relatively similar at both loadings, the t-test analysis demonstrated that level of eight proteins among 322 proteins commonly expressed were significantly different. Detection of these proteins, which were related to biofilm formation and acetate activation, suggested that M. thermophila could regulate acetate utilization and biofilm formation in responding to the increasing organic loadings. The findings obtained in this study can facilitate the development of improved methods to enhance methane recovery from wastewater treatment systems.

Keywords: metaproteomics, methanogens, terephthalate

POS-02-050 In-Depth Phosphoproteomic Analysis of Colorectal Cancer Stem Cells

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Colorectal cancer is the third most commonly diagnosed cancer in the world, with more than one million new cases diagnosed yearly, and despite the currently available diagnostic tools and treatments, over 30 % of colorectal cancer patients still suffer relapse episodes after some months or years of remission. In the last years, a new subpopulation of cancer cells has been identified in colorectal tumours, which due to its stem cell-like proliferative properties and its resistance to chemotherapy is responsible for tumour-regeneration. Thorough characterization of this cell population is therefore clinically relevant to reduce tumour aggressiveness and eliminate relapse episodes.

Here, we used a system-wide proteomics approach to undertake the complexity of the cellular mechanisms underlying colorectal cancer stem cells and study the phosphorylation events in signalling transduction cascades. We present an in-depth proteome and phosphoproteome quantitative analysis of colorectal cancer stem-like cell lines with constitutive activation of the Wnt signalling pathway. These analyses were performed by large-scale mass spectrometry analyses aided by extensive liquid chromatography fractionation and phosphorylation enrichment. Our results provide a comprehensive overview of the expressed proteome in colorectal cancer stem cells, and pinpoint key phosphorylation sites governing the main signalling pathways. These data represent a solid step towards a better understanding of the cellular processes that govern tumourigenic processes in this cellular subpopulation.

Keywords: phosphoproteomics, large-scale LCMSMS, colorectal cancer stem cells

POS-02-049 Comprehensive Analysis of Downstream Targets of the Novel Protein E4TF-1 Binding Methyltransferase (EBM)

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A search for novel interactors of the transcription factor GABPα, an ETS-factor, carried out using a Yeast-Two Hybrid Assay, yielded among others a putative methyltransferase called E4TF-1 Binding Methyltransferase (EBM). In order to understand the functional role of EBM, a selection of cancer cell lines were transfected with an EBM construct or siRNA against EBM and up or down-regulated proteins over 2-fold were identified using liquid chromatography followed by tandem mass spectroscopy (LC-MS/MS).

The final aim of this arm of a much larger on-going project is to understand the methyltransferase function of EBM and the metabolic pathways in which it is involved, particularly linked with cancer metabolism and metastatic potential. From knockdown studies it has already been shown that EBM has a marked effect on the cellular proliferation of certain cancer cell lines, possibly offering a tool to control tumour mass or metastasis.

Keywords: methyltransferase, transcription factors, LC-MS/MS

POS-02-051 Global Characterization of the Proteome and Phosphoproteome in Human Glioblastoma Initiating Cells by High-Resolution Mass Spectrometry

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Glioblastoma is one of the most common and aggressive brain tumors with the median survival of twelve months after diagnosis. Despite extensive studies of this malignant tumor, the outcomes of the treatment have not significantly improved over the past decade. To elucidate the underling mechanisms of its tumorigenicity, we performed parallel analyses of the comprehensive proteome and phosphoproteome in glioblastoma initiating cells that are widely recognized as key players in showing resistance to chemotherapy and radiation. Using high-resolution nanoflow LC-MS/MS (LTQ Orbitrap Velos) in combination with GELFREE™ 8100 fractionation system, we identified a total of 8,856 proteins and 6,073 phosphopeptides, respectively. Global protein network analysis revealed that the molecules belonging to ribosome, spliceosome and proteasome machineries were highly enriched at the proteome level. Our in-depth phosphoproteome analysis based on two fragmentation methodologies of CID and HCD detected various phosphorylation sites on neural stem cell markers such as nestin and vimentin, leading to identification of thirty-six phosphorylation sites including eleven novel sites of nestin protein. The SILAC-based quantitative analysis showed that 516 up-regulated and 275 down-regulated phosphorylation sites upon epidermal growth factor stimulation. Interestingly, the phosphorylation status of the molecules related to mTOR signaling pathway was dynamically changed upon EGF stimulation. More intriguingly, we also identified some novel phosphopeptides encoded by the undefined sequence regions of the human transcripts, which could be regulated upon external stimulation in glioblastoma initiating cells. Our result unveils an expanded diversity of the regulatory phophoproteome defined by the human transcriptome.

Keywords: glioblastoma, phosphoproteomics, quantitative proteomics

POS-02-052 Dynamic Phosphoproteomic Profile of Brown Adipose Tissue Stimulated with Cold Temperation

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The main function of BAT is to generate heat and is essential for adaptive thermogenesis and energy expenditure. Growing evidence obtained from human studies indicates that cold-induced activation of BAT increases thermogenesis and energy expenditure. BAT has emerged as an attractive target for the treatment of obesity and associated cardiovascular diseases. To investigate whether protein phosphorylation may play an important role in acute cold exposure, we exposed C57BL mice to low temperatures, BAT of mice were collected at different time points (0,5,10,30min) after applying cold exposure. We have characterized the phosphoproteome of brown adipose tissue using high-accuracy mass spectrometry and report the identification of 9,182 phosphoproteins and 6,859 class 1 phosphosites. Using quantitation technology, we found 148 potentially differentially expressed phosphorylated proteins. Motif-X analysis of the phosphorylation sites identified three significantly enriched kinase motif. KEGG analysis of the phosphorylated proteins enriched multiple pathways such as insulin signaling, PPAR signaling and glycolysis/glucoeogenesis. These results may provide new evidence for a novel mechanism that protein modification is rapidly regulated in response to cold stress in mice BAT.

Keywords: dynamic phosphoproteomic profile, brown adipose tissue, cold temperation

POS-02-053 Unveiling Phosphoproteome by pY-Enhanced Phosphopeptide Enrichment Coupled with High Resolution Capillary LC-MS

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Reversible protein phosphorylation plays significant roles in cellular signal transduction. Recently, large-scale phosphoproteomics based on LC-MS and Ser/Thr/Tyr -selective phosphopeptide enrichment has become a powerful tool to reveal the signaling mechanisms comprehensively. However, multidimensional LC-based 'standard' approaches require tremendous amount of samples as well as the long LC-MS measurement time to increase phosphoproteome coverage. In addition, it is often so difficult to obtain sufficient information on phosphorylated tyrosine due to the masking effect caused by high-abundant pS/pT-containing peptides. Previously, we have developed one-dimensional LC-MS system with meter-scale monolithic silica columns for deep proteome analysis. In this study, we applied this system to phosphoproteomics together with improved sample preparation to achieve more comprehensive and efficient profiling of phosphoproteome. Phosphopeptides were enriched from tryptic digest of 125 ug HeLa cells using lactic acid-modified titania chromatography, and directly analyzed with LC-MS/MS using a meter-scale monolithic silica capillary column. We identified 9,937 unique phosphopeptides and localized 8,435 phosphorylated sites by single LC-MS run. To improve the phosphotyrosine identification, beta-elimination of phosphorylated serine/threonine or immunoprecipitation using anti-pY antibody followed lactic acid-modified titania chromatography. The sequential combination of enrichment methods enabled us to identify approximately 1,400 phosphorylated tyrosines from pervanadate-treated HeLa cells with over 90% selectivity. The approach for pY-enhanced proteomics was applied to phosphoproteomics of 12 mouse organs. Totally over 1,000 phosphorylated tyrosines in addition to pSs/pTs by titania chromatography were identified, and the obtained phosphoproteome distribution profiles in mouse organs indicates the presence of a large number of organ-specific phosphosites.

Keywords: monolithic chromatography, phosphoproteomics, phosphotyrosine

POS-02-054 SILAC Based Quantitative Phosphoproteome Analysis of INS-1E Pancreatic Beta-Cells to Unravel the Mechanism of Insulin Secretion

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Insulin secretion is a highly regulated biological process involving phosphorylation cascade as the major post-translational modification (PTM) regulation. Glucose is the most important nutrient that induces insulin secretion by pancreatic β-cells, this process is called glucose stimulated insulin secretion (GSIS). Using SILAC-based spike-in standard strategy, we performed quantitative phosphoproteomics research to investigate the phosphorylation regulatory mechanism involved in the process of GSIS. We established the largest dataset of β-cell phosphorylation sites by identifying 8,953 sites with confident localization, among which 771 sites were regulated during the process of GSIS. Glucose regulated the activity of several kinases during the process of insulin secretion, including PKC, PKD, AMPK and so on. Besides, we found the phosphorylation status of several secretory granules associated proteins was also regulated. These proteins were key effectors of vesicle exocytosis by participating in various related processes, such as vesicle docking, priming and fusion. Our results unraveled the phosphorylation regulatory network of insulin secretion at system level, and provided important clues for subsequent functional investigation of pancreatic β cells.

Keywords: SILAC, quantitative phosphoproteome, insulin secretion, pancreatic beta-cells

POS-02-055 Establishment of LC/MS/MRM Assay for Glycopeptide Quantification of Human Serum Prostate Specific Antigen (PSA)

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For quantitative determination of glycoforms of prostate specific antigen (PSA) in human serum we established multiple reaction monitoring (MRM) on LC-MS of various PSA glycopeptides. PSA is widely used as a diagnostic marker for prostate cancer, but even other nonmalignant prostatic diseases such as benign prostatic hyperplasia show an increase in the level of serum PSA. PSA is a glycoprotein produced by the prostatic epithelium, which possesses a single *N*-glycosylation site, and is known to alter the pattern of glycoforms between prostate cancer and benign prostatic hyperplasia. We already performed a detailed analysis of the PSA peptides using MALDI-QIT-TOF MS and confirmed differences in glycan structures on PSA between sera of prostate cancer patients and normal seminal plasma. We thought that discrimination of the PSA glycoforms can help precise diagnosis of the cancer and planed to develop the quantitative analysis of the glycopeptides having a variety of glycan structures using the MRM assay, which has both high sensitivity and high selectivity. We added the known amount of PSA to the control serum and prepared the glycopeptides of PSA by enzymatic digestion after enrichment by antibody. A Triple Quadrupole Mass Spectrometer LCMS-8080 (Shimadzu Corporation, Japan) was used to measure the mixture of the PSA glycopeptides. We have developed the chromatography condition for those glycopeptides and optimized MRM transitions as well as ionization parameters. Finally nine distinct glycopeptides derived from PSA can be quantitatively determined in the mixture from 0.5 pmol of PSA.

Keywords: prostate specific antigen (PSA), glycopeptide, MRM assay

POS-02-056 Enhanced Protein N-Acetylation Analysis by SCX and Dimethyl Labeling and Its Application to Discrimination of Protein Isoforms

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Protein N-terminal acetylation is one of the most common modifications occurring co- and post-translationally on either eukaryote or prokaryote proteins. However, compared to other post-translational modifications (PTMs), the physiological role of protein N-terminal acetylation is relatively unclear. To explore the biological functions of protein N-terminal acetylation, a robust and large-scale method for qualitative and quantitative analysis of this PTM is required. Enrichment of N^a-acetylated peptides or depletion of the free N-terminal and internal tryptic peptides prior to analysis by mass spectrometry are necessary based on current technologies. This study demonstrated a simple strong cation exchange (SCX) fractionation method to selectively enrich N^a-acetylated tryptic peptides via dimethyl labeling without tedious protective labeling and depleting procedures. This method was introduced for the comprehensive analysis of N-terminal acetylated proteins from HepG2 cells under oxidative damage by *tert*-butyl peroxide (*t*-BHP). Several hundreds of N-terminal acetylation sites were readily identified in a single SCX flow-through fraction and the protein N-terminal acetylation patterns with and without oxidative damage were simultaneously determined when the stable isotope dimethyl labeling was introduced. Moreover, the N^a-acetylated peptides of some protein isoforms were simultaneously observed in the SCX flow-through fraction, which indicated that this approach can be utilized to discriminate protein isoforms with very similar full sequences but different N-terminal sequences. Compared to other methods, this method is relatively simple and can be directly implemented in a two-dimensional separation (SCX-RP)-mass spectrometry scheme for quantitative N-termini proteomics using stable-isotope dimethyl labeling.

Keywords: N-terminal acetylation, dimethyl labeling, SCX-SPE

POS-02-057 Elimination of the Last Valid Excuse for Not Testing Every Cellular Proteome Dataset for Viral Proteins

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Real proteome samples from human cells may contain proteins from other species because of infection, transfection or just contamination. Despite recent advances in deep proteomics, the peptides from these "alien" proteins are currently ignored during the MS/MS database search at best, or misattributed to human proteins at worst. But since infection or contamination may affect the results of a biological experiment, detection of alien proteins is very important. To test the ability of contemporary proteomics to reliably detect alien proteins in human samples, we reprocessed mass spectra from an authoritative deep proteomics study of eleven common cell lines (Mann et al.). After searching for human proteins, the attributed to them MS/MS spectra were removed from the dataset, which was then researched for viral proteins at 1% FDR. Viral proteins were positively identified in two cell lines. In HEK293, three proteins coded by human adenovirus 5 were expectedly found (this cell line has been established by transformation of normal human embryonic kidney cells with sheared DNA of adenovirus 5). More surprisingly, in LnCAP cell line, two proteins coded by xenotropic MuLV-related virus were detected. This virus has recently caused a huge controversy, first being associated with prostate cancer and chronic fatigue syndrome and then discarded as a laboratory contamination. With label-free quantification, the viral protein abundances were estimated as 10^6 - 10^7 copies per cell, which stresses their importance in the cellular life cycle. Summarizing, there is no excuse anymore for not testing every cellular proteome dataset for viral proteins.

Keywords: human cell lines, viral proteins, label-free quantitative proteomics

POS-02-058 Applying SWATH-MS to Dissect the Variability and Heritability of the Human Plasma Proteome

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The plasma proteome has attracted a lot of attention in translational medicine and biomarker discovery studies. However, fundamental questions such as the variability of the plasma proteins in a population, their inheritability and their longitudinal pattern over years remain unexplored. This is due to the lack of suitable analytical methods that can consistently identify and quantify a large number of proteins among individuals in large sample cohort. We demonstrate that our newly developed Data independent Acquisition (DIA) method, SWATH-MS [1], provides the unique and unprecedented chance to address these difficulties [2] for population proteomic studies.

Using 232 plasma samples from monozygotic (mz) and dizygotic (dz) twins that were collected with 2-7 year intervals, we aimed to systematically investigate and decompose the technical (peptide- and protein-level) and biological variance (such as genetic and individual-/common-environmental and longitudinally unstable factors) in human plasma proteome profiles. The results indicate that SWATH-MS identified and quantified at least 2500 unique stripped peptides (regardless of charge-state and modification) at an FDR of 1%, corresponding to more than 400 proteins at a high degree of reproducibility in the plasma samples. The data showed an unprecedented degree of reproducibly, with the median CV of 11.2% for technical replicates. Overall, monozygoctic twins showed significantly higher proteomic concordance in plasma compared to di-zygotic twins (P=2.97E-37). An accurate estimation protein heritability allowed by twin strategy is currently underway.

References:

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Keywords: plasma proteome, SWATH-MS, population proteomics

POS-02-059 Comparative Proteomics of *Thermococcus onnurineus* NA1: Insights into the Sulfur Metabolism of a H2-Producing Hyperthermophilic Archaeon

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The hyperthermophilic archaeon, *Thermococcus onnurineus* NA1, has been shown to produce biohydrogen (H_2) using CO, formate or starch as growth substrate. This strain can also utilize elemental sulfur (S^0) as a terminal electron acceptor for heterotrophic growth and reduce it to H_2S. However, metabolic characteristics during growth on sulfur are not studied yet. To gain insight into the sulfur metabolism, the proteome of *T. onnurineus* NA1 cells sampled under sulfur culture condition has been quantified and compared with those under formate, CO and starch culture conditions. Using a label-free nano-UPLC-MS^E-based comparative proteomic analysis, we found that approximately 38.4 % of the total identified proteome (589 proteins) were significantly up-regulated (≥1.5 fold) under sulfur culture condition. Many of these proteins are functionally associated with sulfur reduction, CO_2 fixation and amino acid metabolism. As expected, SurR, Mbh and other key enzymes for H_2-production and-recycling were generally down-regulated in sulfur-grown cells, consistent with a role of SurR as a redox switch in response to S^0. In addition, enzymes involved in oxygen detoxification such as SOR, FdpA, NROR and rubrerythrin, were also markedly down-regulated in sulfur-grown cells. Our data suggest that O_2 resistance-linked H_2 production and sulfur metabolism are oppositely controlled through regulatory actions by oxidized SurR in the presence sulfur. Our results revealed that many previously uncharacterized proteins play a key role in sulfur metabolism and in other metabolic pathways, and provide further insights into the metabolic strategies adapted by hyperthermophilic archaea under sulfur-rich environment.

POS-02-060 Proteomic Analysis of *Mycobacterium tuberculosis* Infection of Human Macrophages

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The etiological agent of Tuberculosis, *Mycobacterium tuberculosis* (Mtb), is one of the most devastating infectious agents known today, responsible for nearly 3 million deaths annually. Mtb primarily infects alveolar macrophages that provide the first line of defense against microbial invasion. Normally, macrophages' engulfment of foreign bodies results in the formation of phagosome which matures in a process involving acidification and fusion with the endosomal network.
Mtb pathogenicity depends on the microbe's ability to inhibit phagosome acidification and maturation after entry into human macrophages. We have previously shown that the secreted Mtb protein tyrosine phosphatase, PtpA, is essential for Mtb pathogenicity and that it interferes with both host phagosome acidification and fusion with the lysosome.
To further identify host cellular processes disrupted by Mtb infection and, more specifically, PtpA, we analyzed human proteome response to Mtb using iTRAQ. This enabled us to quantify over 2500 human macrophage proteins affected by infection. We followed Mtb infection over 18 hours and observed modulation of host protein expression over time. The difference between the Δ*ptp*A mutant and the parental wild-type strain was more pronounced at early stages of infection where we observed an increase in up and downregulated proteins. Confirmation of these modulations was done by Western blot and qPCR analysis. Overall, these analyses further defined the role of Mtb PtpA in modifying host signaling pathways during infection.

Keywords: mycobacterium tuberculosis, macrophage infection, proteomic analysis

POS-02-062 Towards Comprehensive Proteomic Characterization of Human Saliva and Investigation of Its Diagnostic Potential

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Among human body fluids with health/disease diagnostic potential, whole saliva is gaining attention as a potential alternative to plasma/serum. To examine its diagnostic potential, we have undertaken large-scale mass spectrometry-based analysis of saliva to obtain a comprehensive inventorying of its proteins and their PTMs.
Given the wide dynamic range of protein abundance, we initially performed protein dynamic range compression (DRC) of human saliva using hexapeptide libraries. Next, DRC was integrated into a novel three-dimensional peptide fractionation workflow involving sequential steps of IEF, SCX, and RP fractionation prior to mass spectrometric (LTQ-Orbitrap) analysis to obtain nearly 1 million MS/MS spectra from 200 total runs. Obtained spectra were searched using Sequest and MaxQuant preprocessing followed by Protein Pilot. Our integrated workflow generated the largest high-confidence catalog yet of >2200 proteins in human saliva at <1% FDR (Bandhakavi et al., 2009 J Proteome Res; Jagtap P et al., 2012 Proteomics). We present this data here and compare the salivary proteome to that of plasma in terms of diagnostic potential.
We also significantly expanded the known N-glycosites and phosphosites in human saliva via a combination of protein level DRC and PTM enrichment at peptide level (Bandhakavi et al., 2011 J Proteome Res, Stone MD et al., 2011 J Proteome Res). These data will be compared to the total salivary proteome and fluid-specific phosphorylation signatures illustrated.
Ongoing orthogonal approaches to dig deeper into the salivary proteome will be discussed (unpublished results) and results from preliminary quantitative analyses identifying breast cancer associated changes in human saliva also presented.

Keywords: saliva proteomics PTMs, dynamic range compression, 3-D peptide fractionation

POS-02-061 Improvement of Rat Urinary Proteomics by a Differential Precipitation of Proteins

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Body fluids contain proteins/peptides that reflect physiological or pathological state of humans and animals and urine could be collected in large quantities non-invasively. This makes it as one of the ideal samples for liquid chromatography mass spectrometer (LC-MS) based protein biomarker discovery. We studied rat urinary proteomics as an experimental model for biomarker discovery. We identified major urinary protein (MUP), Urinary protein 1, 2 and 3 (UP1, UP2 and UP3) as high abundant proteins (HAP) in male rat urine. Because these HAP interfered the detection of lower abundant proteins we tried to remove them from samples. Initially, we made antibodies against them for their removal by the immuno precipitation, but were not successful. Then we tried to test differential protein precipitation conditions to remove HAP, including organic solvents (acetone, acetonitrile, and ethanol with different concentrations), temperature, and shaking. The concentration of the solvent gave the most effective removal and the best result was obtained by precipitation with ethanol 45-50% at 4 ℃ without shaking. Lower concentration of organic solvent avoided a precipitation of HAP with relatively lower MW (11K-21K) although the protein recovery was low (14%). Then trypsin digested proteins were analysed in the LC-MS/MS (Advance nano-LC (Michrom) and LTQ Orbitrap-XL (Thermo)).Total number of detectable peptides was counted by the Progenesis LC-MS software (Nonlinear), which reached to 25000 by ethanol 45%. The total identified proteins by the MASCOT search reached 455 by ethanol 50%. This enabled the increase in number of detectable peptides/ proteins and the detection limit of some of the previously reported protein biomarkers. The increase in the number of detectable proteins was observed in the entire mass range. Thus, this method can increase the chance of detecting protein biomarkers although the total protein recovery is low.

Keywords: urinary proteomics, rat, high abundant protein

POS-02-063 Rapid and Deep Coverage in Single-Dimension Shotgun Human Proteomics

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On this study, we improve the current limits of 1D shotgun mass spectrometry in today's "deep proteome" research. Multi-parameter optimization was performed without any hardware modification of the commercial LC-MS instrumentation provided by a vendor. With a 50 cm long separation column and 3 h LC gradient, under the optimized experimental conditions, we were able to identify 4,825 protein groups and 37,550 peptides in a single run, and 5,354 protein groups and 56,390 peptides in a triplicate analysis of A375 cell line. The achieved coverage accounts for around 50% of the expressed human cellular proteome. The major steps to enhance the integral performance of our shotgun 1D analysis included the following. ProteaseMAX buffer was found to be more efficient for cell lysis and protein extraction compared to SDC and urea. Preserving cell debris in cell buffer for protein digestion enabled higher coverage of membrane and nucleus proteins. Tailoring the LC gradient profile yielded higher total number of sequenced proteolytic peptides. The optimal dynamic exclusion window setting in data-dependent MS/MS was found to be 15 s for 3 h LC gradient time, and the optimal *m/z* scan window was 400-1200. Ongoing technology improvement in LC-MS should further increase the role of this method in deep proteome studies, allowing faster and more sensitive analyses.

Keywords: shotgun proteomics, Orbitrap mass spectrometry, reversed phase liquid chromatography

POS-02-064 An Established One Dimensional LC-MS/MS Methods and Using It in Quantification Proteomics

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Large-scale proteomic studies often choose the multiple dimensional liquid chromatography coupled tandem mass spectrometry (MDLC-MS/MS) to identify and quantify proteins. However, MDLC workflow takes multiple steps, large amount of proteins and long running time. In addition, peptides and proteins identified across fractions would diminish the protein IDs and accurate quantification. Here, we developed a 1DLC-MS method with 50cm column which can identify 4605 protein groups in only one 400 min gradient. Then, we used HUVEC (Human Umbilical Vein Endothelial Cells) cells with/without MLN treatment to test the method's stability, each of the HUVEC lysate was run for three times which shows high reproducibility in identifying proteins. As known to all, MS1 quantification method is more accuracy than MS2 method. Comparing MLN treated cell lysate to DMSO control cell lysate, we identified 180 proteins expressing differently with MS1 quantification which were related to cell death and apoptosis. These quantification results are in accordance with the previous reports. Therefore, our one dimensional LC-MS/MS proteomics methods proved to a reliable methods in protein identification and can obtain reliable data in label free quantification research.
Keywords: one dimensional LC, long column, quantification proteomics

POS-02-065 Quantitative MS^E Proteomics as a Tool for the Determination of Clinically Relevant Proteins in Wheat Grain

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The amounts of clinically relevant disease-related proteins in wheat grain are largely unknown. Developing the methods for quantitative measurement of clinically relevant proteins could support advancements in understanding exposure thresholds and clinical study design. The aim of our study was to use a data-independent mass spectrometry (MS^E) approach for quantifying gliadin and glutenin proteins in wheat grain. The biological replicated analysis yielded concentrations for 34 gliadin and 22 glutenin proteins and detected several peptides carrying four previously discovered epitopes that belong to gamma gliadin B precursor. The technical coefficients of variation ranged from 0.12 to 1.39 and indicates that MS^E proteomics is a reproducible quantitative method for the determination of gliadin and glutenin content in the highly complex matrix of protein extracts from wheat grain.
Keywords: wheat, grain, allergy

POS-02-066 Phosphoproteome Analysis of *Lotus japonicus* Seeds

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Elucidation of the regulatory mechanisms of germination is essential in order to increase agricultural productivity. In this effort, analyses of signaling pathways are considered to be indispensable. In *Arabidopsis thaliana* and rice, it is reported that protein phosphorylation and plant hormone signaling pathway regulates seed germination. In legumes, however, there have been few reports on the regulation of seed germination at the protein level. *Lotus japonicus* is a model legume because of its small genome size, short life cycle, and synteny with other legume species. Recently, the complete nucleotide sequence of the *L. japonicus* genome has been determined, and a database has been constructed. Consequently, it is now possible to perform genome database-driven proteome analysis in this organism. Meanwhile, the development of mass-spectrometry and phosphopeptide-enrichment techniques made it possible to comprehensively analyze phosphoproteins. In this study, we profiled phosphoprotein expression in the cotyledons and hypocotyls of *L. japonicus* seeds after absorption of water. By proteomic analysis of seeds following water absorption, we identified a total of 721 phosphopeptides derived from 343 phosphoproteins in cotyledons, and 931 phosphopeptides from 473 phosphoproteins in hypocotyls. Kinase-specific prediction analyses revealed that different kinases were activated in cotyledons and hypocotyls. In particular, many peptides containing ATM-kinase target motifs, X-X-pS/pT-Q-X-X, were detected in cotyledons. Moreover, we found that expression of ATM kinase gene homolog is upregulated specifically in cotyledons by real-time PCR analysis, suggesting that ATM kinase homolog plays a significant role in cell proliferation in the cotyledons of *L. japonicus* seeds. This dataset might be useful in studying the regulatory mechanisms of seed germination in legume plants.
Keywords: legume, *lotus japonicus*, phosphoprotein

POS-02-067 The Wheat Chloroplastic Proteome

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Chloroplast has fastidious curiosity for plant biologists due to their intricate biochemical pathways for indispensable metabolite functions. We have attempted to understand the photosynthesis in wheat under abiotic stress such as salt imposed and water deficit during vegetative stage. Those studies provide interesting results leading to a better understanding of the photosynthesis and identifying the stress responsive proteins. Proteomics analysis was conducted using two complementary approaches such as 2-DE coupled to high through put mass spectrometry. These proteins are localized in the chloroplast (607 proteins), chloroplast stroma (145), thylakoid membrane (342), lumens (163), and integral membranes (166), which were predicted by some freeware program. Although, 89% proteins has been accomplished for the key chloroplast pathways in wheat, such as for photosynthesis. Photosynthesis and transpiration rate, stomatal conductance, and relative water content decreased whereas the level of proline increased under salt and water stress. Statistically significant positive correlations were found among the content of hydrogen peroxide, activity of catalase, and superoxide dismutase under salt stress in wheat. Twenty one protein spots were differentially expressed during salt treatment. Sixty five unique proteins assigned in the differentially abundant spots by mass spectrometry such as Cyt b6-f, germin-like-protein, the γ-subunit of ATP synthase, glutamine synthetase, fructose-bisphosphate aldolase, S-adenosylmethionine synthase. Twenty differentially expressed proteins were detected in the chloroplasts and analyzed with high through-put mass spectrometry. Under these stress conditions, 9 proteins were up-regulated in their protein abundance while the levels of 11 proteins were unevenly affected such as chloride carrier/channel family, H^+-transporting two-sector ATPase.
Keywords: wheat chloroplast, salt stress, water stress

POS-02-068 Unraveling the Proteome of Sugarcane Leaves by Mass Spectrometric Analysis

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Despite the importance of sugarcane as the world's largest commercial crop, the proteomes of its roots, stalks and leaves are poorly known. In a study by a Brazilian research consortium focused on the sugarcane genome approximately 90% of the expressed sugarcane genome (237,954 ESTs) was sequenced. However, it is known that mRNA levels are not always directly correlated with protein expression. Therefore, the study of the sugarcane proteome is important to identify proteins that are actually expressed and to compare different cultivars. As a first effort to explore the proteome of sugarcane leaves, we selected two species (*Saccharum officinarum* [SO] and *S. spontaneum* [SS]) and a hybrid cultivar (SP80-3280 [SP]) that differ from each other in their capacity to produce sucrose and to resist to pathogens. In order to obtain proteins in optimal conditions for proteomic analysis, we carried out a phenol/SDS protocol for protein extraction. Proteins from SO, SS and SP showed highly similar profiles at molecular masses between 10 kDa and 100 kDa on SDS-PAGE under reducing conditions. The in solution trypsin digestion of extracted proteins followed by LC-MS/MS analysis of the peptide mixture using the EASY nLCII nano-chromatography system coupled to LTQ Orbitrap Velos resulted in the identification of more than 800 different proteins in SO, SS and SP leaves (1% false discovery rate at peptide/protein levels; MaxQuant software; SUCEST database), which are involved in pathways of protein, DNA, carbohydrate and redox metabolism, and light harvesting. Our preliminary label-free quantitative comparison of identified proteins indicates a close relationship between the proteomes of SO and SP.

Keywords: sugarcane, proteome

POS-02-069 Quantitative Subcellular Proteomics Reveals that Phytohormone Mediated Mechanism is Involved in the Flooding Tolerance of Soybean

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Climate change poses tremendous global challenges for agriculture and environmental stressors severely diminish crop productivity. The flood that is one of them causes a serious problem for soybean cultivation because it markedly reduces growth. To analyze the flooding tolerance mechanism of soybean, the flooding-tolerant mutants were isolated from gamma-ray irradiated soybeans and analyzed using proteomic techniques. In wild-type under flooding stress, levels of proteins related to development, protein synthesis/degradation, secondary metabolism, and the cell wall changed; however, these proteins did not markedly differ in the mutant. The root tips of mutant were not affected by flooding stress, even though the wild-type had damaged root. Physiological and proteomic results indicated that ubiquitin/proteasome-mediated proteolysis did not occur in the root tip in the mutant. Furthermore, especially involvement of abscisic acid (ABA) in soybean response to flooding stress was analyzed by proteomic and biochemical techniques because our subcellular proteomics suggested relationship of ABA in responses. Growth was resumed after water removal when ABA was added during flooding treatment though it was not possible to grow after flooding without ABA. Nuclear proteomics revealed that mRNA splicing and transcription related proteins increased under ABA treatment compared with flooding treatment; however, importin and chromatin remodeling factor related proteins decreased. Out of them, these transcription related proteins were analyzed at the mRNA expression level, indicating they were significantly down-regulated under ABA treatment compared with those under flooding without ABA treatment. ABA mediated regulation of these proteins might be involved in the enhancement of flooding tolerance of soybean.

Keywords: plant, phytohormone, quantitative subcellular proteomics

POS-02-070 Proteomic Analysis of the Seed Development in *Jatropha curcas*: From Carbon Flux to the Lipid Accumulation

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Seed development is one of the most important and complex biological system in higher plants. To characterize the metabolic signatures of lipid accumulation in seeds, comparative proteomic technique was employed to profile protein changes during the seed development of *Jatropha curcas*. Temporal changes in global proteome were examined using gel-based proteomic technique at six developmental stages for lipid accumulation. One hundred sixty one differentially expressed proteins were identified by mass spectrometry. These proteins were classified into 10 functional categories, and the results demonstrated that proteins related to energy and metabolism were notably accumulated in the developing stage of *J. curcas* seeds. The elevation of proteins for energy and metabolism involved in the carbon flux to lipid accumulation occurs primarily from early to late stage in seed development. The expression of mRNAs encoding key proteins was evaluated using quantitative RT-PCR. Glycolysis and oxidative pentose phosphate pathways were the major pathways of producing carbon flux, and the glucose-6-phosphate and triose-phosphate are the major carbon source for fatty acid synthesis. Lipid analysis revealed that fatty acid accumulation initiated 25 days after flowering at the late stage of seed development of *J. curcas*. Furthermore, $C_{16:0}$ was initially synthesized as the precursor for the elongation to $C_{18:1}$ and $C_{18:2}$ in the developing seeds of *J. curcas*. Together, the metabolic signatures on protein changes in seed development provide profound knowledge and perspective insights into understanding lipid network in *J. curcas*.

Keywords: Jatropha curcas, seed development, lipid accumulation

POS-02-071 Mutation of Nucleotide Pyrophosphatase/Phosphodiesterase 1 Stimulates Accumulation of Starch in Rice Seedlings Under High CO_2 Concentration

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Nucleotide pyrophosphatase/phosphodiesterase (NPP) is a new enzyme family that belongs to a large group of structurally related and functionally divergent nucleotide hydrolases. We identified six *NPP* genes in rice genome and cloned their cDNAs. NPP1 was shown to be the enzymes localized in the plastids and endomembrane system, which had a hydrolyzing activity against ADP-glucose. The retro-transposon *Tos17*-inserted mutant of *NPP1* was backcrossed twice with the wild-type and generated the *npp1* homozygote. The expression of ADP-glucose pyrophosphatase activities in *npp1* was reduced to approximately 20% compared with the wild-type. We examined the growth, starch and sucrose accumulation in the seedlings of *npp1* under different conditions of CO_2 concentration and temperature. The seedling growth of *npp1* was slightly rapid compared with the wild-type under high CO_2 and temperature. The accumulation of starch and sucrose in rice seedlings was significantly enhanced under high CO_2 conditions. Noteworthy, the starch accumulation in *npp1* seedlings was much stimulated in comparison with that in the wild-type under high CO_2 conditions, particularly at 1,600 ppm CO_2, 28°C, 14h light/23°C, 10h dark. To elucidate the stimulating mechanism of growth and starch accumulation in *npp1* seedlings under high CO_2 and high temperature, a series of quantitative shotgun proteomic analyses with iTRAQ labeling were carried out. High CO_2 and high temperature similarly enhanced the expression of protein synthetic system in the *npp1* seedlings. Interestingly, several 14-3-3 were highly expressed in the *npp1* seedlings under high CO_2 condition. It is strongly suggested that 14-3-3 is involved in the accumulating mechanism of starch in the *npp1* seedlings.

Keywords: rice nucleotide pyrophosphatase/phosphodiesterase, CO2, starch

POS-02-072 Identification and Characterization of MARK, MAMP Responsive Phosphoprotein for Appropriated ROS Kinetics, in *Arabidopsis*

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Plants utilize pattern recognition receptors (PRRs) to recognize microbe-associated molecular patterns (MAMPs) for broad resistance to microorganisms. A number of PRRs identified to date found to be receptor kinases or their interactors indicating that phosphorylation signaling pathways play an important role in MAMP-triggered immunity (MTI).
In order to identify novel components that regulate MTI, we performed differential phosphoproteomics and identified 569 MAMP responsive phosphoproteins (MRPs) whose phosphorylation status significantly changed in response to flg22 and/or chitin treatment. Since most of the MRPs have not been reported yet to take part in plant immunity, we have isolated T-DNA insertion lines for these proteins and characterized flg22-induced ROS (reactive oxygen species) production in the isolated mutants. As a result, we have identified 38 MRPs that are required for appropriate ROS bursts, and designated as MARK (MRP for appropriated ROS kinetics).
MARK1 is a land plant-specific protein with unknown function. The *mark1* mutant lines exhibit enhanced ROS production upon flg22, elf18, chitin, and pep1 treatments. In addition, *mark1* showed increased MAPK activation and callose deposition upon flg22 treatment. Moreover, *PR1* gene expression in *mark1* mutants was found to be up-regulated under normal growth conditions. These results strongly suggested that MARK1 function as a negative regulator of plant immunity. However, contrary to expectations, *mark1* mutants were more susceptible to a virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) compared to the wild-type plants. This surprising result indicates that MARK1 has an important role in activating a defense system against *Pst* DC3000 which is independent or downstream of previously identified MAMP and SA signaling pathways.
Keywords: plant immunity, phosphoproteomics

POS-02-073 Analysis of Mechanism for Light Irradiation-Mediated Enhancement of Soybean Flooding Tolerance by Comparative Proteomics and Transcriptomics

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Flooding has a negative impact on soybean growth. We found that light irradiation alleviates the injurious effects in flooded etiolated seedlings. Irradiation of light to etiolated seedlings under flooding did not induce greening through chlorophyll synthesis. Dissolved oxygen level in the floodwater was decreased depending on the duration of flooding and it was comparable to that of dark flooded seedlings. The result indicated that oxygen evolution through photosynthesis did not occur in the seedlings. To clarify the alleviation mechanism, comparative proteomic and transcriptomic analyses were performed. Proteomic changes in radicle of the seedlings were analyzed by Gel-enhanced LC-MS/MS in combination with separation using ProteoMiner combinatorial peptide ligand libraries. The ProteoMiner separation contributed to increase 26% of proteome coverage. Light irradiation-mediated significant alterations of 636 proteins in abundance were identified. These alterations were assessed using transcriptomic data from microarray analysis. The alterations of abundance of 177 proteins were positively correlated with their mRNA levels. The data showed that light irradiation caused remarkable decreases of phytochrome A, glycolytic proteins, cell wall proteins and increases of photosystem II subunits. These results suggest that the light irradiation suppresses excessive activation of glycolysis in flooded seedlings through phytochrome-mediated light signaling, that possibly contributes to enhance tolerance.
Keywords: soybean, flooding stress tolerance, GeLCMS

POS-02-074 Analysis of Flooding Stress Response Mechanism in Soybean Using Gel-Based and Gel-Free Proteomics Techniques

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Flooding stress is prevalent over many agro-ecological systems globally, threatening food security. Anaerobic conditions due to flooding effect gene expression. Proteome comparison of flood affected and normally grown soybean provides a key for biomarker identification and its analysis. Soybean is considered sensitive to flooding stress. Analysis of flooding-responsive proteins using proteomics approaches provides information about metabolic pathways of soybean seedlings affected by flooding. Selected accessions of soybean (*Glycine max*) were grown in excess water condition to give flooding stress. Roots were used as a source for protein. Roots from plants grown in well watered condition were used as a control. After sowing, 2-day-old seedlings were flooded. And then, root was collected at 1, 2, 3, 4 and 5 days after flooding. Gel-based approach was adopted for proteomic analysis to get evidence for protein framework of influenced plant organs. Gel-free quantification of changed proteins was also done in roots, hypocotyls, and cotyledons. Metabolic simulation was carried out to understand metabolic profiles and regulatory proteins in pathway analysis. Simulation models provided a framework for visualizing different physiological responses, interacting when plants respond to flooding stress. The study will be helpful to identify the unknown pathways contributing to survival mode of plant under flooding stress. **Acknowledgement:** This work was supported by JSPS Invitation Fellowship for Research, Number L-13552.
Keywords: flooding, soybean, gel-based approach

POS-02-075 Functional Proteomics Reveals Tanshinone IIA Induced Apoptosis of Activated Hepatic Stellate Cells

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Activated hepatic stellate cell (HSC) is the predominant event in the progression of liver fibrosis which is a severe health problem arising from chronic injury of the liver, and selective removal of HSC might be a potent strategy in therapy. *Salvia miltiorrhiza Bunge (Labiatae)* has been widely used in Chinese medicine for the treatment of microcirculatory diseases. In our study, ethanol extract of Salvia miltiorrhiza roots (SMEE) obviously ameliorates liver fibrogenesis in DMN- administrated rat model. Meanwhile, tanshinone IIA (Tan IIA), the pure compound extracted from SMEE, significantly suppressed HSC viability and finally mediated cell apoptosis. We applied proteome tools to reveal that increase prohibitin is involved in cell cycle arrest under Tan IIA treatment whereas knockdown of prohibitin could attenuate Tan IIA-induced apoptosis. Tan IIA also induced the translocation of C-Raf, which was followed by activating MAPK and inhibiting AKT signaling in HSC. Again, MAPK antagonist inhibited ERK phosphorylation and downregulated Tan IIA-induced expression of Bax and cytochrome c. It also abolished Tan IIA-modulated cleavage of PARP. Our results indicated that Tan IIA might effectively prevent hepatic fibrosis by triggering apoptosis of HSC through promoting ERK-Bax-caspase pathways via C-Raf/prohibitin complex.
Keywords: hepatic stellate cell, Salvia miltiorrhiza, liver fibrosis

POS-02-076 Comparative Proteome Analysis of Roots in Drought-Sensitive and Drought-Tolerant Rapeseed and Their Hybrid F1 Line Under Drought Stress

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Plant growth is controlled by several factors, among which water plays a vital role. Water deficit is one of the most significant stresses in agriculturally important crops, affecting growth, development, and yield. Rapeseed, which is the third leading source of vegetable oil, is sensitive to drought stress during the early vegetative growth stage. To investigate the initial response of rapeseed to drought stress, drought-sensitive (RGS-003) and drought-tolerant lines (SLM-046), and their F1 hybrid line were used. Seven-day-old rapeseed seedlings were treated with drought stress by restricting water for 7 days, and proteins were extracted from roots and analyzed using gel-baseed proteomics technique. In the sensitive rapeseed line, 35 proteins were differentially expressed under drought stress, and proteins related to metabolism, energy, disease/defense, and transport were decreased. In the tolerant line, 32 proteins were differentially expressed under drought stress, and proteins involved in metabolism, disease/defense, and transport were increased, while energy-related proteins were decreased. Six protein spots in F1 hybrid were common among expressed proteins in the drought-sensitive and -tolerant lines. Notably, tubulin beta-2 and heat shock protein 70 were decreased in the drought-sensitive line and hybrid F1 plants, while jasmonate-inducible protein and 20S proteasome subunit PAF1 were increased in the F1 hybrids and drought-tolerant line. These results indicate that (1) V-type H^+ ATPase, plasma-membrane associated cation-binding protein, HSP 90, and elongation factor EF-2 have a role in the drought tolerance of rapeseed; (2) The decreased levels of heat shock protein 70 and tubulin beta-2 in the drought sensitive and hybrid F1 lines might explain the reduced growth of these lines in drought conditions.

Keywords: rapeseed, proteomics, drought stress

POS-02-077 Differential Omics Approach on a Metabolic Network Outlined Flooding-Response Mechanisms in Soybean

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The agricultural legume soybean has been important as food for maintenance of human health and crop for sustainable agriculture. Flooding stress is one of the natural conditions that exhibit a severe negative influence on the productivity of arable farmland. Plants respond to such environmental stresses through differential expression of a subset of genes, which changes the 'omics' profile. Linking gene expression, protein abundance, and metabolite accumulation to the phenotype of plants is one of the main challenges for improving agricultural production. The 'omics' approach can be enhanced by mapping multiple 'omics' data on a metabolic network. We mapped 92 metabolites, 75 proteins, and 921 mRNAs whose levels differed between the control and flooding conditions in the early seedling stage of soybean. Based on the generated map, genes encoding glycolysis-related enzymes were up-regulated indicating that activation of glycolysis and fermentation cascades are key elements in the early responses of soybean to flooding stress. In the TCA cycle, proteins and metabolites accumulated under flooding, whereas mRNA expression was down-regulated, suggesting that the TCA cycle is strongly influenced by flooding stress. Although protein destination/storage related proteins were accumulated, mRNA level did not change under flooding stress. This result indicated that the proteins remained by the flood stress as a result of the growth delay though these proteins should disappear originally. Our mapping results indicated disrupted flows, accumulated metabolites and differential transcriptions, caused by the flooding stress, in the metabolic network, and outlined flooding-response mechanisms in soybean.

Keywords: omics, soybean, flooding

POS-02-078 Investigation of Rice Proteomic Change in Response to Microgravity

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Gravity is one of the environmental factors that control development and growth of plants. Space environment, such as space shuttle missions, space labortories and space stations, etc. provide unique oppotunities to study the microgravity response of plant. During the Shenzhou 8 mission in November 2011, we cultured rice cali on the spaceship and the samples were fixed 4 days after launch. The flying samples in the static position (micro g, μ g) and in the centrifuge which provide 1 g force to mimic the 1 g gravity in space, were recovered and the proteome changes were analyzed by iTRAQ. In total, 4840 proteins were identified, including 2085 proteins with function annotation by GO analysis. 431 proteins were changed >1.5 fold in space μ g /ground group, including 179 up-regulated proteins and down-regulated 252 proteins. 321 proteins were changed >1.5 fold in space μg / space 1 g group, among which 205 proteins were the same differentially expressed proteins responsive to microgravity. Enrichment of the differnetially expressed proteins by GO analysis showed that the ARF GTPase activity regulation proteins were enriched when compared the space μg with space 1 g sample, whereas the nucleic acid binding and DNA damage repairing proteins were enriched when compared the space μg and ground sample. Microscopic comparison of the rice cali showed that the space grown cells are more uniformed in size and proliferation, suggesting that cell proliferation pattern was changed in space microgravity conditions.

Keywords: rice, microgravity

POS-02-079 In-Depth Proteome Analysis of Developing Seeds of *Jatropha curcas*

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The potential of *J. curcas* seeds as a source of raw material for the production of biodiesel is hampered by a lack of understanding regarding the enzymatic machinery responsible for the biosynthesis of fatty acids, triacylglycerol and toxic phorbol esters. Using a GelLC-MS/MS approach, we undertook a spatiotemporal proteome analysis of the endosperm, inner integument and plastids isolated separately from the developing endosperm and developing inner integument, that resulted in the identification of 1453, 1471, 1103 and 1873 protein groups, respectively. Besides expanding considerably the repertoire of *J. curcas* proteins so far identified, our results give important insights regarding the carbon flow in the developing seeds, in the anabolism and catabolism of fatty acid and in the deposition pattern of seed reserve proteins. The comparison of the proteomes of plastids from endosperm and from inner integument indicated a striking functional specialization of each plastid type, where the former is clearly geared to the synthesis of fatty acids and amino acids while the latter is geared to the synthesis of secondary metabolites. We also identified in the inner integument, several proteins which may be involved in the transfer of nutrients from maternal tissues to the endosperm and embryo, especially an enzymatic array made of proteolytic enzymes and proteasome components which are thought to mediate programmed cell death in this organ. Apart from curcin, no other protein of known toxic properties was identified in either of the sub-proteomes. This global proteome analysis enlightens the mechanisms that control *J. curcas*seed development.

Keywords: Jatropha curcas, oilseeds, plant proteomics

POS-02-080 Proteomic Analysis of the Japanese Crested Ibis

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We studied a proteome of the **Japanese Crested Ibis** (Aves, Pelecaniformes, Threskiornithidae, *Nipponia nippon*) who died in March 10, 2010 at the Sado Japanese Crested Ibis Conservation Center. The proteins extracted from brain, trachea, liver, heart, lung, proventriculus, muscular stomach, small intestine, duodenum, ovary and neck muscle of the Crested Ibis were subjected to LC-MS/MS analyses with LTQ Orbitrap XL. By searching **NCBI *Gallus gallus databases***, **4,258 cases of GI number** were obtained as the sum of **11 tissues** of the Crested Ibis. In order to interpret the obtained proteomics data, it was verified in detail with the data which obtained from the brain of the Crested Ibis. It has been reported that drebrin A is specifically expressed in adult chicken brain. We identified **drebrin A** as a brain-specific protein in the shotgun proteomic analyses of the Crested Ibis. Furthermore, Western blotting analysis of the protein preparations from 10 tissues of the Crested Ibis and hen using anti-drebrin antibodies showed intensive expression of approximately 100 kDa polypeptides of **drebrin** in both brains. The present data may contribute the initial and fundamental steps to understand the Crested Ibis proteome.

Keywords: Japanese Crested Ibis, 4,258 cases of GI number, drebrin A

POS-02-081 Proteome Analysis of Roots of Wheat Seedlings Under Aluminum Stress

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The root apex is considered the first sites of Aluminum (Al) toxicity and the reduction in root biomass leads to poor uptake of water and nutrients. Aluminum is considered the most limiting factor for plant productivity in acidic soils. Aluminum is a light metal that makes up 7% of the earth's crust dissolving ionic forms. The inhibition of root growth is recognized as the primary effect of Al toxicity. Seeds of wheat cv. Keumkang were germinated on petridish for 5 days and then transferred hydroponic apparatus which was treated without or with 100 μM and 150 μM $AlCl_3$ for 5 days. The length of roots, shoots and fresh weight of wheat seedlings were decreased under aluminum stress. The concentration of K^+, Mg^{2+} and Ca^{2+} were decreased whereas Al^{3+} and $P_2O_5^-$ concentration was increased under aluminum stress. Using confocal microscopy, the fluorescence intensity of aluminum increased with morin staining. A proteome analysis was performed to identify proteins, which are responsible to aluminum stress in wheat roots. Proteins were extracted from roots and separated by 2-DE. A total of 47 protein spots were changed under Al stress. Nineteen proteins were significantly increased such as S-adenosylmethionine, oxalate oxidase, malate dehydrogenase, cysteine synthase, ascorbate peroxidase and/or, 28 protein spots were significantly decreased such as heat shock protein 70, O-methytransferase 4, enolase, and amylogenin. Our results highlight the importance and identification of stress and defense responsive proteins with morphological and physiological state under Al stress.

Keywords: wheat, aluminium stress, proteomics

POS-02-082 Transcriptome and Quantitative Proteome Analyses Reveal Molecular Mechanism Associated with the Low Silk Production of Silkworm *Bombyx mori*

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The silkworm *Bombyx mori*, a model insect in the lepidopteran, has an important academic value for silk protein production. The increasing of the silk production has always been the main goal in manufacture and focal point of the sericulture science. We used a breed with a low silk production which derived from the transgenic experiment as the analytical material (named as ZB) and took the advantage of iTRAQ, in-gel, gel-free proteomic approaches, high-throughput RNA sequencing technology along with the COG, GO, KEGG bioinformatics methods to analysis the differentially changes of proteins and transcripts between ZB breed and control breed L25 from the aspects of transcriptome and proteome. The results indicate that the molecular mechanism of reduced production are related to the decrease of proteins involved in the protein absorption, fat absorption, DNA replication, nucleotide repair, RNA level of processing and transport, and the protein synthesis/processing. Moreover, the increased programmed cell apoptosis pathway also contributes to this mechanism.

Keywords: proteomics, transcriptomics, silkworm Bombyx mori

POS-02-083 Effects of Growth Hormone on the Salmon Pituitary Proteome

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Pituitary, an endocrine organ, synthesizes and secretes various peptide hormones. Each pituitary hormone is released into the bloodstream and each acts on different target organs in order to strictly regulate a variety of biological functions. Simultaneously, the synthesis and secretion of these hormones are controlled by hormonal feedback regulation systems.

Growth hormone 1 (GH1), one of a pituitary hormone, plays a key role in the regulation of growth. Both excess GH1 treatment and overexpression of a GH1 transgene promote growth of salmon, but these animals exhibit physiological abnormalities in viability, fertility and metabolism, which might be related to pituitary function. However, the molecular dynamics induced in the pituitary by excess GH1 remain unknown. In this study, we performed iTRAQ proteome analysis of the amago salmon pituitary, with and without excess GH1 treatment, and found that the expression levels of proteins related to endocrine systems, metabolism, cell growth and proliferation were altered in the GH1-treated pituitary. Specifically, pituitary hormone prolactin (2.29 fold), and somatolactinα0.14 fold) changed significantly. This result was confirmed by proteome and transcriptome analyses of pituitary from the GH1-transgenic (GH1-Tg) amago salmon. The dynamics of protein and gene expression in the pituitary of GH1-Tg amago salmon were similar to those of pituitary treated with excess GH1. Our findings suggest that not only excess GH1 hormone, but also the quantitative changes in other pituitary hormones, might be essential for the abnormal growth of amago salmon. These data will be useful in future attempts to increase the productivity of fish farming.

Keywords: GH, pituitary, salmon

POS-02-084 Differential Protein Expression and Chemoprotection of Fenugreek Leaf Extract on Normal Rat Liver Cells Treated with Cadmium Chloride

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Cadmium (Cd) is a toxic metal known to alter cellular activities that eventually leads to cancer formation in the cell. Many chemo-preventive agents have been used against metal toxicity and Fenugreek is well known for its medicinal value. In this research, chemoprotective effect of Fenugreek Leaf Extract (FLE) and the differential protein expression of $CdCl_2$ treated normal rat liver cells were examined. Normal rat liver cells (CRL-1439) were treated with $CdCl_2$ alone (25 μM) and/or pretreated 4h with FLE (0.005μg/ml) before treated with $CdCl_2$ (25 μM). Treated cells were then incubated at 37 °C in a 5% CO_2 incubator for 36 h or 48 h. The viability result of the cells treated with $CdCl_2$ alone was reduced to 37.1%, while the viability of the FLE cotreated cells was increased to 102% respectively, in comparison to the control cells (100%). The protein expression analysis of normal rat liver cells treated with $CdCl_2$ alone showed 9 up regulated protein spots (≥1.5 fold) and 2 down regulated protein spots (≤ -1.5 fold), while the FLE co-treated cells showed 3 up regulated protein spots (≥1.5 fold) and 15 down regulated (≤ -1.5 fold) protein spots respectively in comparison to the control cells. Our findings showed the chemo-preventive effect of (FLE) and differential protein expression of $CdCl_2$ alone treated cells in comparison to FLE co-treated cells. Target proteins responsible for $CdCl_2$ toxicity may be used as a biomarker to identify $CdCl_2$ exposure in human and animal.

Keywords: fenugrek, liver proteome, cadmium chloride

POS-02-085 S100P Up-Regulated in Hepatocellular Carcinoma Cell Line Treated with Thai Herbal Formula

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Cancer is considered to be one of the leading causes of death. Chemotherapy and radiation cause many side effects, resulting in many cancer patients looking for alternative and/or complementary treatments. In Thailand, there are many herbal formulas were used as traditional medicines. In this study, the investigation of their cytotoxic and antioxidant activities using MTT assay and DPPH radical scavenging assay, respectively, on six human cell lines. This formula was most potent in HCC-S102 and was therefore selected to study the protein alteration by proteomic analysis. The results revealed 5 proteins present only in treated cells, 2 proteins present only in non-treated cells and twenty-eight proteins with more than 1.5-fold change. S100P, was the protein that found only in treated cells and was validated by antibody. It plays a role in cancer progression. Ingenuity Pathway Analysis demonstrated that the main pathway involved was that for cancer, correlating with three main proteins, E2F4, MYC, and ESR1. However, further studies should be performed to understand the role of these altered proteins, especially S100P, and the mode of action of folk herbs in cancer treatment.

Keywords: cancer, proteomics, S100P

POS-02-086 Proteomic Approach to Antioxidative Effects of Food Factors Using Fluorescence Labeled Difference Gel Electrophoresis

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Oxidative stress is a phenomenon, in which cellular antioxidant defense mechanism is insufficient to maintain the reactive oxygen species (ROS) level below the threshold limit. This phenomenon has been associated with several human diseases like atherosclerosis, tumor promotion, carcinogenesis, Parkinson's disease and Alzheimer's disease. Oxidative stress leads to protein carbonylation inside the cell due to the formation of aldehyde groups on basic amino acids side chain such as arginine and lysine. In this study, we established a novel proteomic analysis technique, in which protein carbonyls were derivatized with fluorescent dye hydrazide followed by two-dimensional gel electrophoresis. By the use of the new fluorescent dyes, it is now possible to quantify, detect and / or identify the protein calbonyls by 2D-PAGE. The influence of the oxidative stress is analyzed by 2-D differential gel electrophoresis (2D-DIGE). This method allows rapid identification of oxdative stress changes between two samples on the same 2D-gel without influences of gel-to-gel variations. Thus, this method covers a dynamic detection range of more than 3 orders of magnitude. The differential oxidized proteomes of cells treated with and without hydrogen peroxide and/or food factors were resolved and quantitated with two-dimensional differential gel electrophoresis followed by MALDI-TOF/MS identification. We believe that this technique would help us to identify the relationship among oxidative stress and antioxidant activities of functional foods. This method would provide a means toward clarifying a comprehensive view of oxidative modifications of proteins within various diseases and understanding the mechanism of these diseases.

Keywords: antioxidant, 2D-DIGE, carbonylation

POS-02-087 Proteomic Analysis of Stress-Related Proteins Associated with Desiccation Tolerance in Developing Rice Seeds

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During seed development, the water content of seeds decreases remarkably. Generally, the water content of mature dry seeds is less than 20% on a fresh weight basis and such severe desiccation would kill cells from vegetative parts of the plant. However, mature seeds can germinate after imbibition since the embryos are protected by mechanism of desiccation tolerance. In order to understand the mechanism of desiccation tolerance in seeds, the fluctuations of stress-related proteins in the desiccation phase of rice seeds were analyzed by a real-time RT-PCR and gel-based proteomic approach. Based on the changes in water content of developing rice seeds, we defined stages from the beginning of dehydration (10 to 20 days after flowering) and the desiccation phase (20 to 40 days after flowering). The proteomic analysis revealed that late embryogenesis abundant proteins, small heat shock proteins and antioxidative proteins accumulate at the beginning of dehydration and remain at a high level in the desiccation phase, indicating that these proteins are involved in acquisition of desiccation tolerance. The fluctuation in levels of mRNA encoding some stress-related proteins did not precisely reflect the change in levels of these proteins. Therefore, proteomic analysis, which accurately evaluates changes in protein levels, is a more efficient technique than analysis of transcripts for inferring the physiological roles of stress-related proteins in rice seeds.

Keywords: plant proteomics, desiccation tolerance, seed development

POS-02-088 Analysis of Labor-Related Functions of the Cephalic and Thoracic Exocrine Glands of the European Honeybee by Using Large-Scale Proteomics

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The European honeybee is a model social insect. Female honeybees differentiate into two castes: reproductive queens and sterile workers, depending on the amount of royal jelly (RJ) fed during larval stages. Workers shift their labors from nursing to foraging in an age-dependent manner. RJ is a protein-rich larval food secreted from exocrine glands: hypopharyngeal glands (HGs) and mandibular glands (MGs) of young workers. Recently, we used shotgun proteomics to show that the RJ proteome is derived not only from HGs but also from postcerebral glands (PcGs) and thoracic glands (TGs) (Fujita *et al.*, *JPR* (2013)). Aiming at characterizing labor-dependent functions of the worker's exocrine glands, we here performed a comprehensive proteomic analysis of PcGs, TGs and MGs of nurse bees and foragers. Semi-quantitative analysis based on spectral counting classified a total of 2,955 proteins identified as expressed in a gland- and/or labor-selective manner. KEGG pathway analysis showed that proteins related to 'Genetic Information Processing' were more abundant in PcGs of nurse bees than foragers, whereas those related to 'Energy metabolism', especially, v-type proton ATPase related proteins were more abundant in TGs than the other two glands, suggesting a possible role of pH gradient in TG functions, i.e., enhanced secretion that requires ATPs.

Keywords: honeybee, royal jelly, exocrine glands

POS-02-089 Characterization of Muscadine Berry Proteome Using Label and Label Free Mass Spectrometry Approaches

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Muscadine grapes (*Vitis rotundifolia*) are well known for variety of nutraceutical and enological characteristics. Our earlier results indicated potential anticancer activity of berry extracts on human cancer cell lines. These extracts contained variety of phenolics associated with anticancer activity. Until recently, most studies were focused on viniferas grapes, while little information is available on muscadine grape berries. Advances in mass spectrometry enhanced the ability to reveal more identified proteins in tissues. The objective of this research was to investigate the proteome profile of muscadine berry using gel based and gel free separation methods. Total proteins of pericarp were extracted from different stages of berry development and ripening. Two-dimensional electrophoresis resolved approximately 350 proteins. Gel free iTRAQ label followed by mass spectrometry identified 600 proteins, while label free mass spectrometry revealed over 1600 proteins. Over 150 proteins showed differential expression during the berry development. We contemplate that, gel free and label free method of protein identification revealed more proteins in the pericarp tissue. Functional annotation revealed the proteins involved in pathways related to defense and secondary metabolite synthesis. Protein-interaction studies of these differentially expressed proteins revealed several orthologous proteins showing interactions in *Arabidopsis* interactome database. Further investigation on interaction network will determine the role of differentially expressed proteins associated with the biosynthesis of nutraceutical compounds.

Keywords: grape berry, iTRAQ label, mass spectrometry

POS-02-090 Microbiota Profiling to Improve Quality and Safety of Dairy Products

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The aim of this project is to develop and increase knowledge of key factors influencing the presence of clostridial spores in milk and factors affecting, directly or indirectly, technological characteristics and fermentation of cheese, make it more or less susceptible to the development of defects (swelling). Global microbiota isolated from Grana Padano cheese was characterized by 1D and 2D gel electrophoresis and shotgun mass spectrometry. The main goals of this project are :1) investigate differences in microbiota profile in different stages of maturation with or without cheese antibacterial treatment (e.g. Lysozyme treatment); 2) investigate microbiota changes linked to late blowing effect. Using a complementary bioinformatic approach (Unipept[1] and MEGAN[2]) we described the mechanism of bacterial competition and the changing in relative composition of microflora during maturation also with or without add of antibacterials. We expect that our building of metaproteomic information of Grana Padano cheese will enhance safety and quality of this product. And also shed light to understand the relationship between metabolism of starter and contaminant bacteria and cheese composition.

Acknowledgements
Work supported by FILIGRANA project, financed from MiPAAF D.M 25741/7303/11 - 01/12/2011

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Keywords: metaproteomics, food quality, microbiota

POS-02-091 Proteomic Characterization of Ascorbate Peroxidase in Soybean Under Abiotic Stress

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Soybean is an important dicot crop for the high content of oil and protein in its seeds. Abiotic stresses like flooding and drought limit soybean production particularly at an early stage of development. Ascorbate peroxidases (APXs), which was found using proteomic technique as increased and decreased proteins under drought and flooding stresses, respectively, are known antioxidant enzyme that plays key role in abiotic stresses. To investigate the changes in APX in soybean under drought and flooding stresses, Western blotting, enzyme activity assay and biophoton emission techniques were used. Flooding stress was imposed by adding excess amount of water in the sand and drought by withholding water supply. Under flooding stress, a decrease in APX was detected with time. Completely opposite trend was evident in hypocotyl and root of plants exposed to drought. Western blotting and APX activity results are complementary to each other. Biophoton emissions further confirmed the increasing and decreasing trend of APX under drought and flooding stress, respectively. In the present investigation, the increased photon emission is related to the decreased expression of APX under flooding stress. When APX activity becomes low, there might be low scavenging of ROS that produced in excess in response to flooding. This higher ROS level ultimately causes higher emission of biophotons. In contrast, when APX activity is high, there would be more scavenging of ROS which results in low biophoton emission as detected in case of drought. In conclusion, the biophoton emission patterns confirmed the findings of decreasing and increasing APX expression under flooding and drought stress respectively.

Keywords: soybean, abiotic stress, ascorbate peroxidases

POS-02-092 Overexpression of Sucrose Synthase 1 (sus1) in Transgenic Canola Carrying AtDREB2A Transcription Factor

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Stress responsive transcriptional regulation is an adaptive strategy of plants that alleviates the adverse effects of environmental stresses. To analyze the function of *DREB2A* in canola plants, *DREB2A-CA* gene after removing PEST sequence, which acts as a signal peptide for protein degradation, were over expressed in canola plants. The expression of *DREB2A-CA* in transgenic plants was confirmed by southern blotting analysis. We compared the protein expression patterns between leaves of transgenic and normal canola plants by 2-dimensional protein gel electrophoresis. The protein spots which showed expression different between transgenic and normal plants were cut out and analyzed by matrix-assisted laser desorption/ionization time-of-flight/time-of-flight high-resolution tandem mass spectrometry. Forty inducible proteins were identified by this method. Among identified proteins, western blotting and RT-PCR further confirmed overexpression of SUS1 and COR14 in transgenic canola at translation and transcription levels, respectively. We also measured the amount of sucrose and relative water content in transgenic plants under salt stress condition. Since SUS1 is known to confer tolerance to salinity by osmotic adjustment through catalyzes the breaking down of sucrose to fructose and UDP glucose, our data suggest a possible correlation between SUS1 overexpression and accumulation of sucrose in transgenic canola carrying AtDREB2A transcription factor under salt stress.

Keywords: protemics, overexpression, DREB2A

POS-02-093 Two-Dimensional Gel Electrophoresis Analysis of Flavonoid Sulfate Effects on *A. thaliana* T87 Cells

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Background: Cytosolic sulfotransferases (SULTs) are one of the phase II enzymes and catalyze the transfer of a sulfonate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS), a universal sulfate donor, to a hydroxyl or an amino group of acceptor substrate compounds. Recently we reported that there are some flavonoid sulfotransferases in *Arabidopsis thaliana*. However, physiological functions of flavonoid sulfates are totally unknown. For understanding of functions of flavonoid sulfates in *A. thaliana*, we attempted to treat naringenin or naringenin sulfate, a representative flavonoid, to T87 cells and performed the proteomic analysis by two-dimensional differential gel electrophoresis (2D-DIGE).

Methods: *E.coli* cells haboring a SULT enzyme were grown and induced with IPTG for enzyme expression. LB media were changed to M9 media containing substrates. After a 24-h sulfation, the media were collected and purified by HPLC. The chemical structure of flavonoid sulfates was analysed by LC-MS/MS. Prepared naringenin sulfate was treated to T87 cells and analyzed by two dimensional difference gel electrophoresis (2D-DIGE). The differentially expressed protein spots were identified by peptide mass fingerprinting method using MALDI-TOF/MS.

Results and discussion: It was suggested that a sulfated product was equal to naringenin-7-sulfate by LC-MS/MS. As a result of proteome analysis, we have identified for the first time the differential expression of proteins involved in basal metabolism such as glycolysis, amino acid and nucleic acid synthesis. Furthermore, the detoxification enzymes for reactive oxygen species (ROS) were also identified, suggesting that naringenin might play a role in oxidative stress response in *A. thaliana*. On the day, we will also introduce the function of flavonoid sulfates in plants.

Keywords: sulfotransferase, flavonoid

POS-02-094 Starch Glycomic and Proteomic Aspects of Grain Chalkness

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It is widely recognized that global warming is one of the most serious environmental problems. In recent years, chalky rice grain has been frequently occurred by abnormally high temperature during grain-filling. Although various factors are thought to be involved in the occurrence of chalky rice grain, the precise mechanism is not proposed yet. We conducted quantitative proteomic analysis of chalky sites of rice kernels caused during grain-filling. Our samples scraped out chalky sites and clarity sites from a rice grain harvested by the rice field of 2009 and 2010. The obtained results indicated that the expression of reactive oxygen scavenging enzymes and heat shock proteins were increased at the chalky sites of rice grain compared with the clarity sites. Noteworthy, the expression of enzyme proteins operated in starch degradation was markedly increased. Furthermore, Amy II-3 which is expressed in the endosperm, there has been an increase in expression at any pattern in chalky sites of rice grain compared with the normal site. However, there was no significant difference in the expression level of the starch synthesis-related enzymes, such as starch-branching enzyme and starch synthase. In addition, the chain length distribution of starch in the chalky sites of ice grain was indistinguishable from that in the normal site. Based on the above results, we concluded that the occurrence of chalky rice grain is probably induced by the stimulation of starch degradation rather than the abnormality of starch synthesis system.

Keywords: agriculture proteome, rice, iTRAQ

POS-02-095 Proteomic Analysis of Soybean Root during Recovery After Flooding

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Soybean being important legume crop is frequently exposed to flooding that reduces its growth and yield. To understand the mechanism involved in post-flooding recovery in soybean, gel-based and gel-free proteomic techniques were used. Two-day-old soybeans were exposed to flooding stress for 2, 4, and 6 days and morphological changes were measured at 2, 4, and 6 days after water removal. During recovery stage, growth reduction was severe in root of 4-days flooded soybeans as compared with 2-days flooded; whereas it was completely inhibited in 6-days flooded. Based on morphological measurements, soybeans recovered from 2 and 4 days flooding treatment were selected for proteomic analysis. Gel-based proteomics identified a number of proteins that may be involved in flooding recovery processes in the roots of soybean seedlings. The observed changes in protein expression suggested that regulation of root growth through cell wall modification and the synthesis of S-adenosylmethionine-related metabolites are components of the recovery process. Gel-free proteomics techniques were also performed in recovery stage after flooding. These results suggest that alteration of root cell structure through modulation of cell wall metabolism and reorganization of the cytoskeleton likely play an important role in the recovery processes induced by flooding injury.

Keywords: soybean, flooding, proteomics

POS-02-096 Label-Free Quantitative Proteomics of Nuclear Proteins in Soybean Under Flooding Stress

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Flooding is a serious problem in soybean because it results in reduction of growth and productivity. To understand how response to flooding is altered in nuclear protein of soybean, proteomic technique was used. Two-day-old soybean plants were treated with flooding for 2 days and nuclear proteins were enriched from soybean root tips. Flooding responsive proteins were identified using label-free quantitative proteomic technique. Of total 94 proteins, 19 proteins were increased and 75 proteins were decreased under flooding stress. These identified flooding responsive proteins were functionally classified. The increased proteins included 8 proteins involved in protein synthesis, posttranslational modification and protein degradation; whereas the decreased proteins included 5 ribosomal proteins which also involved in protein synthesis. Furthermore, the decreased proteins included 34 proteins involved in transcription, RNA processing, DNA synthesis and maintenance of chromatin structure. Among these proteins, proteins which changed more than 10 times were a novel protein and two poly ADP-ribose polymerases. Analysis of the mRNA expression levels of these proteins showed a similar tendency to their protein abundance changes. These results suggest that poly ADP-ribose polymerase might play a role in response to flooding stress in soybean.

Keywords: soybean, flooding, proteomics

POS-02-097 Acceleration of Germination of Rice Seeds by Soaking with Red Onion

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Although brown rice grains contain more nutritional components, such as vitamins and gamma aminobutyric acid (GABA), than the ordinary milled rice grains, brown rice is not considered suitable for table rice because of its dark appearance and hard texture. Pre-germinated brown rice (PGBR) is improved compared with ordinary brown rice due to the improved texture of grains. GABA is a promising compound in terms of bio-functionality and many researchers are trying it to develop GABA-rich functional foods like a PGBR.
We developed a novel rapid germination method to improve the quality of PGBR and to save the time needed for germination. This method is useful because red onion inhibits infestation of microorganisms during the germination. PGBR is prepared by soaking in 2% red onion solution for 16h at 35˚C, which increased GABA content, glucose content, and germination ratio was increased 2.3 times, 2.9 times and 2.3 times those in PGBR by soaking in de-ionized water (pH5.8) at 35˚C for 16h. Moreover, it became possible to fortify PGBR with bio-functional components, such as quercetin, contained in onion.
A series of quantitative shotgun proteomic analyses with iTRAQ labeling were carried out. Germination (with/without red onion) enhanced the expression of CBL-interacting protein kinase 23 in the germinated rice grains. Interestingly, FIB (Fish bone) and putative alliinase were highly expressed in the rice grains germinated with red onion. It is strongly suggested that phytohormones and protein kinase are involved in the physiological mechanism of the germination of rice.

Keywords: rice, germination, red onion

POS-02-098 Proteomic Analysis Applied to Meat Science: Characterizing Post Mortem Changes in Japanese Wild Deer Meat

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A predictable meat quality is essential to the meat industry, with tenderness and juiciness being the most important quality. Proteomic analysis was used to investigate the relation between changes in postmortem proteome of myofibrillar proteins and tenderness development. We characterized the changes that occurred in muscle proteins of Japanese wild deer (*Cervus nippon centralis*) during postmortem storage. Muscle samples were taken at slaughter and aged for 0, 3, 5, 7, 9 and 14 days of postmortem storage at 2˚C and analyzed by SDS-PAGE. In addition we analyzed the degree of tenderness, free amino acid concentration and fragmentation of myofibrils. Eight bands were characterized by SDS-PAGE and statistical analysis and sequenced by MALDI-TOF MS/MS. During post mortem storage, the 30kD band showed a tendency to increase and was associated with increased tenderness, free amino acid concentration and fragmentation of myofibrils. Ten peptides corresponding to the 30kD band were identified as troponin-T. In addition some degradation products from myofibrillar proteins were included in the 30kD component. The results demonstrate that the 30kD proteins are related to tenderness development during postmortem storage and these proteins may be good candidates for meat quality markers on Japanese wild deer meat.

Keywords: Japanese wild deer meat, post mortem changes, myofibrillar proteins

POS-02-099 Post-Translational Regulation of Nutrient Signaling and Metabolism Mediated by 14-3-3 Proteins in Plants

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Nutrient availability, in particularly the balance of carbon (C) and nitrogen (N) is one of the most important factors for regulating plant metabolism and development. However detailed mechanisms regulating C/N balance response are not well understood. We previously isolated a novel ubiquitin ligase, ATL31 as essential C/N regulator, which targets 14-3-3 proteins for direct ubiquitination and degradation by proteasome in *Arabidopsis thaliana* (Sato et al., 2009/2011, Plant J). 14-3-3s are reported to interact with various phosphorylated proteins and regulate the key enzyme activities involved in nutrient metabolism and signaling. Now we are evaluating the global effect of 14-3-3 proteins in C/N response with proteomics approach. Our 14-3-3 interactome study with IP/MS analysis demonstrated the dynamics of 14-3-3 interactors in response to C/N condition. In addition, we are trying to integrate phosphorylation signal with 14-3-3 interactome analysis. The details of these proteomics study for 14-3-3 function will be reported.

Keywords: 14-3-3 protein, phosphorylation, plant nutrient metabolism

POS-02-100 Comprehensive Identification of OsSUMO Binding Proteins in Rice Exposed to High Temperature Stress Condition

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SUMO (Small ubiquitin-related modifier) is a post-translational modification factor, and is highly conserved among eukaryotic genomes and the SUMO modification to the substrate protein is increased under high-temperature stress conditions. Rice genome has multiple genes encoding SUMO paralogs, and it is little known about the difference in sumoylated proteins between SUMO paralogs in rice. In this study, we established DsRed:SUMO1/2/3 transgenic calli, and identified the heat-stress responsive SUMO interacting proteins by LC-MS/MS. We analyzed co-immunoprecipitated SUMO interacting proteins by SDS-PAGE, and all transgenics showed significant increase of SUMO interacting proteins under high-temperature condition. The protein banding pattern of elution extract from DsRed:SUMO1 is similar to that of DsRed:SUMO2 whereas that of DsRed:SUMO3 is different from that of DsRed:SUMO1/2. The results suggested that the SUMO1/2 substrates might be different from SUMO3 substrates. To identify the target proteins of SUMO paralogs, size fractionated protein bands are digested and analyzed by LC-MS/MS. The difference of the substrate between paralogs will be discussed from these results.

Keywords: SUMO, thermostress, rice

POS-02-101 Development of an Inflammation-Specific MRM Assay for the Discovery of a Novel Pharmaceutical for the Treatment of Human Vascular Diseases from Derivatives of the Escin Chestnut Extract

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Escin, the extract derived from the horse chestnut (*Aesculus hippocastanum*) is a mixture of tens of structurally closely related triterpene saponin compounds which has shown potential in treatment of a number of human vasculature related ailments, and could be a source of novel drug compounds. Non-targeted and targeted mass spectrometry-based proteomic assays were used to search for newly synthesized escin derivative compounds with protective effects on the vascular endothelium under inflammatory conditions. Initial global non-targeted iTRAQ analysis was carried out on HUVEC cells exposed to the escin extract in combination with TNF. This global proteomic analysis revealed mechanistic information on the action of this herbal remedy and also provided protein markers of the inflammatory response of the endothelium cells under exposure to the escin mixture. Targeted proteomic MRM assays were then developed based on these markers to verify the global analysis results and create a protein panel assay that could be used to select specific escin derivatives. The MRM assay was composed of a 24-protein panel with members from the haemostasis, inflammatory and apoptosis pathways and could quickly select compounds that were protective of the endothelium. The specificity, accuracy and high-throughput of the targeted MRM assay proved superior to the global proteomic analysis and this 24-protein panel could be used in other applications such as compound selection in anti-inflammatory drug studies.

Keywords: escin, human endothelial cells, MRM

POS-02-102 Isotope Labeling-Based Quantitative Proteomics of Developing Seeds of Castor Bean (*Ricinus communis* L.)

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Castor bean (*Ricinus communis* L.) is an important source of ricinoleic acid for industrial applications; furthermore its oil has potential as biodiesel. Lack of knowledge regarding the molecular mechanisms involved in the fatty acid synthesis and its assembly in triacylglycerols coupled with the presence of highly toxic and allergenic proteins, ricin and 2S albumin respectively, have limited a wider exploitation of this species. We used a mass spectrometry-based quantification approach employing isotopic (ICPL) and isobaric (iTRAQ) labeling, to investigate the enzymes involved in the pathways of triacylglycerol metabolism and the deposition pattern of seed storage proteins, toxins and allergens during castor bean seed development. Additionally, we have used off-line hydrophilic interaction chromatography (HILIC) as a step of peptide fractionation preceding the reverse phase nanoLC coupled to a LTQ Orbitrap. Merging all technical and labeling replicates data, we were able to identify a total of 1,875 proteins expanding so far the number proteins identified from developing castor bean seeds. Cluster and statistical analysis resulted in the determination of 975 protein trend patterns and relative abundance of 618 proteins, respectively. Results give important insights into certain aspects of the biology of castor seed development such as carbon flow, anabolism and catabolism of fatty acid and the pattern of deposition of reserve proteins, toxins and allergens such as ricin and 2S albumins. We also found, for the first time, that some genes of reserve proteins are differentially expressed during seed development.

Keywords: Ricinus communis, developing seeds, quantitative proteomics

POS-02-103 Proteomic Identification of Diverse and Dynamic Metabolic Network Upon Reduction of Cellular Oxalate Level in Crop Plant

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Oxalic acid, a two-carbon dicarboxylic acid plant metabolite present ubiquitously as an inert end-product of carbon metabolism is increasingly recognized as a food toxin with negative effects on human nutrition. Oxalates in animals, including human, mostly originate from diet and excess ingestion of oxalate result in a variety of kidney-related disorders, besides neurolathyrism and coronary disease. Decarboxylative degradation of oxalic acid is catalyzed, in a substrate-specific reaction, by oxalate decarboxylase (OXDC), forming formic acid and CO_2 (Chakraborty et al. 2002 PMID: 12192069). To investigate the role of OXDC and the metabolic consequences of oxalate downregulation in a heterotrophic, oxalic acid-accumulating fruit, we generated transgenic tomato *(Solanum lycopersicum)* plants expressing an OXDC *(FvOXDC)* from the fungus *Flammulina velutypes* specifically in the fruit. E8.2-OXDC fruit showed upto a 90% reduction in oxalate content, which correlated with concomitant increases in calcium, iron and citrate. Comparative proteomic analysis revealed a spectrum of OXDC-regulated proteins and pathways previously unknown as OXDC targets and suggested that metabolic remodeling was associated with the decrease in oxalate content. Examination of E8.2-OXDC fruit proteome indicated that OXDC-mediated regulation is influenced by G-protein and Ca^{2+} signaling. We also propose a working model of OXDC-mediated regulation involving metabolic pathways and cross-talk between various cellular processes in feedback dependent activation or repression of vacuolar trafficking and cellular homeostasis (Chakraborty et al. 2013 PMID: 23482874). Collectively, our study provides insights into OXDC-regulated metabolic networks and may provide a widely applicable strategy for enhancing crop nutritional value.

Keywords: comparative plant proteome, oxalic acid, metabolic remodeling

POS-02-104 Proteomic Profiling of Ganglioside-Associated Microdomain in Malignant Melanomas

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Melanoma is difficult to cure because of its malignant properties. Ganglioside GD3 levels are highly elevated in melanomas. It has been shown that GD3 enhances cell proliferation and invasion using GD3 synthase-transfectant cells of a GD3-negative (GD3-) mutant line SK-MEL-28 N1. p130Cas, paxillin and FAK were identified as highly tyrosine-phosphorylated molecules involved in the increased cell proliferation and invasion with GD3 expression. However, remaining issue to be clarified is how GD3 interacts with known/unknown molecules in the vicinity of cell membrane. To clarify these mechanisms, we isolated the glycosphingolipid-enriched microdomain (GEM)/rafts with sucrose density-gradient ultra-centrifugation of Triton X-100 extracts from GD3+ and GD3- cells. We also labeled cell surface molecules present in the vicinity of a target molecule in living cells with EMARS reaction (Honke et al.). Isolated molecules as components in GEM/rafts and EMARS products were comprehensively analyzed with LC/MS (LTQ-Orbitrap). In the GEM/rafts, 73 membrane proteins were identified in GD3+ and GD3- cells. Among them, 50 membrane proteins were common. In the EMARS with GD3, 9 molecules such as neogenin, integrin α3, β1 and MCAM were identified as GD3-interacting molecules. They were also identified in the GEM/raft fraction. To check the results of LC/MS, we performed immuno-blotting and confocal microscopy analysis. Neogenin and MCAM were found in GEM/raft fraction of GD3+ cells and labeled with EMARS reaction in immuno-blotting. They were co-localized with GD3 in confocal microscopy analysis. Differences in the molecular profiles identified in GEM/rafts and as EMARS products suggest the heterogeneity in GEM/rafts.

Keywords: lipid raft, glycosphingolipid, EMARS

POS-02-105 Physiological and Proteomic Characterization of Outer Membrane Vesicle of *Pseudomonas putida* KT2440

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Outer membrane vesicles (OMVs) have been found from various pathogenic gram-negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii*. In this study, we isolated OMV from a representative soil bacterium, *Pseudomonas putida* KT2440, which has biodegradability to various aromatic compounds. *P.putida* KT2440 was obtained from ATCC. Bacterial cultivation was performed according to previous methods (Yun et al. 2011). OMVs of *P. putida* KT2440 were purified from the bacterial culture supernatants by using a modified method from Kwon et al. LC-MS/MS analysis was performed according to a previous method(Park et al. 2006). Proteomic analysis of OMV showed outer membrane proteins (OmpF, OmpW, et al), TonB-dependent receptors, outer membrane ferric siderophore receptor, and 17 kDa surface antigen as major outer membrane proteins of OMV of *P. putida* KT2440. OMV of *Pseudomonas putida* KT2440 showed pathological effect on the culture cells originated from human lung cell. Production and its protein components of OMV were dependable to the carbon sources of culture media. Production of OMV was significantly increased in rich medium (LB) compared with in minimal medium containing benzoate and succinate as sole carbon sources, respectively. However, porins (benzoate-specific porin, BenF-like porin, et al) and enzymes (catechol 1,2-dioxygenase, benzoate dioxygenase, et al) for benzoate degradation were uniquely found in OMV prepared from *P. putida* KT2440 cultured in benzoate. This result suggests that production and components of OMV of *Pseudomonas putida* KT2440 were controlled by the environmental stimulus or nutrients.

Keywords: Outer membrane vesicles (OMVs), Pseudomonas Putida KT2440, LC-MS/MS, degradation

POS-02-106 2D-DIGE Analysis of Cell Surface Proteins Reveals Characteristic Proteome Profiles for Effects of Sulfasalazine in Human Synovial Sarcoma Cell Line, SW982

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Background and objective. Sulfasalazine (SASP) is currently used as a disease-modifying antirheumatic drug, however, the action mechanism of SASP has not been fully understood. To promote understanding of the mechanism, we studied changes of protein profiles by SASP and its metabolites, sulfapyridine (SP) and 5-aminosalicylic acid (5ASA), focusing on cell surface proteins of human synovial sarcoma cell line, SW982.
Methods. Cell surface proteins were isolated from SW982 cells treated with SASP, SP and 5ASA and from non-treated ones by using covalent binding to a thiol-cleavable amine-reactive biotinylation reagent (Sulfo-NHS-SS-Biotin). Then the cell surface proteins were separated by 2-dimensional difference gel electrophoresis (2D-DIGE). Protein spots intensity of which was changed by the agents were detected by using Progenesis SameSpots software.
Results. A total of 860 protein spots were visualized by 2D-DIGE across all the gels. Compared to the non-treated cells, we found significant changes (more than 1.3 folds or less than 1/1.3 fold, $p<0.05$) in the expression levels of 37 protein spots (14 up-regulated and 23 down-regulated) in the cells treated with SASP, 24 spots (21 up-regulated and 3 down-regulated) in the cells treated with SP, and 29 spots (3 up-regulated and 26 down-regulated) in the cells treated with 5ASA. Twelve spots were overlapped among them (5 between SASP and SP, 7 between SASP and 5ASA).
Conclusion. We found that multiple cell surface proteins were affected by SASP. The affected cell surface proteins would be related to the action mechanism of SASP. Identification of the affected cell surface proteins, which is on-going, would help understanding of the action mechanism of SASP.

Keywords: rheumatoid arthritis, sulfasalazine, SW982

POS-02-107 Proteomic Analysis of Outer Membrane Vesicles (OMV) from Multidrug-Resistant *Acinetobacter baumannii* DU202

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Acinetobacter baumannii is an important nosocomial pathogen, particularly in intensive care units of the hospitals. Multidrug-resistant (MDR) *A. baumannii* are difficult strains to treated with antibiotics, and treatment failure in infected patients is of great concern in clinical settings. Outer membrane vesicles (OMVs) are used by bacteria as a secretion mechanism that leads to the delivery of various bacterial proteins and lipids into host cells, thus eliminating the need for bacterial contact with the host cell. MDR *A. baumannii* are DU202 also known to produced OMVs. OMV fractions was separated by ultracentrifuge with the Quix-stand. Comparative proteomic analysis of membrane fraction and OMV fraction are used by 1DE-LC-MS/MS proteomic approaches. Proteomic analysis of OMV showed outer membrane proteins (Omp38, OmpH, et al), putative glucose-sensitive porin (OprB-like), putative outer membrane protein, and TonB-dependent siderophore receptor as major outer membrane proteins of OMV of *A. baumannii* DU202. In total, 248 (LB), 264 (tetracycline) proteins were identified and 221(imipenem) were classified as outer membrane, periplasmic, or plasma membrane proteins. Through this study, we will have a better understanding of resistant mechanisms of MDR *A. baumannii* to active antibiotics in a proteomic perspective. OMV of *A. baumannii* showed pathological effect on the culture cells originated from human lung cell.

Keywords: Acinetobacter baumannii DU202, Outer membrane vesicles

POS-02-108 Identification of Protein Complexes in the Golgi from Rat Liver by High Resolution Native Gel and nanoLC-ESI-Q-TOF

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Golgi apparatus plays a critically important role in the protein sorting and modification. However, few multiprotein complexes (MPCs) are known in the Golgi apparatus so far. In this paper, high resolution native gel coupled with nanoLC-ESI-Q-TOF was used to identify the MPCs in the Golgi apparatus. To this end, golgi apparatus was isolated from the rat liver first, and then the protein complexes in the golgi fraction were extracted and approximately 100 μg proteins were run in 2-7% native gel at pH 7.2 with 1 mM calcium. After finishing running the gel, the gel was stained by Coomassie Blue R and cut into 50 slices. Each slice was performed in-gel digestion and tryptic peptides were subjected to LC-ESI-Q-TOF. MS/MS data generated by data-dependent acquisition via the Q-TOF was searched against a SwissProt database using Spectrumill search engine. The total intensity for each protein is calculated from sum of intensity of corresponding identified peptides and utilized to construct the protein abundance profiles across the gel slice. Hierarchical clustering using Pearson correlation coefficient was performed to find the protein complexes in the golgi. Through clustering, we found 60 potential protein complexes in the golgi, which helps elucidate the golgi function. In the further study, protein complexes needs to be validated through the biological means such as co-immunoprecipitation and microscopy.

Keywords: golgi apparatus, protein complexes, native gel

POS-02-109 A Plasma Membrane Proteomic Analysis of Mouse and Human Cardiovascular Proteins

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We employed cationic silica-bead coating coupled with shotgun proteomics to enrich and identify cell-surface associated proteins from primary mouse neonatal and human fetal ventricular cardiomyocytes, endothelium, and smooth muscle cells. Human coronary artery smooth muscle and endothelial cells, and human cardiac muscle derived cardiomyocytes were cultured. Human fetal ventricular myocytes were acutely dissociated. Membrane proteins were cross-linked to cationic silica beads to isolate cytosolic proteins and a membrane fraction attached to the beads. Samples were analyzed by LC-MS MuDPIT strategies on a Thermo LTQ or LTQ Orbitrap. Shotgun proteomics identified >3,000 mouse and >2,500 human proteins. Organelle enrichments were confirmed by immunoblotting. Mapping of orthologous proteins between mouse and human resulted in 1717 proteins. In the cardiomyocytes, QSpec statistical analysis calculated differential spectral counts between proteins found in the membrane enriched and membrane depleted fraction and provided a dataset of 555 cardiomyocytes proteins. Bioinformatic integration with transmembrane helix predictions, Phenotype Ontology (PO), and available microarray data identified a rank ordered set of cardiac-enriched surface proteins; select examples of which the subcellular location were further confirmed using confocal microscopy, immunogold electron microscopy, and sucrose density gradients. For several of the highly ranked membrane proteins, lentiviral-based shRNA knock-down demonstrated significantly altered Ca^{2+} transient amplitude, release rates, and uptake rates. Initial knockouts in *Danio rerio* (zebrafish) support the cultured myocyte studies. In conclusion, we have provided the first comprehensive analysis of membrane cell surface-associated proteins in all three major cardiac cell types, and provided a pipeline to validate surface proteins in cardiomyocytes that might be involved in Ca^{2+} dynamics.

Keywords: cardiomyocyte, membrane proteomics, cell surface

POS-02-110 An Improved Subcellular Protein Atlas

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Knowing the exact subcellular location of a given protein is of great importance as it indicates protein function and leads to a better understanding of how proteins interact in networks and signaling pathways. The Subcellular Protein Atlas aims to systematically localize the human proteome using an antibody-based approach as part of the Human Protein Atlas project.
To further increase the quality of the subcellular localization data, several validation approaches have been developed. This includes the use of siRNA for validation of antibody binding and protein localization (1), fluorescently tagged proteins (FP) as a complementary technique(2) and paired antibodies targeting the same protein.
In addition, an effort has been made to enable localization of rarely expressed proteins. The panel of human cells has increased to fifteen cell lines of different origin, from which the most suitable is selected based on transcript levels (RNA-seq) of the target gene (3). We have also demonstrated the value of using FP expressing cells for localization of proteins not naturally expressed in the cell panel (2).
In total, over 12000 proteins have been localized. As a result of the new validation strategies and the extended cell panel with RNA-seq data, a new version of the Subcellular Protein Atlas will be released. Here, we present the content and results of the Subcellular Protein Atlas as well as discuss the path to a complete coverage of the human proteome.

Stadler, *et al,* (2012), *J Proteomics*
Stadler, *et al,* (2013), *Nat Methods*
Danielsson, *et al*, (2013), *J Proteome Res*

Keywords: subcellular localization, organelles, Human Protein Atlas

POS-02-111 Proteome Analysis of Normal Human Circulating Microparticles

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Introduction
Microparticles (MPs) are small membraneous vesicles (<1 μm) released into the circulating blood from apoptotic or activated cells as part of normal homeostasis. Their numbers, origin, and composition change in pathology.
Methods
We here characterize the normal MP proteome using nano-LC-MSMS on an LTQ-Orbitrap with optimized sample collection, preparation, and analysis of 12 different normal samples. Analytical and procedural variation was estimated in triply processed samples analyzed in triplicate from two different donors. In addition, two samples were also fractionated by OFFGEL isoelectric focusing prior to LC-MSMS for more in depth analysis of the MP proteome.
Results & Conclusions
Using conservative parameters, 536 different unique proteins were quantitated from single run samples and 1204 proteins were identified by the combination of OFFGEL fractionation and LC-MSMS. Of these, 334 were present in all samples and represent an MP core proteome. Technical triplicates showed <10% variation in intensity within a dynamic range of almost 5 decades. Label-free quantitation of cytoskeletal protein showed linear relations with MP numbers quantified by flow cytometry. Differences due to variable MP numbers and losses during preparative steps could be normalized using cytoskeletal MP protein intensities. Our results establish a reproducible LC-MS/MS procedure, provide a simple and robust MP preparation method, and yield a baseline MP proteome for future studies of MPs in health and disease.

Keywords: microparticles

POS-02-112 Subcellular Fractionation and Bottom-Up Proteomics for Proteome-Wide Protein Localization

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Protein expression varies greatly between cells and between tissues in an organism. Within the cell, most proteins have a distinct localization, closely related to their respective function, *e.g.* as a membrane transporter, nucleic acid binding protein, metabolic enzyme or structural protein. There are several experimental methods for measuring the location or spatial distribution of proteins. Immunohistochemistry with well-characterized antibodies has been applied on a proteomics scale by the Human Protein Atlas (HPA) effort. As part of this consortium, the subcellular localizations of a large and growing number of human proteins are now being experimentally obtained through immunofluorescent staining and confocal microscopy. As an alternative to protein labeling, staining and microscopy methods, proteome-wide spatial information can be obtained by subcellular fractionation and state-of-the-art bottom-up proteomics methodology. We will here describe an experimental workflow and - critically - an informatics pipeline to generate subcellular localization for thousands of proteins in a single experiment, using cardiomyocyte progenitor cells and other examples for illustration. We will compare, and discuss how to compare, bottom-up proteomics data with data from the HPA and other primary information sources. In addition to experimental methods, protein localization can also be inferred or predicted from localization signals or homology. We will also show how to compare experimental protein localization data from bottom-up proteomics with predictions from protein sequences. Finally, we will describe how subcellular fractionation is a powerful tool to generate rich spectral libraries for improved peptide identification in bottom-up proteomics.

Keywords: organellar fractionation, bioinformatics, bottom-up proteomics

POS-02-114 Proteomic Analysis Identifies CaFer1, A Dehydration-Responsive Ferritin in Chickpea (*Cicer arietinum* L.) Secretome, That Promotes Stress Tolerance

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Cell-to-cell communication or cell signaling is maintained by a dynamic biochemical network, involving the extracellular matrix, plasma membrane and cytoskeleton, which is the major determinant that dictates cell fate decision in response to different stress conditions. In an attempt to dissect the molecular circuitry of stress tolerance in plants, we examined the extracellular matrix proteome of a grain legume, chickpea. A critical screening of the proteome led to the identification of a dehydration-responsive ferritin, CaFer1. We investigated the gene structure and organization of *CaFer1*. A detailed analyses demonstrated that *CaFer1* might be involved in dehydration response, besides *Fusarium oxysporum* infection. Expression of *CaFer1* was found to be regulated by dehydration and high salinity, and by treatment with ABA, suggesting that the stress-responsive function of CaFer1 might be associated with ABA-dependent network. Functional complementation of yeast mutant Δ yfh1, deficient in frataxin, substantiates its role in oxidative stress. We used both CaFer1-overexpressed plants and loss-of-function mutants to decipher the role of CaFer1 in iron homeostasis and plant growth and development. Overexpression of CaFer1 in transgenic Arabidopsis seedlings conferred dehydration, salinity and oxidative stress tolerance, and also contributed to improved shoot and root architecture. Our results suggest that the extracellular CaFer1 plays a key role in iron-buffering and multivariate stress tolerance in plants.

Keywords: extracellular matrix/ secretome, ferritin, iron homeostasis

POS-02-113 Proteome Analysis of *Gloeobacter violaceus* PCC7421: Fractionation of Total, Soluble and Insoluble Proteins

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Unicellular *Gloeobacter violaceus* PCC 7421 is a Gram-negative photoautotrophic cyanobacterium. From the phylogenetic study *G. violaceus* was divided at very early point of the phylogenetic tree and contained very primitive and unusual features like lack of thylakoidal membrane and missing of some photosystem components. Genome sequence was completed in 2003 (Cyanobase, Kazusa Institute). But the research area was very limited due to the difficulties in cell culture and some of unique characteristics. In this study we performed proteomic analysis of *G. violaceus* through the following two approaches. First, we separate the total, soluble and insoluble fractions of *G. violaceus* and performed the two-dimensional electrophoresis (2-DE). The soluble and insoluble fractions were separated by repetitive ultracentrifugation. Second, insoluble fractions were analyzed by FT-ICR mass spectrometer. After 2-DE separation in-gel digested spots were identified by the MALDI-TOF/TOF analysis about 115, 73 and 21 proteins were identified from total, soluble and insoluble fractions, respectively. Also insoluble fraction was digested using both chemical and tryptic in-solution digestion methods to obtain the more peptides with transmembrane regions. Following the FT-ICR analysis 785 proteins were identified and among them 139 proteins with 1 or more (~14) transmembrane domains were analyzed using TMHMM program. Considering the number of theoretical proteins (965) with 1~ TM about 14.4% of that proteins were found. Through these proteomic analyses we could determine the protein preparation methods and provide the 2-DE map of *G. violaceus.* These results will be helpful to get the metabolic information of *G. violaceus.*

Keywords: *Gloeobacter violaceus* PCC 7421, Two-dimensional electrophoresis (2-DE), MALDI/TOF/TOF analysis

POS-02-115 Profiling and Semi-Quantitative Analysis of Cell Surface Proteome in Human Mesenchymal Stem Cells

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Mulitpotent mesenchymal stem cells (MSCs) derived from human bone marrow are promising candidates for the development of cell therapeutic strategies. MSC surface protein profiles provide novel biological knowledge concerning the proliferation and differentiation of these cells, including the potential for identifying therapeutic targets. Basic fibroblast growth factor (bFGF) affects cell surface proteins, which are associated with increased growth rate, differentiation potential, as well as morphological changes of MSCs *in vitro*.

Cell surface proteins were isolated using a biotinylation-mediated method and identified using a combination of one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and mass spectroscopy (MS). The resulting gel lines were cut into 20 bands and digested with trypsin. Each tryptic fragment was analyzed by liquid chromatography-electrospray tandem MS. Proteins were identified using the Mascot search program and the IPI human database. Noble MSC surface proteins (n=1001) were identified from the cells cultured either with (n=857) or without (n=667) bFGF containing medium in three independent experiments. The proteins were classified using Fatigo software to elucidate their function. We also confirmed proteomics results using Western blotting and immunofluorescence microscopic analysis.

The nature of the identified proteins makes it clear that MSCs express a wide variety of signaling molecules including those related with cell differentiation. Among the latter proteins, four Ras-related Rab proteins, Laminin-R, and three 14-3-3 proteins that were fractionated from MSCs cultured on bFGF containing medium are implicated in bFGF-induced signal transduction of MSCs. Consequently, these finding provide an insight into the understanding of the surface proteome of hMSCs.

Keywords: mesenchymal stem cell, surface protein, bFGF

POS-02-116 Segment-Specific Membrane Protein Profiling of Human Colon

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The epithelial cells in the human colon play an important role in protecting the underlying tissue by continuous mucus secretion, and are involved in immune interaction, ion exchange and water reabsorption and secretion. Some aspects of epithelial cell function are known to differ along the length of the colon, although a complete characterization at protein level is lacking. We therefore analyzed plasma membrane proteins of isolated colonic epithelial cells isolated from four colonic segments along the large intestine: the ascending, transverse, descending and sigmoid colon. The cells were obtained from mucosal biopsies of healthy control patients (n=4) undergoing routine colonoscopy. Label-free quantitative proteomic analysis was performed using high-resolution mass spectrometry. A total of 2253 proteins were identified of which 1073 proteins in all four segments of the four patients, of which 552 contained transmembrane-spanning domains. Most membrane proteins were expressed in equal amounts along the colon, while proteins associated with bacterial sensing, cation-transport and sialtransferases decreased in the proximal to distal direction, where as microbial defence and anion-transport associated proteins increased in towards the distal end. The presented work is the first comprehensive quantitative dataset of membrane protein expression along the human colon, and will add to the knowledge of the physiological function of the different parts of the colonic mucosa.

Keywords: membrane proteomics, label free, colon

POS-02-117 Membrane Proteomics Study of *Clostridium acetobutylicum*: Discovery of the Proteins Regulating the Transition from Acidogenic to Solventogenic Phase

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To explore the regulation function of cell membrane in the growth phase shift, the response of membrane proteins in *Clostridium acetobutylicum* towards the acidogenic phase and solventogenic phase were conducted in this study. The bacterial membrane proteins at the two stages were prepared by differential ultracentrifugation and their peptides were generated from in-gel digestion. After labeled with iTRAQ reagents, the peptides were separated by differential pH two-dimensional reverse-phase HPLC, and then delivered to ESI MS/MS for protein identification. The data elicited from mass spectrometry revealed total of 311 proteins identified with two unique peptides. Furthermore, as compared to acidogenic phase, 35 proteins are up regulated and 44 proteins are down regulated at solventogenic phase. Bioinformatics analysis suggested the differential proteins were mainly related to transporters and metabolic enzymes, indicating the involvement of membrane in metabolism pathways. Two key enzymes in the solvent generation pathway, 3-hydroxybutyryl-CoA dehydrogenase (BHBD) and butanol dehydrogenase (BDH), were found up regulated at solventogenesis, and the enzyme activity of BHBD was proved to be much higher in solventogenesis than in acidogenesis. It was noticed that one of the phosphotransferase system proteins, HPr, is up regulated at solventogenesis, which was previously reported having regulation activity. More importantly, HPr was found to have interactions with BHBD and BDH, as revealed by blue native PAGE analysis, which might indicate the regulation of HPr to butanol synthesis pathway. These preliminary analyses give some new clues of the function of cell membrane in the growth phase shift and solvent synthesis.

Keywords: membrane protein, iTRAQ

POS-02-118 Purification and Proteomic Profiling of Kidney Vascular Endothelial Cell Plasma Membrane Proteins

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Vascular endothelial cells (VECs) play crucial roles in physiology and pathologic conditions in tissues and organs. Since most of these roles could be attributed to VEC plasma membrane proteins, and VECs have organ- and tissue-specific features, we aimed in this study to identify plasma membrane proteins of VECs of the kidney. Rat kidneys were perfused with cationic colloidal silica nanoparticles (CCSN) to label the VEC plasma membrane. The plasma membrane coated with CCSN nanoparticles were purified by Necodenz density gradient centrifugation. The coating of VEC plasma membranes with CCSN were confirmed by either light microscopy or elector microscopy, and the successful purification of VEC plasma membrane was confirmed by Western blotting analysis using several membrane markers including cavelolin (plasma membrane), LAMP1 (lysosome), cytochrome c (mitochondria), and Ran (nucleus). The isolated preparations of plasma membrane were separated on SDS-PAGE gels, and in-gel digested with trypsin for mass spectrometry using a LC-iontrap tandem mass spectrometer. The proteomic analysis identified 582 proteins with a high-confidence. These proteins included 16 known VEC plasma membrane such as ICAM-2, and 8 novel VEC plasma membrane proteins including Deltex 3-like protein, and phosphatidylinositol binding clathrin assembly protein (PICALM). Gene Ontology (GO) analysis indicated that 84% of identified proteins could be annotated with plasma membrane proteins, and that many key functions of plasma membrane, in particular those of endothelial cells, were significantly enriched. These results suggested that the CCSN method is a reliable technique for isolation of VEC plasma membrane, and contribute to provide a comprehensive profile of proteome of plasma membrane of VECs of the kidney.

Keywords: kidney, vascular endothelial cell, plasma membrane proteins

POS-02-119 A Membrane Proteomics Signature Reveals Surface Factors Associated with Human Embryonic Stem Cell Pluripotency

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Human embryonic stem cells (hESCs) are promising source in regenerate medicine for tissue repair and replacement due to their unique capability of self-renewal and differentiation into variety of adult cell types. Membrane proteins play critical roles in regulating hESCs and cell surface markers are extensively used for stem cell classification and purification as well as monitoring the differentiation stages. An in-depth view of the stage- and lineage-specific expression of hESC membrane proteome will potentially discover efficient stemness-related markers, enhance our understanding on the underlying mechanisms of stem cell differentiation and provide opportunities toward isolation of homogenous primary stem cell population. To reach this end, we applied our developed gel-assisted digestion-based quantitation approach to define a membrane proteomic profile of hESCs and 16-day embryoid body outgrowth. In summary, 4691 proteins were identified and 843 proteins showed ≥ 1.4-fold differential expression during hESC differentiation. The proteomic data revealed that some cell surface markers, previously discovered by gene expression array, have unaltered expression during stem cell differentiation, which may be due to differences in protein turnover and regulation of the abundance of cellular mRNA and proteins. Furthermore, we proposed a panel of cell surface proteins as the basis for the definition of a membrane proteomics signature of hESCs. Among them, two proteins were validated as overexpressed marker proteins in hESCs and affected the maintenance of hESC pluripotency via the WNT signaling pathway.

Keywords: membrane proteomics, stem cell

POS-02-120 Proteomic Profiling of Exosomes Reveal the Intercellular Transfer of Oncogenic Cargo

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Exosomes are 40-100-nm-diameter nanovesicles of endocytic origin that are released from diverse cell types. Depending on the originating tissue, exosomes contain a subset of the host cell-specific molecular cargo including proteins, lipids and RNA. The affordability of accessing exosomes in bodily fluids and the presence of host-cell specific cargo have created immense interest in utilizing exosomes for biomarker analysis. Here, we describe an immunoaffinity capture method using the colon epithelial cell-specific A33 antibody to purify colorectal cancer cell (LIM1215)-derived exosomes. Mass spectrometry-based proteomic analysis revealed a significant enrichment of proteins containing coiled coil, RAS, and MIRO domains in the exosomes. A comparative protein profiling analysis of LIM1215-, murine mast cell-, and human urine-derived exosomes revealed a subset of proteins common to all exosomes such as endosomal sorting complex required for transport (ESCRT) proteins, tetraspanins, signaling, trafficking, and cytoskeletal proteins. A conspicuous finding of this comparative analysis was the presence of host cell-specific proteins such as A33, cadherin-17, carcinoembryonic antigen, epithelial cell surface antigen (EpCAM), proliferating cell nuclear antigen, epidermal growth factor receptor, mucin 13, misshapen-like kinase 1, keratin 18, mitogen-activated protein kinase 4, claudins (1, 3, and 7), centrosomal protein 55 kDa, and ephrin-B1 and -B2. Furthermore, uptake assays revealed that exosomes with the oncogenic cargo can be uptaken by the target cells and influence the signaling pathways. The LIM1215-specific exosomal proteins identified in this study may provide insights into colon cancer biology and potential diagnostic biomarkers.

Keyword: exosomes

POS-02-121 Introducing the Mitochondrial Italian Human Proteome Project Initiative (mt-HPP)

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Mitochondria carry a maternally inherited genetic material, called the mitochondrial genome (mtDNA) which can be defined as the 25th Human Chromosome. Mutations in the mtDNA have been shown to be associated with dozens of unexplained disorder and the information contained in the mtDNA should be of major relevance to the understanding of many human diseases. Within this paper we describe the Italian initiative to the Human Proteome Project dedicate to Mitochondria as part of both programs: Chromosome-centric (C-HPP) and Biology/Disease (B/D-HPP). The presently active research lines involving : oncological, cardiovascular and neurodegenerative disease models will be given. The fundamental tasks of this initiative will be described in the light to develop a strong international collaborative framework. Key milestones will include the following tasks:
1) Definition of Proteomics-based technologies for the identification and quantitation of mtDNA-encoded proteins (mtProteins). This task will include an effort to develop separation, SRM strategies and top-down investigations to tackle:
i. Single nucleotide polymorphism variants
ii. Post-translational modifications
iii. mtProtein biomarkers in specific diseases
2) Definition of a comprehensive protein interaction map for the mtProteins:
i. mtProtein-mtProtein interaction and oligomeric status in the membrane
ii. mtProtein-nProtein (nuclear encoded Protein) interaction map
iii. mtProtein-p/scProtein (pathogen/symbiont coded Protein) interaction map
3) Definition of an integrated OMICS model with a particular focus on metabolite fingerprint - proteome map:
i. Correlation of mtProtein profile, metabolite fingerprint and RNA patterns
ii. Correlation of nProtein localized in the mitochondria, metabolite fingerprint and RNA pattern
iii. Correlation of mtProtein and nProtein localized in the mitochondria profiles with ionomics.

Keywords: Human Proteome Project, mitochondria

POS-02-122 Exploring the Capabilities and Specificities of CESI-MS for Bottom-Up Proteomics and Peptide Mapping of Proteins Through a Comparison to nanoLC

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The CESI platform describes the sheathless hyphenation of capillary electrophoresis (CE) and electrospray ionization through the use of a separation capillary, the outlet portion of which has been made porous by hydrofluoric acid. The specific geometry of the tip enables the generation of stable spray at extremely low flow rates, even below 10 nL/min. CESI therefore presents the advantages of nanoESI. Using various samples, the capabilities of CESI-MS were broadly assessed for the analysis of complex mixtures of peptides. To serve as a reference, nanoLC-MS was performed in parallel for most of the samples. Initially, a yeast tryptic digest was analyzed in triplicate at different loading amounts (2ng to 2 μg). Depending on the separation dimension, the number of peptide identifications followed significantly different patterns. With loading amounts below 100 ng, significantly higher identification rates were obtained with CESI, demonstrating a significantly higher intrinsic sensitivity of the system. However, on the high end of the loading curve, LC provided increased identification rates as compared to CESI. A careful study of the data demonstrated that the main limitation of CESI was not overloading but the limited time separation window enabled by the bare fused silica capillary. The complementarity between LC and CESI was further studied. The physico-chemical properties of the different peptide populations were further compared and will be presented. After having broadly assessed the capabilities of CESI with a yeast sample, other samples of varied complexity (bovine milk, human cell lysate, and individual proteins) were further analyzed. Preliminary observations suggest strong capabilities of CESI for the analysis of phosphorylated peptides but also for the peptide mapping of individual proteins. These observations could be due to the intrinsic characteristics of CE and the general enhancement of ionization efficiencies and will be assessed further.

Keywords: sheathless capillary electrophoresis mass spectrometry, Ionization efficiencies, NanoESI

POS-02-123 New Developments on a Benchtop Quadrupole-Orbitrap Mass Spectrometer

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In accordance with this year's conference theme, "the Evolution of Technology in Proteomics", we describe new technological and performance developments on the existing bench-top quadrupole-Orbitrap mass spectrometer platform. For widely-used bottom-up proteomics applications, the number of peptides identifiable per unit time increases with greater MS sensitivity and scan speed. Modifications to the existing instrument further enhanced ion transmission during precursor isolation, a critical step for generating high-quality tandem MS spectra. Additionally, precursor isolation transmission windows were optimally shaped to improve quantitative accuracy and speed when using isotope-labeling methods, like SILAC, and to support sophisticated use of data-independent acquisition. To fully utilize the benefits of improved ion transmission, a compact high-field Orbitrap is employed to obtain higher scan speeds. The benefits of these technological advances are demonstrated in a head-to-head comparison between a series Q Exactive™ and the research prototype instrument using a standard HeLa tryptic digest. For high-resolution analysis of proteins heavier than 15 kDa, this research prototype also implements a dedicated mode of operation for improved analysis of large molecules, as demonstrated by baseline-resolved carbonic anhydrase at 29 kDa. This work also addresses the challenge of MS-based lipidomics: to fully resolve exceedingly complex structures of nearly identical masses. To meet this challenge, the length of the Orbitrap transient was adjusted to enable mass resolving power exceeding 240,000. As exemplified on real-life samples, these features pave the way for broader acceptance of Orbitrap-based instruments, both in high-end proteomic applications and in routine LC/MS analysis.

Keywords: Orbitrap, high resolution, accurate mass

POS-02-124 Development of Methods for Information-Driven MS/MS (ID-MS/MS) for Increased Identification Rates in Bottom-Up Proteomics of Human Blood Serum

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Shotgun bottom-up proteomics is used for biomarker discovery or therapeutic target analysis. The approach is limited for biofluid samples with a wide range of protein concentrations, preventing a comprehensive overview within a single analysis. To increase analytical depth and coverage, an iterative process of excluding previously identified peptides was applied. A set of subsequent LC-MS/MS runs with exclusion of already detected peptides allowed identification of proteins of lower abundance and decreased redundancy.
Sigma male AB serum was depleted of Serum Albumin and IgGs before tryptic digestion. Digested serum was used as a test sample to evaluate the efficiency of peptide exclusion in subsequent LC-MS/MS runs on a UHR-Q-TOF instrument. The effects of column load, analysis repetition with exclusion of already identified peptides and different acquisition strategies were assessed. All data were transferred to a data analysis software (ProteinScape, Bruker Daltonics) which allowed pooling of analyses and, following the database search (Matrix Science MASCOT MSMS ion search in Swiss Prot DB at 1.5% False Discovery Rate FDR), generation of peptide exclusion lists and the compilation of a non-redundant protein list.
The identified peptides from the iterative runs were added to an increasingly longer "Scheduled Precursor Exclusion List" (SPExL) which was extracted automatically from MASCOT search results during data analysis and applied to the MS-control software to re-acquire data from the same sample. Using a 90 min gradient with an 800 ng column load, the first run lead to 199 identified proteins. The first round SPExL run led to the identification of 179 new proteins for a total of 369, the 2nd round SPExL to additional 68 proteins, and a 3rd round SPExL increased the list to further 62 proteins. The increase of the total number from 199 to 467 unique proteins was obtained by three additional injections

Keywords: bottom-up proteomics

POS-02-125 Development of High-Sensitivity nanoLC-MS/MS Systems Using Narrow-Bore Meter-Long Monolithic Silica Capillaries at Low Flow Rate

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In shotgun proteomics, protein mixtures are digested by trypsin to increase the sensitivity in MS, although the increase of the sample complexity, caused by the digestion, leads to decrease in identification efficiency. Therefore, the front-end separation methods such as nanoLC have been inevitably employed to reduce the sample complexity prior to MS analysis. Recently we reported that nanoLC-MS/MS systems with meter-long monolithic silica capillary columns of 100 μm inner diameter without pre-fractionation identified the expressed proteome of *Escherichia coli* on a microarray scale.
In this study, we prepared the monolithic silica capillary column with smaller inner diameter to increase the sensitivity in LC-MS/MS analysis at lower flow rate. By optimizing the preparation protocol, we successfully synthesized C18-modified monolithic silica skeleton within 2 meter long fused silica capillaries of 25 μm or less inner diameter. Comparing to the 100 μm inner diameter monolithic silica column, the 25 μm inner diameter column provided 3-fold higher sensitivity without decrease of the separation efficiency when 1 μg of HeLa digests were analyzed at linear velocity of 1 mm/sec with 8-hour gradient. Since LC columns with smaller inner diameter at lower flow rate increase the sensitivity in nanoLC-ESI-MS/MS analysis, this miniaturized high-sensitivity system can be applied to a limited amount of samples such as patient tissues.

Keywords: monolithic silica column, high sensitivity

POS-02-126 Disease Model Cell System: A Novel Proteomics Tool to Elucidate Protein Networks with Spatio-Temporal Information

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Omics and bioinformatics approaches provide much information regarding key factors that cause diseases and their interactions. Despite such accumulated data, spatial information, -the localization of proteins and the structure of intracellular organelles and cytoskeletons- under disease condition remains insufficiently developed. Cell-based assays with fluorescence microscopy method provide one solution to evaluating spatial information. We have been developing various types of cell-based assays by using GFP-visualization technique and semi-intact cell system with its resealing technique. Semi-intact cells are cells, whose plasma membrane is permeabilized with streptococcal pore-forming toxin, streptolysin O (SLO). Since the intracellular structures remain virtually intact, semi-intact cells are called "cell-type test tube." By changing the cytoplasmic proteins with the exogenously-added cytosol, which was prepared from the cells or the organs different in cell cycle, differentiation, or disease state, we can alter the intracellular condition and can reconstitute the various biological processes that occur specifically under different state in semi-intact cells. Furthermore, the permeabilized plasma membrane in semi-intact cells can be resealed by the clearance of SLO-mediated pores in the presence of calcium ion. Most current cell-based assays make use of so-called "normal" cells, which do not reflect intracellular disease conditions. Using the system, we established "disease model cells", into which the cytosol prepared from liver of disease model mouse was introduced to change the intracellular condition to the disease state. In this paper, we introduce the powerful cell-based assay system of diabetic model cells, which enables the investigation of perturbed protein networks under diabetic conditions while retaining the morphology of intracellular structures.

Keywords: cell-based assay, disease model cell

POS-02-127 Travelling Wave Ion Mobility Assisted Duty Cycle Enhancements for Targeted and Non-Targeted Proteomics Experiments

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The duty cycle of ToF analysers is dependent on the *m/z* acquisition range; however, the integration of TWIM in QToF geometries can afford significant duty cycle improvements. In this High Duty Cycle (HDC) mode, the pusher number is synchronised with the pusher delay time and related to TWIMS drift time. The system can be programmed to optimise this parameter for a specific charge state over the entire mass range.
Protein digests and synthetic peptide mixtures were analysed by direct infusion, LC-MS or LC-IM-MS. A nanoscale LC system was configured with a trapping and an analytical column. A RP gradient changed the composition from 1 to 40% in 30 or 90 min, depending on sample complexity. The MS was operated in non-targeted DDA, whereby species are selected for CID fragmentation, or in a targeted MS/MS mode, enabling quantitation. HDC mode was tuned to enhance the signal of multiply charged precursor ions or singly charged fragment ions. The ToF analyser was programmed to enhance the charge state of interest by selection from an *m/z vs.* drift time distribution the region populated by these ions. The output of this selection produced a calibration file for IM enabled MS methods.
We will show that a signal improvement with HDC mode enabled for both multiply charged precursors and singly charged fragment ions of five to seven fold is achieved during the infusion of Glu-Fibrinopeptide B. Injection of an *E. coli* digest onto the LC system were performed with the instrument operating in IM enabled DDA mode. The enhancement in fragment ion signal lead to more confident identifications. For targeted MS/MS analyses of a four protein digest spiked into an *E. coli* digest, the LOD can be extended by at least an order of magnitude when the exact mass traces for several fragment ions are combined. We show from a dilution series of the four proteins that LOQ is also extended with HDC on compared to HDC off.

Keywords: DDA, duty cycle, targetted MS/MS (PRM)

POS-02-128 Sheathless Capillary Electrophoresis Mass Spectrometry as a Versatile and Powerful Tool for the Characterization of the Primary Sequence and Glycosylations of Monoclonal Antibodies

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Aiming at improving the capabilities of the analytical toolbox available for monoclonal antibody (mAb) characterization, a novel platform, combining sheathless capillary electrophoresis (CE) with fast and highly resolving mass spectrometry (MS) has been assessed for primary sequence and glycosylation characterization of mAbs. Initially focusing on peptide mapping, the versatility of the platform has been demonstrated through separating and unambiguously identifying, within a single run, very different molecules ranging from small di or tripeptides to very large peptides (MW> 8000 Da). Further taking advantage of the high compatibility between the speed of the CE and the duty cycle capabilities of the mass spectrometer in MS/MS mode, 100% sequence coverage has been obtained for the two tested molecules. As glycosylation represents such an important attribute of mAbs, it was further important to assess the capabilities of the platform for the study of glycoforms. In this context, it was found that the sensitivity of the platform was an enabling parameter. Major and minor glycoforms with intensity differing by more than three orders of magnitude were identified. To this end, the electrophoretic migration of glycopeptides was used as an additional confirmation to that of MS and MS/MS data to confirm their identification. Their relative abundance was further studied. With a total analysis time of about 45 min (from injection to injection), the capabilities to concomitantly achieve 100% sequence coverage and an in-depth glycosylation characterization, the considered CE-MS platform proves to be a very valuable approach for the fast and thorough study of mAbs and thereby a very promising platform for bottom-up proteomics in general.

Keywords: sheathless capillary electrophoresis mass spectrometry, peptide mapping, glycopeptides

POS-02-129 Improving Label-Free Quantitation of Plasma and Serum Proteins Using a High-Resolution Hybrid Orbitrap Mass Spectrometer

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Label-free mass spectrometry (MS) is an increasingly preferred method for biomarker discovery workflows applied to serum and plasma samples. High resolution and mass accuracy are critical components to successfully identifying and quantifying peptides in a label-free experiment. Here we present a modified data-dependent MS acquisition method as well as a real-time intelligent acquisition strategy for HRAM targeted quantification that improves sensitivity, speed and mass accuracy in our workflow by at least 25%, providing increased protein identifications, protein coverage and better label-free quantitation at lower signal thresholds. Twelve non-human protein standards of varying amounts ranging from 10 to 200 fmol were spiked into human plasma samples digested with trypsin. The protein standards were selected to encompass a broad range of masses and hydrophobicities. Samples were analyzed on a high-resolution hybrid Orbitrap mass spectrometer coupled to nanoLC. Initial discovery experiments were run in a Top-20 data-dependent fashion. Proteome Discoverer and Pinpoint software packages were used to analyze both the qualitative and quantitative data. The spectral library resulting from initial runs was used to create a targeted inclusion list and reference information to perform qual/quan determination in real-time. Data were acquired and peptide coverage and relative quantitation were measured for each of the twelve standard proteins. By modifying the data-dependent acquisition parameters and implementing real-time intelligent acquisition peptide coverage was improved for each protein and the relative quantitation of peptides was improved at lower signal thresholds.

Keywords: label-free, quantitation, biomarker discovery

POS-02-130 Methodological Development of Gel-Filter for the Enrichment of Low-Molecular Weight Proteins from Serum and Comparative Study with Differential Solubilization Method

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The human serum proteome has been extensively studied to find protein and peptide biomarkers. Due to the large dynamic range of protein amounts in serum, identifying and detecting changes in small molecular weight but physiologically and pathologically informative proteins (molecular weight < 30kDa) and peptide concentrations by varied enrichmental methods and mass spectrometry analysis are constrained by the complexity of the serum proteome, especially several kinds of high abundant and large molecular weight proteins. In this study, we developed a system as gel-filter method with multiple layers of tricine-SDS-PAGE-based gels to block the abundant proteins with high-molecular weight (HMW) and specifically enrich low molecular weight (LMW) proteins. Furthermore, the enrichment efficiency of serum LMW proteins and the reduction abundance of larger proteins were evaluated in comparison with other two publically available methods including Glycine-SDS-PAGE method and differential solubilization (DS) method. The result showed that gel-filter method could efficiently remove HMW protein and enabled the highest number identification of LMW proteins within three strategies, with 332 LMW proteins identified against 191 and 130 for DS and Glycine-SDS-PAGE method, respectively. Moreover, a number of proteins (≤ 30 kDa) known to be present in serum in ＜ 10ng/mL were observed by gel-filter method. Our result indicated that gel-filter method offered a rapid, highly reproducible and efficient approach for screening biomarkers from serum through proteomics study.

Keywords: serum, low-molecular weight protein, mass spectrometry

POS-02-131 Western Blot Quantification of GFAP in Organotypic Brain Slices Using Stain-Free Gel Technology or Housekeeping Proteins as Loading Control

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Western blotting is often used to validate a biomarker discovered in a proteomics study. Quantitative analysis of biomarkers by western blot relies on accurate loading control. Organotypic brain slices might be useful alternatives to animal models to study Alzheimer's disease. Increased levels of glial fibrillary acidic protein (GFAP) is a marker for astrocyte activation. We compared two loading control methods to quantify GFAP in neonatal transgenic P301S mice, adult wild type (WT) C57BL/6 x C3H mice, and adult P301S mouse organotypic brain slices after 1, 7 and 15 days *in vitro* (DIV) by western blotting. The first method used a Stain-Free gel technology, where the gels are irradiated by UV-light after protein separation to specifically modify tryptophan residues. The resulting fluorescent proteins were quantified in the gel before and after the transfer as well as on the blot membrane to normalize the protein load and to judge the transfer efficiency. The second method uses the immunodetection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to normalize the protein load. Both methods showed a tenfold increase in GFAP level in neonatal mice and a decrease in GFAP level in the adult WT and transgenic tissue over the cultivation time. Stain-free normalization provided more reliable data with a lower error than GAPDH normalization. For example the GFAP level in the WT tissue exhibited a high standard deviation (~50%) using GAPDH normalization. This study demonstrated that the stain-free technology provides a convenient and reliable normalization to accurately quantify a biomarker by western blotting in proteomics studies.

Keywords: organotypic brain slice, stain-Free gel technology, Western blotting

POS-02-132 Stain Free Total Protein Quantitation - A Method for Greater Reliability in Western Blot Loading Controls

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Western Blotting is often applied to validate biomarkers discovered in a proteomics study. Reliable assessment of changes in biomarker protein levels by western blot requires measurement of both the biomarker and loading control proteins in the linear dynamic range. Stain free technology is a novel method introduced by Bio-Rad to visualize and quantify proteins in gels and blots. In this study, we compared the linearity of a series of dilutions measured by stain free total protein measurement as well as immunodetection of three housekeeping proteins-β-actin, β-tubulin, and GAPDH-commonly used for loading controls in western blotting. We found that immunodetection-based measurements of β-actin, β-tubulin and GAPDH protein levels neither showed good linearity nor accurately indicate 10-50 μg of HeLa cell lysate loading levels. In contrast, stain free total protein measurements exhibited great linearity in the same loading range and its linearity correlated very well with the immunodetection of a selection of low abundant protein targets: MEK, Akt and Erk. Our study demonstrated that stain free total protein measurement serves as a more reliable loading control than housekeeping proteins, particularly in the range of loading most commonly used for cell lysates, 10-50 μg.

Keywords: western blot, normalization, immunodetection

POS-02-133 Higher Data Confidence in Western Blot Analysis - Stain-Free Technology as Novel Normalization Tool

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Proteomic technologies like two-dimensional electrophoresis are valuable tools in semi-quantitative protein profiling studies to understand molecular events in cells. The de-regulated protein spots on 2-D maps are usually identified and characterized by mass spectrometry. In addition, the quantitative protein profiling data need to be confirmed by a second, independent method like Western-blotting. Western blots are used to specifically measure the relative quantities of proteins of interest in complex biological samples. However, quantitative measurements can be subject to error due to process inconsistencies like uneven protein transfer to the membrane. These non-sample related variations need to be compensated for by an approach known as normalization. Two approaches to data normalization are commonly employed: housekeeping protein (HKP) or total protein normalization (TPN). In this study we evaluated the performance of Stain-Free (SF) technology as a novel TPN tool for western blotting experiments in comparison to GAPDH as a representative of the HKP normalization strategy. The target protein (TP) used for this study was MCM7, a DNA licensing replication factor, which was shown previously to be down-regulated by 20% in irradiated lymphoblastoid cells (LCL). We studied the relative expression level of MCM7 with a multiplex western blotting approach based on fluorescently labeled secondary antibodies and found that Stain-Free technology appears to be more reliable, robust and more sensitive to small effects of protein regulation when compared to HKP normalization with GAPDH. Stain-Free technology offers the additional advantages of providing check points throughout the western blotting process by allowing rapid visualization of gel separation and protein transfer. These technical advantages and improvements enable faster time-to-results and higher data confidence.

Keywords: stain-free technology, Western blotting, data normalization

POS-02-134 Analysis of an Apidaecin 1b Resistant *E. coli* Strain Using Differential Gel Electrophoresis

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Multi-drug resistant bacterial pathogens represent a major health threat all over the world that spread continuously into the general public. Besides the development of novel antibiotics it is also important to understand the underlying resistance mechanisms in detail for already approved antibiotics and also for compounds in clinical or preclinical phases. Common mechanisms rely often on altered protein expression rates of efflux proteins or enzymes that metabolize the antibiotics. These proteins can be identified by comparing the protein patterns of resistant strains and non-resistant strains by differential gel electrophoresis (DIGE). *Escherichia coli* strain BL 21 AI was cultured with increasing concentrations of apidaecin 1b, an antimicrobial peptide expressed in honey bees (*Apis mellifera*). After ten passages the minimal inhibitory concentration (MIC) increased from originally 2μg/mL to 128μg/mL. Cytosolic proteins (soluble fraction) were isolated after sonication of the cell culture. Each 2-DE analysis consisted of six biological replicates per strain and each experiment was done twice at different time points to confirm the obtained results. Protein samples were labeled with dyes G200 and G300, mixed, and separated by isoelectric focusing (IEF) followed by SDS-PAGE. A laser scanner and a charge-coupled device were used for image acquisition. The 2-DE patterns of the two different image sets were compared with Delta 2D. In total 415 spots could be detected with the laser scanner and 372 spots with the charge-coupled device. Significance of the obtained data was tested by using cluster- (HCL) and principal component (PCA) analysis. Significant spots were selected by using T-test analysis with a p-value of 0.01. However, no significant regulated spots could be obtained with both data sets using the described parameter. Comparison of single spot parameter as normalized spot volume and coefficient of variation will be presented.

Keywords: antimicrobial peptide (AMP), resistance mechanisms, differential gel elelctrophoresis

POS-02-135 Immuno-Sequencing (I-Seq): Digital and Sensitive Antibody Based Proteomics Using DNA Barcoding and Massively Parallel Sequencing

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Immunoassays are important and widespread techniques in biological research and clinical diagnostics. On the other hand, massively parallel sequencing platforms (MPS) have recently been applied in a diverse range of methods and have provided powerful sample investigations. Here we describe a novel method denoted Immuno sequencing (I-Seq), which combines the great advantages from both systems, using DNA barcoded antibodies. These bio-conjugates are impressive constructs, providing sensitive and multiplexed detection, thanks to the possibility of PCR-based signal amplification and parallel detection on MPS platforms. We have described a method for fully-automated antibody-DNA conjugation at low amounts, in 96 well-plate format. As a proof of principle, we demonstrated sensitive and multiplexed detection of peptides printed on a customized planar array using the I-Seq principle. The results showed a significant ($p<0,05$) signal to noise ratio regarding the sequencing reads count from expected barcode sequences and internal negatives. A significant correlation based on linear regression ($R2=0,94$) was also observed between the sequencing reads count and printed peptide quantity.

Keywords: antibody-based Proteomics, massively parallel sequencing, antibody-DNA conjugates

POS-02-136 SOMAmer Capture Coupled to Mass Spectrometry for Plasma Protein Quantification

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Proteins can be quantified with high specificity and sensitivity by mass spectrometric measurement of immunoaffinity-enriched proteins or proteotypic peptides. Such assays are either termed mass spectrometric immunoassays (MSIA) or stable isotope standards and capture by anti-peptide antibodies (SISCAPA) depending on whether the quantification is performed at protein or peptide level.
However, capture molecules are still the limiting resource despite extensive activities in the field of affinity reagent generation. Slow off-rate modified aptamers (SOMAmers) are synthetic protein binders, which are isolated from artificial binder libraries. Availability of such binders is virtually unlimited and their production is more cost efficient compared to e.g. antibody binders. We demonstrate a workflow where plasma proteins and their isotopically labeled counterpart are precipitated using SOMAmers. The enriched proteins are digested on-bead and quantified by selected ion monitoring mass spectrometry with peptide redundancy for each protein. Advantages and disadvantages compared to peptide-centric approaches such as SISCAPA will be discussed by means of comparative data generated with SOMAmers.
Keywords: mass spectrometric immunoassay, slow off-rate modified aptamers, plasma proteins

POS-02-137 Affinity Probe Capillary Electrophoresis (APCE) of Insulin Using a Fluorescence Labeled Recombinant Fab as an Affinity Probe

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Most proteins exist as isoforms as a result of post-translational modifications and non-enzymatic chemical reactions, e.g. glycosylation, phosphorylation, deamidation, etc. The pattern of isoforms is often relevant to the temporal function of proteins and the state of cells and organisms. Affinity probe capillary electrophoresis (APCE), which separates and detects isoforms as complexes with a fluorescence-labeled affinity probe (AP), is especially suitable for evaluation of such isoforms in an intricate biological fluid. Recent advances of APCE using a recombinant Fab as an AP are presented in the separation and detection of insulin as a model system. Recombinant anti-insulin Fab was expressed in *E. coli* and labeled at a cysteine residue with a thiol-reactive rhodamine dye. Pure labeled Fab was recovered from a focused band on an IEF gel. Insulin sample and the labeled Fab as an AP were mixed with a carrier ampholyte, and introduced into a fused-silica capillary (50 μm i.d., 120 mm long). Isoelectric focusing was carried out at a voltage of 6 kV, and the capillary was scanned with a fluorescence detector under the focusing condition. The insulin-AP complex focused within 6 min at pH 6.6 with the free AP at pH 7.6. A linear calibration line was obtained for standard insulin for 20 pM to 5 nM. The results demonstrated the potential abilities of APCE in the isoform analysis of proteins.
Keywords: capillary electrophoresis, antibody, capillary isoelectric focusing

POS-02-138 Quantitative Screening of Aptamers via Particle Display

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Nucleic acid aptamers are synthetic affinity reagents that offer many advantages such as chemical synthesis, facile modification, and thermostability. Importantly, aptamers are generated completely *in vitro* and offer the potential for high throughput discovery, making them promising tools for proteomics research. However, conventional selection-based aptamer discovery methods (*e.g.* SELEX) often fail to yield molecules of suitable quality for proteomic analysis because the selection process is vulnerable to various biases. In fact, recent reports have suggested that it may only be possible to generate DNA aptamers for less than 30% of the human proteome without relying on modified nucleotides. Thus there is an urgent need for alternative aptamer discovery technologies that can rapidly generate aptamers for the large number of unaddressed protein targets.
We present a novel screening-based aptamer discovery technology (termed particle display) that overcomes the limitations of selection-based methods and show that aptamers can potentially target a far broader swath of the proteome. We generate a library of aptamer particles that each displays multiple copies of a unique aptamer sequence on its surface. Then we measure the relative affinities of every aptamer particle and selectively isolates those with highest affinities via fluorescence-activated cell sorting (FACS). Using our particle display method, we have generated DNA aptamers for four different proteins with equilibrium dissociation constants (K_d) ranging from 7.04 pM to 2.32 nM, including two recalcitrant proteins for which previous SELEX experiments have repeatedly failed.
Keywords: affinity reagents, aptamer, high throughput screening

POS-02-139 Global Proteome Survey (GPS) Finds New Roads for Translational Biomarker Signatures in Breast Cancer

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Breast cancer is the most common cancer type among woman in the western world. Hence, there is an urgent need for improved clinical tools using specific biomarker signatures for accurate tumor classification, monitoring of tumor progression and predicting patient prognosis. Until today there is no existing clinically used multiplexed biomarker signatures to address these questions and we here present a first attempt in this matter. Earlier studies from our group have presented a novel way of combining affinity proteomics and label-free LC-MS/MS for discovery studies in different kinds of material including tissue - a method termed global proteome survey (GPS). We have now, using GPS, profiled 52 breast cancer tissue samples, making this study one of today's largest breast cancer tissue proteomics studies. Data from our study have generated detailed quantified proteomic maps representing 1388 proteins covering a wide dynamic range, enabling us to decipher in-depth molecular portraits of histologic graded breast cancer tumors reflecting tumor progression. In more detail, a 49-plex candidate tissue biomarker signature discriminating histologic grade 1 to 3 breast cancer tumors with high accuracy was defined. These results were confidently confirmed when compared to mRNA profiles. Finally, we have now designed SRM-assays for our panel of candidate biomarkers which are going through a comprehensive validation with the ultimate long term goal set for an assay ready for clinical tests.
Keywords: breast cancer, biomarkers, mass spectrometry

POS-02-140 Identification of Metastasis Related Membrane Glycoprotein in Triple Negative Breast Cancer Using A Lectin Microarray

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Despite the progresses in the treatment of breast cancer, the majority of the triple negative breast cancer (TNBC) patients are still suffering. It has been shown that the altered glycsosylations of membrane glycoproteins are responsible for tumor progress and metastasis. Here an integrated method combining lectin microarray, SILAC labeling, lectin affinity enrichment and LC-MS/MS was employed to identify the aberrant glycosyaltion on the cell surface and its conjugated membrane proteins of triple negative breast cancer (TNBC) cell lines with different metastatic capabilites. Lectin microarray analysis showed that a galactose-specific lectin Ricinus Communis Agglutinin I (RCA-I) bind the low, middle and high metastatic TNBC cells proportionally to its metastasis capability. This binding was confirmed by flow cytometry analysis. Function study showed that RCA-I could inhibit the cell motility. Furthermore, tissue microarray staining indicated that RCA-I bound to late stage breast cancer tissue more significantly. To dissect the underlying mechanism, a magnetic beads coated with RCA-I was used to capture the glycoproteins from the SILAC labeled low and high metastatic TNBC cells, the IDs of these glycoproteins were revealed by LC-MS/MS. Taken together, our results suggest that increased galactosylation on the surface of tumor cells is linked to their elevated metastatic abilities. and indicate that POTEG, an un-annotated membrane glycoprotein, is an important player in TNBC cell motility, which may could be used as a potential biomarker to predict the tropesis of TNBC metastasis in the future.

Keywords: breast cancer, metastasis, lectin microarray

POS-02-141 Preliminary Study on the Development of Novel Proteomic Analysis by Using Surface Enhanced Raman Scattering (SERS)

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Introduction
Surface Enhanced Raman Scattering (SERS) has been widely applicated in industrial microanalysis, and reserched to apply for various fields, for example biological research. We tried to perform proteomic analysis of the serum samples from the patients with gastric and colon cancer, and benign diseases by using SERS.

Material and Methods
Blood samples were obtained from every 12 patients with gastric and colon cancer, and benign diseases. All blood samples were centrifuged at 1600g for 7 minutes, and serum samples were extracted. Sarum samples were placed on the silver coated base with nanoparticles to detect SERS spectra by low intensity helium-neon red laser beam with 633 nm as wavelength.

Results
SERS spectra were detected in all samples within 4 minutes from irradiation. Limiting dilution of detecting SERS was 1/1000 of serum. SERS spectra patterns were different between cancer and benign disease patients, and maximum peak level of the SERS in the samples from cancer patients were significantly higher than those from benign disease patients. SERS spectra of the samples from colon cancer patients appeared later than those from benign diseases and gastric cancer patients. By using antibody, maximum peak level of SERS were dropped.

Conclusion
We suggest that our novel analytic methodology by using SERS is useful for proteomic analysis.

Keywords: proteomic analysis, surface enhanced raman scattering

POS-02-142 A Quick Profiling System for Cancer Marker Proteins with Diverse Post-Translational Modifications by a Fully Automated 2DE Device, Auto-2D

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We have developed a fully automated two-dimensional gel electrophoresis system (Auto-2D) which makes tissue/cellular/biological fluid protein analyses more simple, quick, sensitive, and reproducible with a small volume of samples, thus, being expected as a convenient clinical analysis tool for the personal diagnosis. In this study, we utilized this system for the analysis of prostate cancer specific proteins. Using this system, thousands of protein spot can be detected in the prostate cancer (PCa) cells with high resolution (isoelectric point; 0.02pH, molecular weight; 2kDa) and high reproducibility in a short time (minimum time; 100min). To establish the better diagnostic method for PCa, the Auto-2D was used for the analysis of the post-translational modification patterns of prostate specific antigen (PSA) in PCa patient sera, seminal plasma, and PCa cell lines, such as hormone therapy sensitive LNCaP cells and insensitive C4-2 cells. In each sample, more than 10 PSA protein spots with high resolution were found in western blotting and validated with MS analysis. The spot patterns of each samples being largely different because of the modification (glycosylation, proteolysis, others) differences between those of normal and PCa samples, suggesting that the PSA modification profiling obtained from this system may provide the molecular information for PCa diagnostics. The resolution, sensitivity and reproducibility for the analysis of this prostate cancer specific PSA spot pattern by the Auto-2D method were significantly higher than those by the conventional methods. These results suggest that this Auto-2D combined with western blotting method will be useful to study the specific protein expression profiles not only for the prostate cancer but also for variety of the biological specimens, and could be introduced as a new clinical laboratory technology.

Keywords: 2DE, diagnostic marker, prostate specific antigen(PSA)

POS-02-143 High-Resolution Separation for Western Blotting by Automated 2-DE and Blotting System

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Protein analysis that combines a two-dimensional electrophoresis (2DE) and a western blotting (WB) method has been used for drug discovery and clinical research. The two-dimensional electrophoresis is the method to separate proteins by their isoelectric point and molecular weight. However, this method always lacks reproducibility of the protein separation, and also is complicated and time consuming. Therefore, we realized highly reproducible and easy two-dimensional electrophoresis. In this system, the plate with immobilized pH gradient (IPG) gel and automated plate delivering machinery make two-dimensional electrophoresis automatic. Our Automated two-dimensional electrophoresis system, Auto2D, has been marketed in Japan since 2011. Furthermore we developed fully automated two-dimensional electrophoresis and electroblotting system. This system makes it possible to detect the highly separated proteins by Western Blotting easily. The electroelution from the top of SDS-PAGE (sodium dodecyl sulfate poly-acrylamide gel electrophoresis) gel and automated membrane-drawing machinery make blotting automatic. Because we adopt the electroelution mechanism, this system achieves fully automation with high transfer efficiency of both low and high molecular proteins. In this study, we try to develop the algorithm to optimize the membrane-drawing speed and the narrow range IPG gel. With the algorithm and the IPG gel, we can expand the target region with high-resolution. This system is able to detect the slight modification of proteins with high-reproducibility in a short time, therefore, we consider that the system can be utilized for diagnosis and industry use.

Keywords: 2 dimensional electrophoresis, Western blotting

POS-02-144 Plasma Proteome Analysis Using LC-MS/MS with Travelling Wave Ion Mobility and an Alternative Computational Solution to Protein Quantitation

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Biomarker discovery involves the analysis of highly complex biological samples. Even using chromatography coupled with mass spectrometry, many species still co-elute, causing masking of ion signals and challenging protein identification and quantitation. Many techniques for incorporating additional separation, such as pre-fractionation and electrophoresis, decrease sample throughput and involve additional sample preparation which can contribute to preanalytical variation. Travelling wave ion mobility coupled with label free data independent acquisition (DIA) requires no additional sample preparation, yet confers significant advantages in proteomic analysis. The inclusion of an ion mobility step into the workflow also allows the number of identified proteins to be significantly increased whilst precursor and product mass accuracies are maintained between the modalities The ion packeting behaviour of travelling wave ion mobility (TWIMS) affords improved and less interfered detection of lower abundant species, however, one obstacle encountered with the analysis of high dynamic range proteomic samples is signal saturation of high abundant ions, causing issues in quantitating the most abundant proteins. This abstract presents an alternative bioinformatic approach which overcomes this by calculating protein quantities from product ion data. Protein quantities calculated with this method are more in line with the widely accepted label free data independent acquisition quantities calculated from precursor ion data. It is thus shown that ion mobility enhances proteome coverage, and with an adapted data processing method, it can be reliably used for quantitation as part of a biomarker discovery pathway.

Keywords: plasma, ion mobility, absolute quantitation

POS-02-145 A Fast Workflow for Identification and Quantification of Proteomes

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The current in-depth proteomics makes use of long chromatography gradient to get access to more peptides for protein identification, resulting in covering of as many as 8000 mammalian gene products in 3 days of mass spectrometer (MS) running time. Here we report a fast sequencing (Fast-seq) workflow of the use of dual reversed phase HPLC-MS/MS with a short gradient to achieve the same proteome coverage in 0.5 day. We adapted this workflow to a quantitative version (Fast quantification, Fast-quan) that was compatible to large-scale protein quantification. We subjected two identical samples to the Fast-quan workflow, which allowed us to systematically evaluate different parameters that impact the sensitivity and accuracy of the workflow. Using the statistics of significant test, we unraveled the existence of substantial falsely quantified differential proteins (FQDPs) and estimated correlation of false quantification rate and parameters that are applied in label-free quantification. We optimized the setting of parameters that may substantially minimize the rate of FQDPs, and further applied them on a real biological process. With improved efficiency and throughput, we expect that the Fast-seq/Fast-quan workflow, allowing pair wise comparison of two proteomes in one day, which may make MS available to the masses and impact biomedical research in a positive way.

Keywords: proteomics, mass spectrometry, proteome coverage

POS-02-146 DMSO Drastically Enhances Electrospray Response Boosting Sensitivity of Proteomic Experiments

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The *de-facto* standard analytical platform for the identification and quantification of peptides and proteins in current proteomics is the coupling of reversed phase liquid chromatography to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). Here, we describe that low percentages of dimethyl sulfoxide in liquid chromatography solvents lead to a strong enhancement of electrospray ionization response of peptides. Overall signal intensity was boosted between 2-4 fold depending on which mass spectrometer is used. This in turn allowed the identification of 35% more peptides and 28% more proteins from 1ug HeLa tryptic digest. Low abundance peptides benefit more from the addition of DMSO than high abundance peptides. Replicate serial dilution analysis of HeLa digests (0.1-1,000 ng digest on column) revealed that the increase in peptide and protein identification was increasingly pronounced when lower sample quantities were analysed. At low sample loadings, sensitivity was increased by 10-fold in the presence of DMSO We observed no adverse effects of DMSO addition with respect to chromatographic performance but the LC gradient composition needs to be adjusted to prevent the loss of hydrophilic peptides. The commonly observed polysiloxane ions from ambient laboratory air were completely absent in the presence of DMSO. Instead, mass deficient background ions arising from DMSO clusters were detected among which, the m/z 401.92 species represents a viable lockmass alternative. The method can be easily implemented on any LC-MS/MS system without modifications to hardware or software and at no additional cost providing substantial practical and economic value to the field of proteomics.

Keywords: electrospray ionization

POS-02-147 Increasing the Quality of Bottom-Up Proteomics Data by Computer-Assisted Liquid Chomatography Gradient Optimisation

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The role of liquid chromatography in LC-MS/MS bottom-up protein discovery experiments is to provide a means by which the multitude of individual peptide species in a complex sample can be sequentially introduced into the mass spectrometer (MS). Without separation of peptides in time, the capabilities of the MS to measure each introduced peptide would be severely decreased, due to; (i) the limitations of the instrument to perform isolation and fragmentation of many distinct precursor ions in a short time, (ii) the increased probability of isobaric interferences reducing the selectivity of the MS measurements, and (iii) the phenomenon of ion suppression, where the intensity of the signal generated by any particular ion can be reduced by the presence of co-eluting ions.

Due to the complex and unknown nature of the peptide species to be measured in many bottom-up proteomics LC-MS/MS experiments, generic LC gradients are often used with little effort devoted to matching the gradient profile used to the nature of the sample.

Here we introduce a simple software tool to assist in the optimization of liquid chromatography gradients. After an initial scouting run of a sample using a simple gradient, the software tool takes input from the database searching program ProteinPilot™ and compares it to the gradient used, calculating the number of high-confidence peptide identifications per unit time. This calculation is then used to generate an optimized gradient profile that can be used to improve the quality of subsequent analyses of the sample.

Keyword: liquid chromatography

POS-02-148 Metal-Chelate Methacylate Monolithic Support for Targeted Affinity Selection of Histidine-Peptides in High-Throughput Proteomics

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In recent years, bottom-up approach has become the popular method of choice for large scale analysis of complex proteome samples. Peptide fractionation determines the efficiency of the bottom-up method and often the resolving power of reverse phase liquid chromatography (RPLC) is insufficient for efficient protein identification in case of complex biological samples. To overcome the inherent limitation of proteomics associated with sample complexity, we evaluated fast flow metal chelate methacrylate monolithic system - CIM (Convective Interaction Media) disk chelated with Cu (II) for targeted affinity selection of histidine-containing peptides. Initially the Cu(II)-IMAC using CIM disk was evaluated using tryptic digest of individual protein and protein mixtures and was found to be highly efficient in capturing histidine containing peptides from tryptic digest of individual as well as protein mixture with a high degree of specificity and selectivity. Affinity capturing of histidine-containing peptides not only resulted in reducing the sample complexity but also enabled identification of additional peptides that were not identified in the LC-MS/MS analysis of the total tryptic digest. Further the efficiency of histidine-peptide enrichment using CIM-IMAC was also evaluated using complex biological samples like cell lysates and serum and the results showed the potential of this approach not only in reduction of sample complexity but also enabled identification of additional low abundant proteins that are not usually detected in the analysis of total digest through the targeted affinity enrichment of histidine-peptides. The lower frequency of occurrence of histidine in proteins makes this method potentially useful for reducing sample complexity and identification of proteins based on affinity selected histidine containing peptides, in large scale proteome analysis.

Keywords: histidine-containing peptides, affinity selection, CIM monolithic disk

POS-02-149 Improving MS^n Performance with a Multitasking Mass Spectrometer

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For data dependent analysis of complex samples, one of the most critical performance measurements is the spectral generation rate. One path to improve the rate is the use of a mass analyzer which scans faster, but this often results in reduced mass spectral performance. Given a spectrometer with a compatible arrangement of components, many "execution optimization" concepts from CPU design, such as multi-processing, pipelining, and out-of-order execution, can enhance repetition rates 2-4x relative to serial execution.This work was performed on a novel hybrid instrument, based on a mass filter, OrbitrapTM, collision cell, ion trap (Q-OT-qIT) architecture. The components are controlled by several independent processing units, such that ion control in one section of the instrument does not impact unrelated ion control in a different section.Operating in a serial fashion, a hybrid linear trap/Orbitrap mass spectrometer generates data at a rate that, although impressive, leaves room for improvement. Without the ability to parallelize some steps in the execution of a data dependent experiment, a survey scan followed by MS/MS of the 30 most abundant species requires approximately 3.3 seconds. Benefits, analogous to multi-core data processing, are achieved through simultaneous operation of the Orbitrap and linear trap analyzers. If the linear trap initiates MS/MS as soon as the Orbitrap begins transient acquisition, ~700 msec can be recovered in each cycle. The steps of injection, isolation, activation, and analysis in the linear trap can be pipelined with the addition of a mass filter and collision cell, transforming "tandem in time" processes to "tandem in space". Using these techniques, a Top 30 experiment can be completed in < 1.1 seconds. For complex biological mixtures, this increase in repetition rate leads to >30% more unique peptides being identified than previously possible.

Keywords: instrumentation, Orbitrap

POS-02-150 Increased Throughput 2D Nanoscale LC Analysis of Human Placental Samples

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Most proteomic samples generate post-digestion peptides with similar hydrophobicities and mass distributions. The complexity of proteomic samples requires orthogonal separation methods to identify and quantify all peptides in a sample. Data-independent analysis yields reproducible fragmentation and peak area information for all detectable peptides. The use of ion mobility during this analysis inserts an orthogonal separation in the gas phase between chromatographic and mass spectral analyses. In this study, 2D chromatography is combined with ion mobility to resolve peptides in multiple dimensions in a high-throughput manner.
Proteins were extracted from human placenta samples into two solubility fractions using TRIzol reagent with sonication. Proteins were reduced, alkylated and digested in-solution with trypsin. Samples were injected in triplicate onto a nanoscale liquid chromatography system and analyzed with a data-independent method using alternating low and elevated collision energy on a quadrupole time of flight instrument with ion mobility. Multidimensional chromatographic methods were employed using high-low pH RP-RP with discontinuous step gradients.
A comparison was made between a traditional 2D method and a faster technique that utilized simultaneous gradients in both dimensions. The faster technique took 68% of the time of a traditional 3-fraction method and the percent savings in time increases as the number of desired fractions increases. Use of the faster method allowed for a 70% increase in the number of ions detected per minute, a 54% increase in the number of peptides identified per minute, and a 46% increase in the number of proteins identified per minute. Incorporation of ion mobility into the analysis yielded an increase in peak capacity of at least another order of magnitude.

Keywords: 2D nanoscale LC-MS

POS-02-151 Data Independent Acquisition (DIA) Analysis on the Q Exactive Mass Spectrometer

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Data independent acquisition (DIA) strategies are becoming increasingly popular due to the high reproducibility and collection of comprehensive data in large-scale protein quantitation experiments. We introduce two data independent acquisition methodologies on the Q Exactive mass spectrometer, DIA and multiplexed DIA (msxDIA). For a "conventional" DIA method on the Q Exactive, MS/MS spectra are collected by selecting broad m/z windows for fragmentation. The unique feature of spectra multiplexing on the Q Exactive enables the use of msxDIA as an alternative DIA method with the advantage of simpler de-multiplexing during data analysis. Here narrow isolation windows are selected randomly, isolated ions are fragmented, and their fragments stored serially-in-time prior to mass analysis in the Orbitrap.
The DIA and multiplex DIA methods are used to quantify protein digest standards spiked into matrix. The complexity of the MS/MS spectra is high and accurate quantification is only possible when fragments are sufficiently separated from interferences. The high resolving power of the Q Exactive is applied to separate the interferences from the target. Two peptides per protein and the three most intense fragment ions (b and y types) per peptide are used for quantification, and eight to ten most intense fragment ions per peptide are used for confirmation. DIA data are processed by Pinpoint software, in which ion chromatograms of the peptide transits are extracted and integrated with a mass tolerance of 5ppm. Data from msxDIA are processed by Skyline software, in which multiplexed MS/MS spectra are de-multiplexed into 5 separated 8 Da isolation windows. Excellent reproducibility is shown for quantification. Limit of detection and limit of quantification are achieved in the amol range, and 3.5 orders of quantification linearity are achieved using both methods for quantification in complex biological samples.

Keywords: Q Exactive, Data independent acquisition (DIA), multiplexed Data independent Acqusition (msxDIA)

POS-02-152 Missing Proteins in Chromosome 16-Spanish HPP

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In the scope of the HPP project, there is a special situation for the proteins that had not spetral and/or expression evidence, those are called "Unknown proteins". The reason for this lack of information could be due to either the low level of the protein expression or the specific cell line/tissue in which is expressed or the specifically development step of their turn up. To overcome this problem, in the Spanish HPP consortium, we are trying to get the spectral and the MRM data from the "unkwnown proteins" for its detection later in human samples. To obtain this information, we analyzed the Chr.16 gene-encoded proteins and located about 280 unknown proteins fro which there is not spectral information. Moreover, for most of them there is not empirical evidence in the Swissprot database, or the evidence exits only at transcript level. For those 280 proteins, currently we have > 50 of them available as cloned plasmids (www.cicancer.org; www.dnasu.asu.edu) ready to be expressed in a *in vitro* transcription traslation systems (IVTT). After testing several different cell-free expression systems, we decided to use human IVTT, because of the high yield, the possibility of scaling up the protein expression adn the full compatibility with S/MRM methods. Using this approach, it is possible to obtain enough protein amounts to design S/MRM methods for the detection and quantification of these proteins. Here, it will presented, as a proof of concept, 15 "unknown proteins" that had been expressed and characterized; in addition, for all of them we obtained at least two prototypic peptides for analysing them in differnt human samples.

Keywords: unknown proteins, S/MRM assays, IVTT expression systems

POS-02-153 A Novel Chemical Tool for Proteomic Analysis of Tranlational Control

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Translational regulation, which controls expression of proteins strictly in time and space, is essential for development, differentiation, and adaptation to environment. For example, during axonal development of neurons, translation of various localized mRNAs at the growth cones is controlled through various guidance cues independently from their cell bodies. To understand the regulatory mechanism, it is quite valid to visualize and identify nascent proteins. As one of the nascent protein labeling technologies, puromycin derivatives with fluorescent dyes or haptens have been developed. However, undesired accumulation in specific intracellular regions and difference in the labeling efficiency caused by the functionalized compounds coupled to puromycin make it difficult to use these derivatives in cell-imaging and proteomic studies. Therefore we developed a useful chemical, named "N3-dC-puro" for the purpose, which is an azide-containing derivative of puromycin. This molecule is incorporated into nascent proteins and can be labeled by click chemistry with alkyne-containing fluorescence dyes or haptens. In this study, we show that the N3-dC-puro can actually label newly synthesized proteins *in vitro* and in HeLa cells. Additionally, we also confirmed that the treatment of the N3-dC-puro do not exert endoplasmic reticulum stress, which inhibits translation *via* phosphorylation of eIF2A, at least within few hours after administration. These results indicate that the N3-dC-puro is a practicable reagent for translational regulation studies.

Keywords: translational control

POS-02-154 Mass Spectrometry Based N- Terminal Sequence Determination with *De Novo* Sequencing Using CID and ETD

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In proteomics studies, proteins sequence carries plenty of proteolytic processing information like post translational modification and degradation site of protein. In order to study unknown protein's functions and the proteolytic pathway, *de novo* N-terminal sequencing of proteins is essential. However, *de novo* sequencing possess a big challenge since the obtained peptide fragmentation spectra generally complicates the correct peaks identification due to the unknown charge origin and charge state. Therefore, many chemical strategies which can provide a specific marker is introduced to simplified the mass spectra. In addition to successfully *de novo* peptide sequencing, complete or complementary series product ions are desired allowing easier interpretation. Herein, various chemical modifications of real samples (Bovine Serum Albumin, α-casein, β-casein, Fetuin and Phosvitin) undergo different enzymes cleavage and then coupled with MS/MS activation methods like collision induced dissociation (CID), electron transfer dissociation (ETD) to set up the best *de novo* sequencing platform.

Keywords: de novo sequencing, N-terminal

POS-02-155 Application of a Peptide-Based Assay System to Study the Human Proteome

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We developed scalable methods for generating and analyzing custom peptide sets of high complexity (1). The peptides are made as peptide-cDNA fusions by *in vitro* transcription/translation from pools of DNA templates. This approach enables large sets of peptides to be custom designed in silico, manufactured cost-effectively in parallel, and assayed efficiently in a multiplexed fashion. We will present applications of peptide-cDNA pools derived from the human proteome in binding and activity-based enzymatic assays.

References

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Keywords: peptide-DNA conjugates, high-throughput peptide screening assay, custom high complexity peptide sets

POS-02-156 Toward Early Stage Detection of AD: Dynamic Binding and Unbinding of Kinesins during Collective Transport

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Early diagnostic of deficiencies in mechanical nanotransport inside cells can revolutionize the diagnosis and management of neurodegenerative diseases. According to recent NIH studies of Alzheimer's disease (AD), 5% earlier detection of AD could lead to a 50% reduction in mortality. In AD, hyperphosphorylated tau protein affects neuronal transport, leads to beta-amyloid peptide deposition, and synaptic malfunction. An early effect of hyperphosphorylated tau protein is a deficient mechanical transport executed by motor proteins such as kinesins along microtubules. This study focuses on a novel computation model for understanding the interaction between kinesin and altered microtubules. Teams of several kinesin molecules are involved in long range transport along microtubules. One of the important mechanisms in this transport is the stochastic binding and unbinding of kinesin to microtubules. In this study, the unbinding probabilities corresponding to each mechanochemical state of kinesin are predicted. Starting from experimental data, the model predicts that most of the unbinding occurs during two of the states of the system, and the unbinding probabilities during the other states are negligible. Overall transport characteristics such as the run length play a crucial role in neurodegenerative diseases and are influenced by binding and unbinding. Our studies show that the run length varies non-monotonically over the forces applied on the cargo. Also, the transport velocity for teams of kinesins is lower than that of a single molecule. These results provide a starting point for the development of biomarkers and methods to detect shortening run lengths as indicators of deficient neural nanotransport.

Keywords: kinesin-microtubule interaction, Alzheimer's disease, detection and biomarkers

POS-02-157 Comprehensive Mass Spectrometry-Based Characterization of Box H/ACA Small Nucleolar Ribonucleoprotein Complexes in *Schizosaccharomyces pombe*

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Recent studies show that diverse types of ribonucleoprotein (RNP) complexes consisting of small non-coding RNAs and a particular subset of proteins play pivotal regulatory roles in a variety of cellular processes. One of such examples is a box H/ACA RNP complex that participates in the pseudouridylation of various RNAs such as rRNAs. The chemical composition of this RNP complex is well characterized in a number of organisms, *e.g. Saccharomyces cerevisiae*; however, little is known about RNA components in an equivalent complex in *Schizosaccharomyces pombe* (*S. pombe*). To characterize the structure/function relationship of this functional RNP complex in further detail, we performed the comprehensive mass-spectrometry (MS) based analysis of the box H/ACA RNP in *S. pombe*. The RNP complex was isolated from the fission yeast cells by the affinity-based purification using tagged Gar1 protein as a bate followed by ultracentrifugation, and the protein and RNA components were characterized by the MS-based "ribonucleoproteomics" technologies (Taoka *et al.*, Nucleic Acids Res. 2009; **37**: e140; Nakayama *et al, Nucleic Acids Res.* 2009; **37**: e47). We report here that (1) the fission yeast complex consists of four protein components, Gar1, Cbf5, Nop10 and Nhp2, and 38 snoRNAs, including 18 known and 20 previously unknown snoRNAs, and (2) at least 16 of the 20 snoRNAs found in this study actually participates in the pseudouridylation of particular sites on the 25S or 18S rRNA and thereby the novel functional cofactors of the box H/ACA snoRNP complex. We will also present the details of the MS-based RNA analysis.

Keywords: RNA, ribonucleoprotein complex

POS-02-158 Improving Throughput for Highly Multiplexed Targeted Quantification Methods Using Novel API-remote Instrument control and State-Model Data Acquisition Schemes

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Targeted quantification has become a very popular technique to verify putative biomarker candidates in large clinical cohorts of samples. These candidates are usually generated following a biomarker discovery experiment or derived from a biological hypothesis e.g., a pathway or biophysical interaction. These lists are usually large, containing upwards of 500-2000 proteins spanning several orders of magnitude representing analytical challenges for conventional SRM assays both in terms of method development and throughput. We propose using high-resolution/accurate mass (HR/AM) MS and MS/MS schemes in conjunction with validated spectral libraries for automated method building, data acquisition, verification, and quantitation in real-time using novel acquisition schemes. HCT116 colon carcinoma cells were grown in heavy and light media, collected and mixed at different ratios to cover a 20-fold dynamic range. All samples were digested and analyzed on a quadrupole Orbitrap mass spectrometer equipped with a nanospray ion source. Data was acquired in two steps to simulate traditional workflows. Initial experiments employed unbiased data-dependent MS/MS acquisition resulting in peptide/protein identification as well as building of a spectral library. A highly multiplexed, targeted protein list was created from the spectral library and used for automated data acquisition and processing real-time to facilitate changes to the acquisition scheme. More than 1000 proteins were selected from the HCT116 cell line and imported into the new algorithm. Experiments performed on the quadrupole Orbitrap facilitate unique product ion collection and detection schemes to perform state-model data acquisition increasing the ability for quantitation. The developments here resulted in the successful qualitative/quantitative analysis for over 5000 peptides representing over 2000 proteins in this complex leukemia cell digest.

Keywords: SILAC, real time, acquisition

POS-02-159 *In Vitro* Phosphorylation-Assisted in Depth Human Serum Proteome Analysis

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Despite the ease in collecting human serum, its use for proteome analysis has not been explored yet in biomarker discovery studies due to the difficulty in detecting low abundant tissue-derived proteins in the presence of ultrahigh abundant serum proteins by LC-MS. To increase the identification efficiency for these low abundant proteins, it is necessary to do extensive pre-fractionation, causing longer analysis time.

Here we introduced a novel approach to identify low abundant proteins based on phosphoproteomics coupled with *in vitro* phosphorylation by spiked kinases. The sample complexity can be reduced without increasing analysis time by enriching phosphopeptides.

Proteins were extracted from human serum and digested by proteases. Then serum digests were phosphorylated *in vitro* by 9 kinases to prepare artificial phosphoproteome, and the resultant phosphopeptides were enriched prior to LC-MS analysis. Through the analysis of whole serum proteome and artificial phosphoproteome, 392 proteins and 408 proteins were identified, respectively. The overlaps between them were 232 proteins, implying that they have different characteristics. In order to clarify the differences between them, abundance profiles of each proteome were analyzed. As a result, lower median values of protein abundance in artificial phosphoproteome were obtained. This suggests that low abundant proteins were enriched in artificial phosphoproteome and it was able to identify lower abundant proteins efficiently using this approach. We will also report the use of six proteins-depleted serum in combination with this approach to increase the proteome coverage.

Keywords: phosphoproteomics, in vitro kinase reaction, human serum

POS-02-160 Comprehensive Quantitative Phosphoproteomic Approach by MS/MS ^{ALL} with SWATH™ Acquisition

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Protein phosphorylation plays a crucial role in many biological processes such as inter- and intracellular signaling, cell cycle, protein synthesis, degradation and apoptosis. To understand these biological processes, it is important to develop analytical strategies to identify and quantify a broad range of phosphopeptides and proteins. Recently MS/MS^{ALL} with SWATH acquisition was reported as an alternative strategy to increase the comprehensiveness of data acquisition. SWATH acquisition is a data-independent acquisition method that generates, in a single measurement, a complete recording of the parent ion and fragment ion spectra of all the analytes in a biological sample. SWATH acquisition provides a powerful workflow for targeted protein / peptide quantitation and will increase the reproducibility and comprehensiveness of data collection and processing. In this study we use SWATH acquisition combined with Immobilized Metal Affinity Chromatography (IMAC) for quantitative phosphoproteomics. Each sample was digested with trypsin and phosphopeptides were enriched using IMAC. Purified phosphopeptide mixtures were analyzed by LC-MS/MS. MS experiments were performed on a TripleTOF® 5600+ system (AB SCIEX) connected with a ekspert™ nanoLC 425 with cHiPLC® system (Eksigent). Qualitative data was processed using ProteinPilot® Software 4.5 beta to identify proteins. Quantitative data of each phosphopeptide were processed using SWATH acquisition microApp in PeakView® software.

Keywords: SWATH, phosphorylation, IMAC

POS-02-161 Large Scale Targeted Protein Quantification Using HR/AM Selected Ion Monitoring with MS/MS Confirmation on A Novel Hybrid, Q-OT-qIT Mass Spectrometer

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A hybrid orbitrap MS mass spectrometer has been applied for targeted protein quantification using HR/AM selected-ion monitoring (SIM) for quantification and pre-defined ion trap MS/MS for simultaneous peptide verification. The throughput has been limited on current hybrid instruments because the pre-defined CID MS/MS cannot be acquired in parallel with Orbitrap SIM. A novel hybrid instrument, based on mass resolving quadrupole, Orbitrap, collision cell, linear ion trap (Q-OT-qIT) architecture enables parallel orbitrap SIM and rapid targeted ion trap MS/MS, maximizing instrument duty cycle. Here we developed a high throughput targeted protein quantification workflow that uses three HRAM SIM scans with wide isolation windows (200 amu) to cover all precursor ions of 450 - 1050 *m/z*. In parallel with each SIM scan, 20 sequential ion trap MS/MS with 10 amu isolation windows are acquired to cover the associated 200 amu SIM mass range. With this data independent approach, quantitative information for all precursor ions detected in three sequential SIM scans is recorded in a single run. Plus, all MS/MS fragment information over the mass range of 450 - 1050 *m/z* is recorded for sequence confirmation of any peptide of interest by querying specific fragment ions in the spectral library. The quantitative performances and throughput of this new approach are evaluated using various samples. This new data independent acquisition (DIA) approach enables accurate and reproducible quantitative results for any precursor ions detected in SIM mode without prior knowledge on the new hybrid instrument.

Keywords: hybrid, Q-OT-qIT mass spectrometer, Parelle SIM-cid msms workflow in DIA fashon, simultaneous qual/quan

POS-02-162 Quality Control Tool and Long-Term Performance of SRM Instruments

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Selected Reaction Monitoring (SRM) is becoming a standard tool for reproducible high-throughput protein quantification. However, for the method to become fully integrated for everyday use, more knowledge is needed about the long-term stability and performance of the liquid chromatography triple quadrupole (LC-QQQ) instrument when operated in SRM mode. Here we present a quality control (QC) system, based on the previously published Anubis SRM analysis software (*Teleman et. al., JPR 2012*), to automatically monitor LC-QQQ performance over time. The QC-setup displays key metrics such as signal intensity, retention time and fragmentation pattern for repeated analysis of a QC-sample, giving a quick and lean overview of the instrument performance. To exemplify the power of the QC-system, we studied 400 QC-sample injections, collected over the course of 6 months. Monitoring 22 peptides, we find that the signal intensity, retention time and fragmentation pattern are mostly stable or varying in a linear fashion, and instrument malfunction could be pinpointed. In conclusion, the provided tool gives laboratories a new way of assessing SRM instrument performance, enabling higher throughput and less wasted sample and instrument time due to running samples on suboptimal instruments.

Keywords: mass spectrometry, quality control, software

POS-02-163 Facile Synthesis of Novel Magnetic Silica Nanoparticles Functionalized with Layer-by-Layer Detonation Nanodiamonds for Secretome Study of an Inducible Hepatitis B Virus Cell Line

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In this study, novel magnetic silica nanoparticle functionalized with layer-by-layer detonation nanodiamonds (dNDs) were prepared by coating single submicron-size magnetite particle with silica and subsequent modification with detonation nanodiamond. The resulting layer-by-layer dNDs functionalized magnetic silica microspheres (Fe_3O_4@SiO_2@[dNDs]n) exhibited well-defined magnetite-core-silica-shell structure and possess high content of magnetite, which endow them with high dispersiblity and excellent magnetic responsibility. As a result of their excellent magnetic property, Fe_3O_4@SiO_2@[dNDs]n microspheres were successfully applied for convenient, fast and efficient pretreatment of low-abundance peptides/proteins from high-diluted sample solution. The signal intensity could be improved by at least two to three orders of magnitude. Even in high salt concentration solution, peptides/proteins could also be isolated effectively. The facile synthesis and the convenient and efficient enrichment process of the novel layer-by-layer dNDs-functionalize microspheres make it promising candidate for isolation of protein in secretome. Combination of the pretreatment approach of Fe_3O_4@SiO_2@[dNDs]n and iTRAQ technology, the host response to HBV using an inducible HBV-producing cell line (HepAD38) was investigated by the comparative protein expression in the secretome of HepAD38 treated with and without tetracycline, which showed a cluster of pro-fibrosis factors were secreted. In a trans-well co-culture cell system, the activation of Hepatic stellate cell (HSC) was observed innovated with HepAD38. This finding shows a new mechanism that HBV could active HSC via hepatocyte paracrine pathway to induce live fibrosis.

Keywords: detonation nanodiamond, magnetite particles, secretome

POS-02-164 Urimem, A Simple Economical Urinary Protein Membrane, Will Facilitate Biomarker Research

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Biological samples from patients are invaluable. Ideally the samples should be preserved for the same period of time as the duration of their corresponding medical records. Urine is a body fluid that can be non-invasively acquired and contains important biological information about the patient. Simple and inexpensive urinary protein sample preservation can be the starting point for comprehensive biological sample storage just like medical record of patients. Here, we propose a method to adsorb urinary proteins to a membrane named Urimem that can then be dried and stored. This method is very simple and inexpensive and requires minimal sample handling. It does not use organic solvents. The proteins on the membrane are dry and are prevented from degradation. The membrane may even be able to be stored at room temperature at least for weeks. The quantity of eluted proteins from a membrane is sufficient for biomarker validation experiments. Comprehensive historical biological information can also be used in retrospective studies to understand the pathophysiology of disease and the relationships among diseases as well as to monitor the long-term efficacy and side effects of treatments. With this information, medical research can be conducted more easily, considerably faster, and more economically, ultimately benefiting the patients who provided the samples. Thus, we believe that it is possible to preserve urinary protein samples from each stage of disease development for every consenting patient in a hospital. This can potentially change the landscape of medical research and medical practice in the future.

Keywords: urine proteome, biomarker

POS-02-165 A Comprehensive Analysis of the Interactions between *Streptococcus pyogenes* and Human Plasma Proteins

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Streptococcus pyogenes is a major human bacterial pathogen responsible for hundreds of millions of cases of superficial skin and throat infections annually. In the transition from these mostly uncomplicated conditions to severe and invasive disease associated with high mortality rates, the bacterium interacts with several human blood plasma proteins to evade host defences and to secure nutrients. Clarifying these interactions and their biological consequences will help to explain the progression from mild to severe infections. In this study, we used a combination of mass spectrometry based technologies to comprehensively quantify the components of the S. pyogenes-plasma protein interaction network. From an initial list of 184 interacting human plasma proteins defined using LC-MS/MS analysis we further subdivided the interacting protein list using selected reaction monitoring depending on the level of enrichment and protein concentration on the bacterial surface. The combination of MS techniques was applied to study differences in protein binding to a *S. pyogenes* strain isolated from the same patient; one isolate was from the throat where it showed no local symptoms, the other was from a deep tissue infection causing severe and life-threatening disease. Comparing the plasma protein-binding properties of the two isolates revealed considerable differences, underlining the pathogenic significance of these protein interactions. The results also demonstrate the power of the developed mass spectrometry method to investigate host-microbial relationships with a large proteomics depth and high quantitative accuracy.

Keywords: SRM, human plasma, *Streptococcus pyogenes*

POS-02-166 Monitoring Protein Synthesis in Living Cells with Fluorescent Labeled tRNA FRET Pairs

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We introduce Protein Synthesis Monitoring (PSM) - a technique to monitor protein synthesis in living cells. In PSM, we transfect cells with tRNAs labeled as FRET donors and acceptors. A FRET signal is generated only when a donor- and an acceptor-labeled tRNA come in close contact (< 7nM), as they do on the ribosome during elongation. The intensity of the FRET signal correlates with the number of ribosomes engaged in protein synthesis, providing a real-time, live-cell assay for measuring rates of protein synthesis. PSM can monitor general protein synthesis using bulk tRNAs, or the synthesis of a specific protein, using specific pairs of tRNA. PSM has sub-micron spatial and sub-second temporal resolutions. Cells continue to live and grow normally, and the synthesized proteins are unchanged since the labeling is on the tRNA itself and not on the amino acid. The cells uptake the tRNAs using liposomes or other common methods. The specificity of PSM arises from the large number of distinct tRNA pairs - 1176 in humans (corresponding to 48 isoacceptors). For about 83% of all proteins, an adjacent pair of tRNAs can be found that is enriched in the synthesis sequence of that protein compared to its frequency in the average, or background protein. The enrichment, or E-factor, can indicate the expected signal to background value for a given protein. With this approach, cells can be monitored for the exact timing of synthesis of a protein of interest, provided it is synthesized at sufficient rates (e.g. a sufficient number of ribosomes is engaged in synthesis of this protein). We have demonstrated specific PSM for monitoring synthesis of a viral protein (NS3) during viral infection using Isoleucine tRNA, and for monitoring synthesis of collagen during fibrosis in mouse fibroblasts using tRNA-Gly and tRNA-Pro. We will discuss these results as well as additional applications of PSM in stem cells, drug discovery, cell sorting and basic research.

Keywords: protein synthesis, ribosome, tRNA

POS-02-167 A Novel Method to Quantify Protein Conformational Changes in Complex Cellular Matrices

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Protein conformational changes often results in an alteration of function and range from small local motions, such as allosteric regulation, to drastic structural rearrangements, such as amyloid formation, where a gain-of-function phenotype is observed. Despite their important role in the functioning of a protein, not much is known about intracellular conformational switches and the consequences thereof, mostly due to the lack of suitable techniques to study protein conformations in their native cellular environment. Structural techniques such as X-ray crystallography or NMR are incapable of dealing with complex matrices, while FRET-based techniques require protein tagging and are not applicable at high-throughput. To overcome this limitation, we developed a novel proteomic strategy, combining the limited proteolysis (LiP) biochemical tool with selected reaction monitoring-mass spectrometry (SRM-MS), to identify and quantify protein conformational switches under different conditions within a complex biological matrix. We assessed the performance of the method using complex cell extracts and model proteins undergoing well-characterized conformational changes of different magnitude. Our approach enabled the extraction of conformational markers for the different protein conformations, that allow to quantify the extent of the conformational change with a ̃10-amino acid resolution, directly in the cellular matrix and across multiple samples. Furthermore, we showed that the method can also be applied in an unbiased manner to identify unknown conformational changes in the proteome of yeast, under different growth conditions. In conclusion, This method enables the quantitative and high-throughput characterization of biological processes regulated or driven by protein conformational changes, thus opening a range of exciting possibilities in systems biology and biomedical applications.

Keywords: protein conformational change, targeted proteomics, protein structure

POS-02-168 Intact Protein LC-MS, How to Overcome the Challenges?

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LC-ESI-MS protein characterization finds two major applications; in top-down proteomics and the analysis of recombinant proteins, antibodies and other biotherapeutics. Reversed-phase separations with narrow-bore columns are particularly interesting, because of the high compatibility with MS of the eluents used, and the capability of analyzing limited sample amounts. Nevertheless, this approach still poses several challenges. Problems such as degradation, broad peak width, poor resolution, recovery, ionization efficiency and sensitivity are often encountered in LC-MS of intact proteins.
In this work, the performance of several prototype reversed-phase column /spray interfaces was evaluated for the separation of intact proteins and monoclonal antibodies (MAbs).In particular, the impact of the reduction of post column dead volume on recovery and sensitivity was assessed. The integration of column and sprayer minimized the post column volume, hence the peak broadening, and allowed the MS to detect peak with higher intensity. The direct integration of column and electrospray needle diminished the losses of proteins occurring in the transfer line; this was beneficial to recovery, and eventually contributed to enhance the overall detection sensitivity.
The impact of different reversed-phase chemistry and stationary phase support (namely a porous particle, a solid core particle and a monolithic structure) to the overall performance was also evaluated. It was observed that the particle-based stationary phases showed a higher loadability compared to the monolith. The loadability for packed columns was up to five-fold higher; however it was strongly protein-specific. The peak capacity on the monolith was higher probably due to the favorable mass-transfer contribution of the non-porous structure.

Keywords: intact proteins, antibodies, LC-MS

POS-02-169 Data Independent LC-IM-MS Strategies for the Multi-Omic Scale Identification and Quantitation on Cytochrome P450 Transfected Hepatocytes

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Drug toxicity is a major reason for candidate pharmaceutical failure during development. It is thus important to realize toxicity potential timely. Many xenobiotics are bioactivated into toxic metabolites by cytochromes P450 (CYP). However, the activity of these enzymes typically falls *in-vitro*. A transformed human hepatocyte cell line (THLE) became available in which metabolic activity of specific CYP isoforms is maintained. The baseline effect of CYP2E1 addition into THLE hepatocytes has been characterized to better understand system biochemistry.
Dedicated sample preparation protocols were applied to isolate metabolites, lipids and (digested) proteins, and three independent replicates of THLE null or THLE +2E1 cells investigated. The same LC-MS system was used for all experiments and application dependent LC conditions applied. MS data were acquired using data independent analyses, whereby the energy applied to the collision cell was switched between a low and elevated energy state during alternate scans. For the proteomics experiments, ion mobility separation was incorporated in the LC-MS method. Multi-omic data were processed and searched and quantified using TransOmics software. Pathway analysis and systems biology experiments were conducted using various informatics tools.
Comparison of the correlation variance and fold change between groups illustrates significant analyte expression. Interpretation by means of clustering, statistical, and data analysis approaches have shown protein, lipid, and metabolite data to be complimentary and confirmative, which is further supported from the resulting pathway analysis output. The data interpretation has shown protein, lipid, and metabolite data to be complimentary and confirmative, which is further supported from the resulting pathway analyses. The most significantly enriched signaling pathways were EIF2, regulation of EIF4 and p70S6K, mTOR, actin cytoskeleton and ILK.

Keywords: multi-omics, pharmacoproteomics

POS-02-170 Proteomic Analysis of Antidepressant-Like Effect of a Kampo (Japanese Herbal) Medicine "Kososan (Xiang-Su-San)" on Brain from Stress-Induced Depression-Like Model Mouse

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A Kampo (Japanese herbal) medicine, kososan (KS), is clinically used to treat the depression-like symptoms. Our previous studies using stress-induced depression-like model mice, demonstrated that oral administration of KS leads to an antidepressant-like effect via the normalization of dysfunction of hypothalamic-pituitary-adrenal axis.[1)] In present study, we have adopted a proteomic analysis to identify brain proteins that are affected by KS treatment of the mouse model using agarose two-dimensional gel electrophoresis and mass spectrometry-based protein identification.
The depression-like model mice were prepared using a combination of modified forced swimming and three different chronic mild stresses (tilting, pouring of water onto the sawdust bedding, and shaking of cages) to ddY mice.[1)] KS (1.0 g/kg/day) was administered orally for 9 days during the stress exposure. Antidepressant-like activity was estimated with the duration of immobility of mice during the forced swimming test (FST). The duration of immobility of the stressed mice during the FST was significantly increased when compared with that of nonstressed mice. Administration of KS significantly reduced the duration of immobility. Proteomic analysis showed the expressions of metabotropic glutamate receptor 2 (mGluR2) and cyclic phosphodiesterase 1 (CNPase1) were reduced on the hypothalamus of depression-like model mice, but the expressions were recovered by the administration of KS. Western blot analysis of the hypothalamus also confirmed these results. Immunohistochemical analysis showed the expression of mGluR2 protein in the hypothalamus paraventricular nucleus was down-regulated in the model mice, but KS ameliorated this alteration to the normal level.
These results suggest that mGluR2 and CNPase1 in hypothalamus is related to the antidepressant-like activity of KS.

1) Ito N. *et al.*, *Phytomedicine* **13**, 658-667 (2006).

Keywords: depression-like model mouse, hypothalamus of brain, Kampo (Japanese herbal) medicine

POS-02-171 Proteomic Analysis of Cardiomyocetes after Inhibition of MMP-2 Gene Expression

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In the development of cardiac injury, the inhibition of matrix metalloproteinase-2 (MMP-2) by MMPs inhibitors protect against cardiac injury. Using a specific MMP-2 inhibitor such as MMP-2 siRNA reduces the MMP-2 expression and protects the contractile protein such as myosin light chain 1 (MLC1) from degradation and contractile dysfunction of cardiomyocytes. The objective of this study is evaluating changes of cardiomyocyte proteome in association with MMP-2 inhibition.
MMP-2 inhibition resulted in the increase of contractility of cardiomyocytes and MLC1 level.
Using arbitrary threshold, the comparison of the cardiomyocyte proteomes between control and MMP-2 suppressed expression groups by two-dimensional electrophoresis revealed 10 significantly different protein spots. Eight protein spots were identified as mitochondrial enzymes involved in energy production and represent 7 proteins. Level of 6 proteins are significantly increased and they represent: dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, ATP synthase beta subunit, cytochrome c oxidase subunit 5A, ATP synthase alpha subunit precursor, electron transfer flavoprotein subunit beta, and NADH dehydrogenas [ubiquinone] 1 alpha subcomplex subunit 5. Level of 1 metabolic enzyme is reduced and it is a fragment of mitochondrial precursor of long-chain specific acyl-CoA dehydrogenase. Also, 2 proteins such as heat shock protein 60 precursor and Cu-Zn superoxide dismutase increased they level after inhibition of MMP-2.
Measurement of ATP synthase activity showed that ATP synthase activity increased in MMP-2 siRNA group in comparison to the control by approximately 30%.

Keywords: cardiomyocytes, matrix metalloproteinase, ATP synthase,

POS-02-172 Proteomic Analysis of Liver Microsomes in *X. tropicalis*

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The Western clawed frog, *Xenopus tropicalis*, is an emerging model animal in life sciences including toxicology and pharmacology. Its genome sequence data has been completed, therefore, comprehensive proteomics research becomes possible in amphibians. In order to identify and characterize liver microsomal proteins, in particular, drug metabolizing enzymes (DMEs), we performed shotgun proteomics analysis of liver microsomes from *X. tropicalis* adults. Liver microsomes were fractionated using a calcium precipitation method, and then their fractions were separated by SDS-PAGE. DMEs-enriched bands corresponding to molecular weights of 55 ~60 kDa were cut off and were in-gel digested by trypsin. Their trypic peptides were subjected to shotgun analysis using nano LC-MS/MS with LTQ-Orbitrap XL mass spectrometer. We identified over two hundred unique proteins including many DMEs. Notably, over 30 cytochrome P450 (CYPs) that are major xenobiotic enzymes in liver, were identified. This is the first report of comprehensive proteomics research about liver microsomes in amphibians. In addition, this proteomic profiling provides us an important reference to analyze the effects of chemicals and drugs on amphibians and discover biomarkers for proteomics-based toxicology and pharmacology using *Xenopus*.

Keywords: Xenopus tropicalis, liver, cytochrome P450

POS-02-173 Monitoring Drug Metabolizing Enzymes and Transporters Using Txp Antibodies and Immunoaffinity MS

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One central issue in pharmacokinetics and -dynamics is the induction of drug metabolizing enzymes and transporters by drugs and drug candidates. The expression of cytochrome P450 enzymes and transporters can increase by the factor 100 after drug administering. The concentration of these proteins can be assessed by mass spectrometric measurement of proteotypic peptides with highest specificity. Current methods include laborious up-front sample preparation in the form of ultracentrifugation or gel electrophoresis.

Here we present an alternative sample preparation employing TXP-antibodies specific to short C-terminal peptide epitopes capable of enriching peptide groups. We generated antibodies targeting common epitopes present in signature peptides derived from members of the cytochrome P450 system. Furthermore we addressed drug transporters. These antibodies were applied in an immunoaffinity step prior to an MRM or MRM-like readout.

The combination of an immunoenrichment step with an MRM readout resulted in a more than 200-fold higher sensitivity than reached by MRM alone. Thus we were able to quantify CYP3A4, CYP3A5, CYP3A7, CYP2A13, CYP2A6, CYP2C9, CYP2C19, CYP2D6, POR, and MDR1 as toxicologically relevant proteins directly from proteolytical digests. The established assays were used to monitor the induction of drug-transforming enzymes and transporters in *in vitro* cell culture experiments using hepatocytes derived from human donors and xenograft mouse models.

Keywords: cytochrome P 450, drug transporter, immunoaffinity-MS

POS-02-174 Evaluation of Nanomaterial-Induced Biological Responses by Toxico-Proteomics Analysis

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Nanomaterials are defined as substances that have at least one dimension of less than 100 nm in size. Although their practical uses are rapidly spreading to wide variety of fields, the debates on safety of nanomaterials has expanded worldwide because they have unique physicochemical properties and exert innovative functions. Therefore, it is urgently needed to identify novel biomarkers that may predict adverse biological effects induced by nanomaterials. Also, it is important to uncover the as yet unknown biological effects of nanomaterials. In this regard, we previously demonstrated by proteomics analysis that some acute phase proteins such as SAA could be useful biomarkers for analyzing the biological effects associated with exposure to silica nanoparticles (nSP) in mice. Here, we attempted to assess the nSP-induced biological effects involved in the expression of the acute phase proteins. Initially, we focused on the changes in the number of blood cells in mice because it is reported that SAA induced neutrophilia in mice. We analyzed the changes in the number of blood cells in mice after intravenous injection of nSP with diameters of 70 nm (nSP70) via tail vein. The number of granulocytes in nSP70-treated mice was significantly higher than those in control mice. Then, flow cytometry analysis showed that proportion of neutrophil was elevated in peripheral blood of nSP70-treated mice. These results suggested that nSP70-induced neutrophilia could induce inflammatory responses by reason that neutrophil play important roles in inflammation. Now, we are trying to examine the association between nSP70-induced neutrophilia and inflammatory responses.

Keywords: toxicoproteomics, nanomaterial

POS-02-175 Discovering Novel Neurotoxins from Snake Venom by Using Mass-Spectrometry-Guided Purification Approach and Pharmacological Assays

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Snake venom is a highly complex mixture of various protein components. One of the important key components in snake venom particularly from family *Elapidae* is neurotoxin (1). Despite being toxic, snake venom neurotoxins are used as probe in biomedical research to study various receptors and ion channels. Application of mass-spectrometry provides an alternative method to extensive use of animals in neurotoxin characterization and discovery process. Instead of spending numerous hours on onventional fractionation-guided pharmacological assays, novel and interesting neurotoxins can now be detected easily from the samples via LC-MS/MS and the respective databases. Thus, allowing focus purification and pharmacological assays to be done on a particular sample. However, venoms of many venomous species have not been studied and thus the toxin database for a particular species may not be complete or available. The complexity of the venom and impurity content of venom are also some of the obstacles that need to be resolved before extending this capability. In our laboratory, we have isolated and identify novel neurotoxin via LC-MS/MS that exhibited neurotoxicity effect in neuromuscular systems from the venoms of *Bungarus fasciatus*. Data will be presented on how the identity of neurotoxin was confirmed by LC-MS/MS and followed by the *in vitro* pharmacological assays for neuromuscular systems to confirm the activities of the neurotoxin.

Keywords: venom, neurotoxins, bioassays, proteomics

POS-02-176 Calcium Oxalate Crystals Induced Changes in Secretion of Proteins from Basolateral Compartment of Renal Tubular Cells That, in Turn, Enhanced Crystal Invasion

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Calcium oxalate monohydrate (COM) crystals cause kidney stone disease by several unclear mechanisms. One of hypotheses was that COM crystals induce changes in various cellular mechanisms, leading to enhancement of crystal invasion in renal interstitial tissue. The present study thus aimed to characterize changes in secretion of proteins from basolateral compartment of renal tubular epithelial cells after exposure to COM crystals. Polarized MDCK cells were cultivated in Transwell containing serum-free medium with or without 100 μg/ml COM crystals for 20 h. Trypan blue exclusion assay revealed comparable cell viability between the two groups of cells. Secreted proteins in culture medium from the lower chamber (basolateral compartment) were then collected, desalted by dialysis against deionized water, and finally concentrated by lyophilization. The recovered proteins from five individual cultures in each group (total = 10 biological replicates were analyzed) with equal amount were resolved in individual 2-D gels and visualized by Deep Purple stain. Spot matching and intensity analysis revealed six protein spots with significantly altered levels in the basolateral compartment of COM-treated cells. The altered secreted proteins were then successfully identified by LC-Q-TOF-MS/MS, including enolase-1, phosphoglycerate mutase-1, actinin, 14-3-3 protein epsilon, alpha-tubulin 2 and ubiquitin-activating enzyme E1. The proteomic data were confirmed by Western blot analysis. Finally, functional analysis was performed to validate the significant role of the identified altered proteins in crystal invasion using crystal invasion assay recently established by our group [Chiangjong W, Thongboonkerd V. Talanta. 101:240-5, 2012]. These data provide an important basis for further elucidation of sophisticated mechanisms of kidney stone disease.

Keywords: calcium oxalate, crystal invasion, kidney stone

POS-02-177 Temporal and Quantitative Proteomics Study Reveals Mitochondrial Dysfunction, Perturbed Secretory Pathway and ER Stress-Mediated Apoptosis as Anticancer Mechanisms of a Chalcone on A375 Melanoma Cells

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Global incidences of melanoma, the most lethal form of skin cancer continue to rise. There remains an urgent need for more efficacious therapeutics due to limited treatment options for advanced melanoma and the development of resistance towards existing drugs. Panduratin A (PA), a cyclohexanyl chalcone found in *Boesenbergia rotunda,* was investigated for its anti-cancer potentials against A375 malignant melanoma cells. The molecular actions of PA in inducing cell cycle arrest and apoptosis were further investigated in the present study. **Methods** Temporal quantitative proteomics by iTRAQ 2D-LC-MS/MS approach was used. Two independent biological replicates of A375 cells treated with 15 μM of PA for four time points (0 H, 4 H, 12 H and 24 H) were subjected to isobaric iTRAQ labelling, followed by 2D LC-tandem MS analysis. Potential targets of PA were validated by western blot and immunofluorescence assays. **Results** Proteomics analysis identified 4888 proteins (corresponds to 1% global FDR) with 296 being significantly deregulated in PA treated A375 cells. These include differentially expressed proteins of mitochondria oxidative phosphorylation (OXPHOS), secretory pathway and endoplasmic reticulum (ER) stress response. Inhibition of ER stress with global protein translation inhibitor, cycloheximide prevent cancer cell death induced by PA. **Conclusion** Collectively, these data suggest that PA perturbs OXPHOS and secretory pathway leading to ER stress. Prolonged ER stress ultimately resulted in apoptosis of melanoma cells. This study provides comprehensive mechanistic insights into the anti-cancer effects of PA and more importantly, its potential as a therapeutic agent for melanoma.

Keywords: melanoma, iTRAQ

POS-02-178 New Insights into the Cellular Response Triggered by Silver Nanoparticles Using Quantitative Proteomics

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Silver nanoparticles (AgNPs) are broad-spectrum antimicrobial agents with proven effectiveness against multidrug-resistant bacteria, HIV and SARS viruses. Due to this feature, AgNP-based products is a fast growing market, including wound dressings, athletic socks, toothpaste, mouthwash, food storage bags, and even dietary supplements. It is therefore necessary to assess whether human exposure to AgNPs is harmful as well as the cellular mechanisms triggered by nano-bio interactions. The present study, therefore, aimed to answer which set of proteins and cellular networks are (dis)-regulated by AgNPs. We treated human intestinal epithelial cells with 10 μg/mL AgNPs (20 or 100 nm) for 24h, and performed an iTRAQ-based quantitative proteomic approach. In total, 3352 proteins were confidently identified (FDR ≤1%) and quantified in the three independent biological replicates. Following ANOVA corrected multiple testing with Benjamini-Hochberg, only proteins with p-value≤0.01 were considered differentially regulated. The 20 nm AgNPs induced regulation of 467 proteins (240 down- and 227 up-regulated) while the 100 nm AgNP statistically changed the abundance of 306 proteins (143 down- and 163 up-regulated). Protein-protein interaction networks and gene ontology analyses revealed that 20 nm AgNPs predominantly induced down-regulation of proteins involved in mitochondrial electron transport chain, and up-regulation of proteins involved in the DNA damage response. Moreover, ROS activity measurements and quantification of protein oxidation by OxyBlot showed that 20 nm particle induced a higher oxidative stress than 100 nm. These observations as a whole correlate well with 3D confocal microscopy demonstrating that 20 nm AgNPs are internalized while 100 nm remains outside the cells.

Keywords: silver nanoparticles, quantitative proteomics, iTRAQ

POS-02-179 Protein-Protein Interaction Analysis Using Oligo-Cysteine Tagged Protein Chip. Detection of Tubulin from Mouse Brain Extract by Oligo-Cysteine Tagged Stathmin Chip

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Protein chip technology is essential for high-throughput functional proteomics. We developed an oligo-cysteine tagged protein chip, where the protein immobilized on the chip contains five tandem cysteine repeats (Cys-tag) at the terminus of the protein. The Cys-tag was designed to allow covalent attachment of proteins to the surface of a maleimide-modified, diamond-like, carbon-coated silicon substrate. This Cys-tagged protein chip has unique characteristics such as high protein density on the surface of the substrate and the difference in orientation due to the position of the tag. In this study, we performed protein-protein interaction analysis using stathmin/op18 as a model protein. Stathmin is a protein having a molecular weight of 18,000 and it is expressed highly at brain, testis, and tumor cells, such as leukemia cells. It functions as a regulator of microtubule dynamics and chaperone function to heat stresses by interacting tubulin heterodimers and BiP/GRP78, respectively. The Cys-tagged protein chips were incubated with mouse brain extracts, trypsinized directly, and then analyzed by using a mass spectrometer (LC/MS-MS). As a result, tubulin α, tubulin β, dynamin-1, and microtubule-associated protein tau were detected only on the protein chips exposing N-terminus of stathmin. However, these proteins were not detected on the protein chips exposing C-terminus of stathmin. It has been reported that the N-terminal of stathmin like domains (SLDs) impede tubulin polymerization. These results are consistent with previous reports and indicate that the difference in the interacting protein occurs depending on the exposing terminus of protein on the chip. Thus, the Cys-tagged protein chip is a powerful novel tool for protein printing method for protein chips that requires very low amounts of protein and can be used for high-performance analysis of protein-ligand interactions.

Keywords: protein chip

POS-02-180 Miniaturization of Multiplexed Planar Recombinant Antibody Nanoarrays for Serum Protein Profiling

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Antibody-based microarrays have been established as a high-throughput affinity proteomic technology for protein expression profiling of crude clinical samples in the search for disease-associated biomarkers. However, in order to enable global proteome analysis, novel miniaturized high-density antibody array layouts needs to be developed. Recently, we have successfully developed the first multiplexed planar recombinant scFv antibody nanoarray platform for profiling of non-fractionated, directly labelled serum samples. Miniaturized nanoarrays (10 μm sized features), displaying a probe density of 38,000 features/cm^2, were produced, using a desktop nanofabrication system based on dip-pen nanolithography technology, and interfaced with a high-resolution fluorescent-based scanner. Well-characterized serum samples were targeted and the results showed that multiplexed profiling could be performed in a sensitive, specific, and reproducible manner. We have now developed and expanded this technology platform further and produced the first 48-plex planar recombinant scFv antibody nanoarrays. The applicability of the platform was then evaluated by performing miniaturized, multiplex serum protein profiling of systemic lupus erythematosus (SLE). SLE is a severe chronic autoimmune connective tissue disease, for which serum biomarkers for diagnosis and prognosis remains to be delineated. The results showed that SLE-associated serum biomarkers reflecting disease could be deciphered, outlining the use of antibody nanaoarrays for disease proteomics.

Keywords: antibody arrays

POS-02-181 Personalised Proteomics by Means of Individualised Protein Microarrays

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Recently, whole genome sequencing of individuals has become. Very soon, it will be applied routinely in clinical settings. Despite remarkable progress in understanding the level of nucleic acids, however, insights into disease biochemistry frequently remain preliminary. Much disease-relevant regulation and activity occurs at the protein level through control of both gene expression and protein isoform variations. Consequently 97% of all current therapeutic agents target proteins. We are aiming at taking advantage of sequence information from individuals for a directed characterisation of disease-specific protein isoforms (mutations, polymorphisms and splice variations), utilising a newly developed technique of producing personalised protein microarrays. First, a tissue's RNA/cDNA is copied onto the microarray by an on-chip PCR amplification, using gene-specific primer pairs that are attached to the chip surface. The arrayed DNA copies then act as templates for an *in situ* cell-free expression, yielding a protein microarray that presents the protein content of a particular tissue of an individual person. This array format provides a basis for the analysis of protein interactions with other proteins, nucleic acids and small chemical compounds. Our overall objectives are the detection of disease-related protein variations, the development of personalised diagnostic methods and the identification of therapeutically relevant compound lead structures. For future therapy, knowledge of protein isoforms and their combinations in individual patients will be critical for therapeutic approaches that target disease-relevant protein conformations, leaving the molecules in healthy tissues unaffected.

Keywords: personal proteome, interaction studies, in situ synthesis

POS-02-182 High Throughput Cell-Based Studies and Protein Microarrays for Biomarker and Target Discovery

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One of the most compelling steps in the post-genomic era is learning the functional roles for all proteins. The DNASU repository accelerates discovery by providing nearly 15,000 full-length clones for human genes enabling high-throughput protein function studies. To exploit this resource, we developed a novel protein microarray method, called nucleic acid programmable protein array (NAPPA). In lieu of printing purified proteins, NAPPA translates proteins in situ from printed cDNAs by extracts containing human ribosomes and chaperones. This obviates the need to purify proteins, produces human proteins in their natural milieu, and ensures protein stability on the array as the proteins are made just-in-time for assay. NAPPA arrays have been used in the discovery of disease biomarkers, protein-protein interactions and enzyme substrates. Recent experiments have focused on the search for autoantibody responses in breast cancer patients. The prevalence of autoantibodies to specific proteins is typically in the 20% range; thus a panel of autoantibodies will be needed to achieve high sensitivity. Using 155 cases/130 controls in a three phase study, including a blinded validation, we have discovered a panel of 28 autoantigens in breast cancer with sensitivities ranging from 5-40% and specificities ranging from 80-100%. We are further advancing this methodology using photolithographically etched discrete silicon nanowells coupled with next generation piezoelectric printing to achieve very high densities. High density protein expression and display, as well as functional protein-protein interactions, was shown in 8000 nanowell arrays, the highest reported density of individual proteins in nanovessels on a single slide.

Keywords: autoantibodies, biomarker, cancer

POS-02-183 Identification of B-Cell Lymphoma Subsets by Protein Profiling Using Recombinant Antibody Microarrays

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B-cell lymphomas are the most common type of Non-Hodgkins lymphomas (NHL), a type of blood cancer with an alarming development rate, with incident rates having nearly doubled since the 1970s. The current methods of classifying and diagnosing these lymphatic cancers are often invasive and generate inadequate information, hampering the clinical management of the patients and thus representing critical unmet clinical needs. In this project, the protein profile of minimally invasive plasma samples from several hundreds of patients with different B-cell lymphomas, including mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL), were analyzed using our recombinant antibody microarray platform. This involves the printing of miniaturized arrays (<1 cm^2) containing numerous individual (<2000/cm^2) recombinant antibody fragments, capable of targeting different proteins in minute amounts (μl scale) of labeled clinical samples in a sensitive, reproducible, and high-throughput manner. Disease-associated biomarkers were deciphered, and the results revealed, as might be expected, large heterogeneity within the different diseases. Interestingly, the analysis generated the first candidate plasma biomarker signatures capable of classifying subsets of the lymphomas, dividing the all the targeted lymphomas into 2 or more subgroups. Of note, the two novel subgroups of DLBCL patients indicated a correlation with survival. Taken together, we have taken the first steps towards deciphering disease-associated multiplex plasma protein panels, in the long-term run paving the way for improved diagnosis and stratification of B-cell lymphoma patients.

Keywords: antibody microarrays

POS-02-184 Protein Chip Methodology to Detect HSP70 Autoimmune Antibodies in the Sera of Hepatitis C Virus-Positive Hepatocellular Carcinoma Patients

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We report an antigen-protein chip to detect antibodies in sera. This chip method was created to detect hepatocellular carcinoma-related autoantibodies in the sera of hepatitis C virus infected individuals. Five cysteine-tagged (Cys-tag) and green fluorescent protein (GFP)-fused recombinant heat shock protein 70 (HSP70), superoxide dismutase 2 (SOD2), and peroxiredoxin 6 (PRDX6), were spotted and immobilized on maleimide-incorporated diamond-like carbon (DLC) chip substrates. Antibodies in diluted sera were trapped by these proteins at each spot on the chip, and visualized by a fluorescence-conjugated anti-human IgG. The total immobilized protein level of each spot was detected with anti-GFP mouse IgG and a fluorescence-conjugated secondary anti-mouse IgG. Heat treatment of the chip in a solution of denaturing and reducing agents, before serum-incubation, improved autoantibody detection. We tested 31 samples of sera from healthy individuals and HCC patients using the chips. The HSP70 autoantibodies were often found at high levels in sera from HCV-positive HCC patients, but not in HCV-negative sera. This antigen-array chip methodology may have useful properties to capture various kinds of antibodies from many serum samples.

Keywords: protein chip, HSP70, hepatocellular carcinoma

POS-02-185 Phenotyping of Various Membrane Markers on Plasma Exosomes from 80 Healthy Donors Using an Extracellular Vesicle (EV) Array

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Exosomes are endosome-derived vesicles between 40 - 100 nm in diameter that are secreted by many cell types. The quantity and molecular composition of exosomes shed from various cell types differs considerably. It is therefore expected that plasma from healthy donors will contain a wide range of exosomes with differentphenotypes, reflecting the phenotype of the cells that produced them.

In this study the newly developed technic of an Extracellular Vesicle (EV) Array was used for capturing, detecting and profiling exosomes in plasma from 80 healthy donors. The EV Array is based on the antibody capture of microvesicles and subsequent detection of the captured exosomes by biotin labeled anti-tetraspanin antibodies (CD9, CD63 and CD81). Antibodies against 21 different exosome biomarkers were used to capture the exosomes. The panel of antibodies contained the well-known exosome markers (CD9, CD63, CD81 and HLA-ABC), and 17 other membrane markers and antigens related to eg. cancer and inflammation.

Using the EV Array, it was possible to detect and profile exosomes for 21 analytes simultaneously using only 10 μl of plasma. In the cohort of 80 healthy donor the amount of exosomes varied from $5x10^8$ to $1.5 x10^9$ exosomes/mL plasma. The distribution of the 21 exosome markers varied greatly among the donors and was visualized using clustering analysis. The clustering clearly demonstrates that donors with a low amount of exosomes expresses exosomes with a higher percentage of the normal exosome markers (CD9 and CD81). This is in relation to donors with a high amount of exosomes that seems to have exosomes with a broader range of markers.

Keywords: exosomes, Extracellular Vesicle (EV) Array, plasma

POS-02-186 Extracellular Vesicle (EV) Array: Microarray Capturing of Exosomes and Other Extracellular Vesicles for Multiplexed Phenotyping

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Based on the technology of protein microarray, we hereby present a highly sensitive Extracellular Vesicle (EV) Array capable of detecting and phenotyping exosomes or other microvesicles from unpurified starting material in a high-throughput manner. The EV Array utilizes the possibilities to detect and profile microvesicles for 21individual surface exposed antigens simultaneously using only small amounts of starting material.

Exosomes are extracellular vesicles (40-100 nm) secreted by various cell types. The quantity and molecular composition of exosomes shed from different cell types differs considerably. Until now, the "gold standard" for quantification, characterization and phenotyping of exosomes is either by WB or FACS. These types of analyses requires considerable amounts of exosomal material (20-30 μg of protein derived from appr. 10^8 cells) and are only capabale of producing phenotypical data on one antigen per experiment. The EV Array exploits the potentials to detect and profile exosomes for 21 antigens simultaneously using unpurified exosomes from cell culture medium from 10^4 cells.

The EV Array is based on the antibody capture of microvesicles and subsequent detection of the captured subtypes of microvesicles by labeled anti-tetraspanin antibodies (CD9, CD63 and CD81 for exosomes). Antibodies used to capture these targeted exosome biomarkers are specific to membrane proteins for: exosomes in general (CD9, CD63, and CD81), and exosomes from cancer cells (EpCam, CD276) and 11 other membrane markers. Compared to NanoparticleTracking Analysis (NTA), EV Array revealed a higher specificity and sensitivity for exosomes, regardless of the pre-analytical circumstances as two-steps centrifugation or freezing/storage of the samples. Among others, the method was used to generate an extensive phenotyping of plasma-derived exosomes from 80 healthy donors.

Keywords: protein microarray, exosome, Extracellular Vesicle (EV) Array

POS-02-187 Multiplexed Analysis of Target Protein and Its Lys Acetylation Using Antibody Microarray

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Background: Lysine acetylation is a reversible and dynamic post translational modification of proteins and plays an important role in diverse biological processes.

Technological limitations have so far prevented comparative quantification of lysine acetylation in different samples.

Results: we developed a microarray with dual antibody sandwich immunoassay for detecting and quantifying acetylation of targeted protein by employing 3D aldehyde protein chip.

In this microarray, the individual protein is captured with protein specific antibody and detected with protein or acetyl antibodies.

BSA and acetylated BSA variations spiked in serum samples were measured in our study.

This method provides an efficient, quantitative and rapid detection of protein abundance and acetylation within targeted protein in parallel.

Conclusion: Here we established a method to detect levels of acetylation by microarray.

This acetyl microarray might be easily applied to large sample sets and would help to identify and validate disease associated acetylation alterations as biomarkers under both normal and pathological circumstances.

Keywords: microarray, acetylation, quantification

POS-02-188 Multiplexed Analysis of Target Protein and Lys Acetylation Using Antibody Microarray

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Background: Lysine acetylation is a reversible and dynamic post-translational modification of proteins and plays an important role in diverse biological processes. Technological limitations have so far prevented comparative quantification of lysine acetylation in different samples. **Results**: we developed a microarray with dual-antibody sandwich immunoassay for detecting and quantifying acetylation of targeted protein by employing 3D aldehyde protein chip. In this microarray, the individual protein is captured with protein-specific antibody and detected with protein or acetyl antibodies. BSA and acetylated BSA (BSA-Ac) variations spiked in serum samples were measured in our study. This method provides an efficient, quantitative and rapid detection of protein abundance and acetylation within targeted protein in parallel. **Conclusion**: Here we established a method to detect levels of acetylation by microarray. This acetyl microarray might be easily applied to large sample sets and would help to identify and validate disease-associated acetylation alterations as biomarkers under both normal and pathological circumstances.

Keywords: microarray, acetylation, quantification

POS-02-189 DNA Methylation Presents Distinct Binding Sites for Human Transcription Factors

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DNA methylation, especially CpG methylation at promoter regions, has been generally considered as a potent epigenetic modification that prohibits transcription factor (TF) recruitment, resulting in transcription suppression. Here, we used a protein microarray-based approach to systematically survey the entire human TF family and found numerous purified TFs with methylated CpG (mCpG)-dependent DNA-binding activities. Interestingly, some TFs exhibit specific binding activity to methylated and unmethylated DNA motifs of distinct sequences. To elucidate the underlying mechanism, we focused on Kruppel-like factor 4 (KLF4), and decoupled its mCpG- and CpG-binding activities via site-directed mutagenesis. Furthermore, KLF4 binds specific methylated or unmethylated motifs in human embryonic stem cells *in vivo*. Finally, functional analysis of KLF4 mutants revealed that its mCpG-binding activity is likely to play a role in neuronal cell differentiation. Our study suggests that mCpG-dependent TF binding activity is a widespread phenomenon and provides a new framework to understand the role and mechanism of TFs in epigenetic regulation of gene transcription.

Keywords: human protein microarray, DNA methylation, transcription factors

POS-02-190 Structural Proteomics of the Human Nucleoporin 107 Complex

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The nuclear pore complex is possibly the largest eukaryotic protein complex and its structure is presently understood only at a connectional level. Here we present an integrated structural proteomics approach to determine the structure of the major scaffolding component of the human nuclear pore, the Nup107 subcomplex. We have analyzed the entire subcomplex consisting of ten proteins by cross-linking mass spectrometry and single particle electron microscopy. We present a pseudo-atomic model that has implications for nuclear pore complex assembly and function.

Keywords: chemical cross-linking, nuclear pore complex, Nup107 subcomplex

POS-02-191 *In Vivo* Crosslinking Combined with Label-Free Quantitative Proteomics to Determine the Subcellular Distribution and Stoichiometry of Proteasome Complexes

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The proteasome is a large protein complex involved in the degradation of intracellular proteins. It thus plays a crucial role in the regulation of many cellular processes and in antigenic peptides presentation. A 20S core particle, that contains the catalytic activity, can be associated to one or two regulatory particles (RPs) of identical or different protein composition. Several RPs exist but their precise subcellular distribution remains to be determined. Here we present an efficient integrated workflow combining *in vivo* crosslinking with cell fractionation and an affinity purification-mass spectrometry strategy using the 20S core particule as a bait and label-free quantitative proteomics to determine the endogenous subcellular distribution of human proteasome complexes[1].
Labile proteins interactions of RPs with the 20S complex have been successfully stabilized by *in vivo* crosslinking using formaldehyde before cellular fractionation while maintaining proteasome activity. Label-free quantitative data were then acquired using a high sequencing speed, high resolution Orbitrap mass spectrometer and analyzed using the home-developped MFPaQ software. In two leukemic cell lines we showed that there was a high proportion of 20S complex not associated with RPs and that the 19S RP was the main associated activator in all cellular compartments (cytosol, microsomes, nucleus). This result was confirmed in total cell extracts of 7 various additional cell lines. However, the determination of the precise distribution of free and RPs associated 20S complexes revealed differences that could be correlated with variations in proteasome activity, including in fractions where proteasome complexes are present in low amounts (nucleus, microsomes). Moreover, this optimized workflow allowed to follow the dynamics and to highlight the specificity of RPs association with the 20S complex.

1. Fabre et al, Mol Cell Proteomics 2013, 12, 687

Keywords: protein complex, human proteasome, crosslink

POS-02-192 Elucidating the Unfolded Protein Response in GPCR Expressing Yeast Host

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G-protein coupled receptors (GPCRs) are a diverse class of therapeutically important proteins and are implicated in regulating nearly every aspect of our physiology. Expression of mammalian G-protein-coupled receptors (GPCRs) is a necessary step toward biophysical characterization and high-resolution structure determination. The Robinson lab has generated various yeast strains that express the human adenosine receptors, including A_2aR, hA_2bR and several chimeras. Previous results from our laboratory indicate that differential levels of protein expression are correlated to the retention of the immature form of the protein within the ER and Golgi complexes. Translocation and activation of UPR has been implicated as one of the critical limiting steps in the production of active GPCRs in *S. cerevisiae*. However, as of yet, the chaperones or other cellular proteins involved in facilitating GPCR production have not been identified. In the present study we aim to elucidate the molecular mechanism of the folding and translocation of the GPCRs in the eukaryotic host. Western blotting was used to compare the activation of various transcription regulators and membrane trafficking factors. Selected UPR indicators were probed by use of western blots. The results indicate that Aftiphillin, AFF4-ELF, and ER-GIC53 are upregulated in the expression of specific GPCRs. Their activation is dependent on the duration of the culture and incubation temperature. Interestingly, there appears to be a sequence dependent interaction of the recombinant protein with the molecular machinery that controls folding and translocation and UPR in the host.

POS-02-193 Molecular Mechanism Underlying IgSF11-Induced Cell Migration

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IgSF11 is a member of the immunoglobulin super family (IgSF) that possesses two immunoglobulin-domains, and is expressed ubiquitously in tissues such as brain, testis, kidney and others. IgSF11 was up-regulated in gastrointestinal and hepatocellular carcinomas, and its down regulation leads to lower grades of malignancy. In zebrafish, a couple of mutated IgSF11 genes hampered migration of melanophore and its survival *in vivo*. IgSF11 also enhanced cell migration on laminin coated dishes *in vitro*. To examine the molecular mechanism by which IgSF11 stimulated cell migration, we first transfected HEK293 cells and other with IgSF11 cDNA to compare cell adhesion, proliferation as well as cell migration. IgSF11 did induce cell migration, however it did not clearly enhance cell adhesion and proliferation. We examined genes expressed differently between transfectants and untransfectants using SILAC coupled to LC-MS/MS. More than 20 molecules including Filamins were lowered substantially, whereas only two molecules including HSP90 were up-regulated to some extent. We next focused on molecules such as Filamin a, Filamin b and HSP90, since these are shown involved in migration of neurons and some type of carcinoma. Immunoblot analysis revealed that Filamin a was lowered by 30-50%, some of which were phosphorylated. Several kinase inhibitors including RSK also showed the additive effect in terms of inactivation of Filamin a. These suggest that some part of IgSF11-induced signal transduction was mediated by Filamin a. We have been examining the possibility that the other molecules may be involved in the same or different type of cells concomitantly.

Keywords: Igsf11, silac, filamin

POS-02-194 Proteomics Approaches to Understand Molecular Basis of Mammalian Circadian Clock

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Many organisms show physiological and behavioral rhythms with a period of approximately 24 h. These rhythms are governed by the circadian clock based on a transcription/translation feedback loop. In mammals, bHLH-PAS transcription factors, CLOCK and BMAL1, bind to E-box elements during the daytime to activate transcription of Per and Cry genes. During the nighttime, translated PER and CRY interact with CLOCK-BMAL1 heterodimer and inhibit its transcriptional activity. The large protein complex including CLOCK-BMAL1 dimer is called "timesome". Although many reports show the importance of posttranscriptional modification of clock proteins for stabilizing circadian period, little is known about the dynamic change of timesome components that control circadian transcription cycle. Here we examined size variations of timesome throughout the day. When nuclear extracts of mouse livers were fractionated by gel filtration chromatography, most of CLOCK and BMAL1 proteins were detected as a large protein complex at the daytime (ZT6) and the nighttime (ZT18) in the fractions of 500-2000 kDa. PER and CRY proteins were also detected in the same fractions with CLOCK and BMAL1 at ZT18, but not at ZT6. These results suggest that components of timesome change dynamically across the day. Intriguingly, we also found that phosphorylated forms of CLOCK and PER2 were fractionated as a larger complex than that containing their unphosphorylated forms, suggesting that phosphorylation events of the clock proteins are regulated by changes in timesome components. In order to identify novel components of timesome, we purified timesome by gel filtration and ion exchange chromatography, and finally affinity purification using anti-CLOCK-antibody. A silver staining of purified timesome components showed purification of a number of proteins including CLOCK and BMAL1. These proteins thought to dynamically change their interactions to control circadian clock.

Keywords: circadian clock, protein complex, phosphorylation

POS-02-195 Mapping the Dynamic Interactome Through the Human Cell Cycle

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The cell cycle is a fundamental and dynamic biological process of eukaryotic cells where cyclin-CDK complexes trigger a series of events that includes DNA replication, mitosis and cytokinesis, ultimately resulting in a cell dividing into two identical copies. Recent studies have focused on a global perspective of the mammalian cell cycle from the view point of transcriptome, proteome, phosphoproteome and genome-wide knockdown. However, how proteins interact in the context of the cell cycle and how such protein-protein interaction governs the event are still elusive.

Recently we developed a high-throughput approach for measuring temporal changes in the interactome using quantitative proteomics and size exclusion chromatography (SEC) (AR Kristensen et al., Nat. Methods 2012). Although SEC itself has been demonstrated as a useful tool for characterizing protein complexes as each component of a protein complex co-elute together, we further extended the approach in combination with SILAC-protein correlation profiling for proteome-wide identification of the protein complexes responded to EGF.

Here we applied this approach to profile human cell cycle interactome in a global, high-throughput manner. We show a quantitative interactome map for about 1,500 proteins throughout the four major cell cycle phages (G1-S-G2-M). This study reveals the dynamic rearrangement of the protein complexes, including MCM replication complex and elongation factors, during the cell cycle. Further, we will discuss how gene expression is retained during the mitosis through a mitosis-specific interaction of a small phosphatase and RNA polymerase II.

Keywords: protein-protein interaction, cell cycle

POS-02-196 A Novel Mechanism of Keratin Cytoskeleton Organization Through Casein Kinase I Alpha and FAM83H in Colorectal Cancer: Interactome Analysis of FAM83H

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Keratin filaments form cytoskeletal networks in epithelial cells. Dynamic rearrangement of keratin filament networks is required for epithelial cells to perform cellular processes such as cell migration and polarization; however, the mechanism governing keratin filament rearrangement remains unclear. Here, we found a novel mechanism of keratin cytoskeleton organization mediated by casein kinase Iα (CK-1α) and a newly identified keratin-associated protein, FAM83H. FAM83H knockdown induces keratin filament bundling, whereas FAM83H overexpression disassembles keratin filaments, suggesting that FAM83H regulates the filamentous state of keratins. Intriguingly, keratin filament bundling is concomitant with the dissociation of CK-1α from keratin filaments, while aberrant speckle-like localization of CK-1α is observed concomitantly with keratin filament disassembly. Furthermore, CK-1α inhibition, like FAK83H knockdown, causes keratin filament bundling and reverses keratin filament disassembly induced by FAM83H overexpression, suggesting that CK-1α mediates FAM83H-dependent reorganization of keratin filaments. Since the N-terminal region of FAM83H interacts with CK-1α, whereas the C-terminal region interacts with keratins, FAM83H might tether CK-1α to keratins. Colorectal cancer tissue also shows keratin filament disassembly accompanied with FAM83H overexpression and aberrant CK-1α localization, and FAM83H-overexpressing cancer cells exhibit loss or alteration of epithelial cell polarity. Importantly, FAM83H knockdown inhibits cell migration accompanied by keratin cytoskeleton rearrangement in colorectal cancer cells. These results suggest that keratin cytoskeleton organization is regulated by FAM83H-mediated recruitment of CK-1α to keratins, and that keratin filament disassembly caused by FAM83H overexpression and aberrant localization of CK-1α may contribute to the progression of colorectal cancer.
Keywords: interactome, cancer, keratin

POS-02-197 Protease Resistant Soy Proteins Interact with the Cell Surface Proteins of an Intestinal Cell Line, Caco-2, and Change the Cell Signaling

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Soy has been known to affect on regulation of food intake and cholesterol-lowering. There are many studies that some substances in soy, such as dietary proteins or isoflavones have effects on these functions. However, it is still clearly not known that which materials do what.
According to our previous study, the soluble soy proteins were not readily digested by proteases, such as pepsin and pancreatine, in the *in vitro* digestion analysis. Structural analysis by homology search suggested that the protease resistant proteins have very compact beta-sheeted structures and a domain sharing similar amino acid sequence. So, we named these proteins as PRFPs (protease-resistant food proteins), and postulated that PRFP can interact with the mucosa membrane on the villi or microvilli in the small intestine to trigger the biological activities mentioned above.
Biotinylated soy PRFP binding to the small intestine of mice with photo-affinity crosslinking and affinity purification with an avidin-agarose bead, let us isolate several promising cell surface proteins. Those proteins were identified by MS/MS analysis.
In order to find the changes in cell signaling in human intestinal cell line, Caco-2, by treating with soy PRFPs, phosphoproteins in the cells were separated on 2-D gels and stained with Pro-Q-Diamond reagent. Differentially expressed spots compared to control (treated with PBS instead of soy PRFPs) were identified with mass spectrometry. Interestingly, many of the proteins have ATP-binding functions.
For further studies, detailed cell signaling pathway/mechanism by soy PRFPs treatment to the cells are under investigation in view of revealing the function of soy proteins in satiety, cholesterol-lowering, and others.
Keywords: protease resistant protein, intestinal membrane protein, cell signaling

POS-02-198 Interactions between SAP155 and FUSE-Binding Protein-Interacting Repressor Bridges c-Myc and P27Kip1 Expression Revealed by GeLC-MS Approach

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c-Myc plays a critical role in cell proliferation, tumorigenesis, apoptosis, and cell cycling, but its precise mechanisms remain largely unknown. p27Kip1 (P27) arrests cells in G1, and SAP155, a subunit of the essential splicing factor 3b (SF3b) subcomplex in the spliceosome, is required for proper P27 pre-mRNA splicing. FUSE-binding protein-interacting repressor (FIR) is a *c-myc* transcriptional suppressor that suppresses TFIIH/P89/XPB (P89) DNA helicase and is alternatively spliced in colorectal cancer lacking the transcriptional repression domain within exon 2 (FIRΔexon2). FIR and FIRΔexon2 form a homo- or hetero-dimer that complexes with SAP155. Our study indicates that the FIR/FIRΔexon2/SAP155 interaction bridges *c-myc* and P27 expression. Knockdown of FIR/FIRΔexon2 or SAP155 reduced P27 expression, inhibited its pre-mRNA splicing, and reduced cyclin-dependent kinase (cdk)2/cyclinE expression. Moreover, spliceostatin A (SSA), a natural SF3b inhibitor, markedly inhibited P27 expression by disrupting its pre-mRNA splicing and reduced cdk2/cyclinE expression. The expression of P89, another FIR target, was ncreased in excised human colorectal cancer tissues. Knockdown of FIR by siRNA reduced P89; however, the effects on P27 and P89 expression are not simply or directly related to altered FIR expression levels, indicating that the mechanical or physical interaction of SAP155/FIR/FIRΔexon2 complex is potentially essential for sustained expression of both P89 and P27. Together, the interaction between SAP155 and FIR/FIRΔexon2 potentially integrates cell-cycle progression and *c-myc* transcription by modifying P27 and P89 expression. Our results indicate that enhanced FIR/FIRΔexon2/SAP155 interaction is pivotal for cancer development and differentiation; therefore, the interaction is a potent target for cancer screening and treatment.
Keywords: c-myc, alternatice splicing, protein interaction

POS-02-199 Dynamic Long-Range Chromatin Control of *Klf4* Transcription by IRF8 in Monocyte Differentiation

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Monocytes regulate host defenses, inflammation, and tissue homeostasis. We have recently shown by employing a genome-wide approach and that the IRF8-KLF4 transcription factor cascade is essential for driving myeloid progenitors to differentiate into inflammatory monocytes. IRF8 binds to multiple sites at the large (120 kb) distal region located 208 kb upstream of the *Klf4* TSS. Yet, how IRF8, presumably via this large distal region, controls *Klf4* transcription remains to be understood. In this study, we performed detailed time-course chromatin immunoprecipitation (ChIP) assays for various histone modifications at the *Klf4* gene locus following IRF8 induction. Upon IRF8 binding to the distal region, the partner transcription factor PU.1 was rapidly recruited, concomitant with the induction of an enhancer chromatin signature H3K4me1. This was followed by the induction of H3K4me3 at the *Klf4* TSS, suggesting the onset of transcription initiation. Interestingly, at the distal region, the "active" enhancer mark H3K27Ac was induced only after the H3K4me3 at the TSS. Then, productive *Klf4* transcription finally occurred concomitant with the elongation signature H3K36me3 at the gene body. These results suggest that IRF8 binding to the distal region may trigger a long-range interaction between the distal enhancer and the promoter regions of the *Klf4* gene, which causes bi-directional, dynamic effects on each other. We plan to confirm this chromatin interaction, and to perform ChIP for RNA polymerase II. Furthermore, we are currently purifying IRF8 protein complexes to identify critical components involved in the above process.
Keywords: myeloid differentiation, chromatin, transcription machinery

POS-02-200 Analysis of Intact Macromolecular Assemblies on a Bench Top Orbitrap MS System

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Intact proteins are routinely measured using ESI-MS instrumentation under acidic, denaturing conditions, destroying large, noncovalent protein assemblies and substrate bound complexes. Under native conditions, fully active protein assemblies can be studied, but these experiments are challenging due to the limited surface area of protein complexes for protonation at physhiological pH. Ion signals are shifted to higher m/z values, which until recently, only TOF instruments were capable of detecting. TOF measurements have limited achievable resolution, making it difficult to resolve specific isoforms or substrate complexes. With a modified bench top Orbitrap instrument we were able to detect ion signals up to m/z 25,000 with high mass resolving power thus achieving remarkable signal distribution and precision for various large protein assemblies. Thermo Scientific Exactive Plus™ mass spectrometer (Bremen, Germany) with a modified pre-amplifier and automated, electronically adjustable gas supply for HCD/CLT gas, was used to measure various proteins and noncovalent protein complexes. The samples were introduced using either a Thermo Scientific nanospray source or an Advion TriVersa NanoMate. 1-10 μM solutions of IgG, phosphorylase b, pyruvate kinase, and GroEL were analyzed.

Different glycoforms of the IgG were baseline-resolved, allowing their accurate assignment. For the phosphorylase b and pyruvate kinase samples a large number of isoforms were detected for their tetramers. While smaller proteins like IgG can be easily analysed under normal conditions, for larger protein complexes like GroEL, mild collision activation in the HCD cell had to be used to ensure complete desolvation in order to determine the accurate mass of the protein complex. Efficient HCD fragmentation of the GroEL complex could be performed by changing the collision gas from nitrogen to xenon when GroEL 13-mer ions up to m/z 25,000 were detected.

Keywords: intact proteins benchtop Orbitrap

POS-02-201 The Monitoring of Protein Complex Dynamics in Response on Apoptosis Using PCP-SILAC

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Apoptosis is an essential, irreversible process within multicellular organisms. Although observed as a discrete phenomenon the molecular mechanism responsible for this ordered decommissioning of cell involves numerous proteins acting to initiate and inhibit apoptotic activity continuously within the cell. This balancing of factors ultimately governs the sensitivity of cells to apoptosis and is mediated largely by protein-protein interactions. Due to the multitude of the numerous pro and anti- apoptotic systems within a cell previous studies aimed at understanding apoptosis have typically monitor apoptosis by studying a limited set of proteins at a time. By monitoring the global changes in protein interactions that result in response to apoptosis a greater understanding of the how these systems coordinate their pro and anti-apoptotic properties could be gained. Thus to both understand and monitor the interactions that govern apoptosis we have sort to undertake a global analysis of the initiation of apoptosis using size exclusion chromatography coupled to protein correlation profiling SILAC (SEC-PCP-SILAC) [1]. This technique allows the assessment of both protein complexes composition and dynamic allowing changes associated with a given stimuli to be monitoring in a high-throughput manner. Using this technology we have examined the initiation of apoptosis of Jurkat cells in response to stimulation of the CD95 signaling pathways. By examining this pathway we have been able to monitor the assembly and remodeling of protein complexes in response to CD95 activation preceding the activation the executive caspases demonstrating the potential of SEC-PCP-SILAC in the study of protein complex dynamics.

1. Kristensen AR, Gsponer J, Foster LJ (2012) A high-throughput approach for measuring temporal changes in the interactome. Nat Methods. 9(9):907-9

Keywords: PCP SILAC, protein complexes

POS-02-202 Structural Analyses for the Effects of Ets1 Phosphorylation on Ets1-Containing TF-DNA Assemblies

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Chemical modifications of transcription factors (TFs) alters the TFs' behaviors on target gene enhancers and thereby changes a transcriptional profile in a cell. Many studies revealed chemical modification of a TF activates or inactivates the modified TF itself. However, gene activation is regulated through complexes comprised of multiple TFs and enhancer DNA, and thus effects of TF's chemical modification on transcriptional regulation need to be understood in the context of higher-order TFs-DNA complexes. To reveal molecular mechanism by which TF's modification regulates TF-DNA complex formation, we focused on Ets1 as a representative TF. Ets1, which plays important roles in hematopoiesis, binds cooperatively to target enhancers with partner TFs, and its DNA binding activity is suppressed by its phosphorylation. Here we show that, upon phosphorylation of Ets1, many of Ets1-containing TF-DNA complexes would dissociate, and some of them would be retained, depending on the partner TFs in the enhancer-specific TF-DNA complexes. In addition, we performed crystallographic analyses of complexes composed of Ets1, Runx1, CBFβ and DNA derived from the *TCR α* gene enhancer, and compared it with the previously reported structures of other Ets1-contatining TF-DNA complexes. From these results, we concluded that allosteric regulation among Ets1, Runx1 and DNA would play a central role in Runx1-dependent counteraction of the inhibitory effect of Ets1 phosphorylation on DNA binding activity of Ets1.

Keywords: transcription factor, allosteric regulation, phosphorylation

POS-02-203 Isolating, Characterizing, and Monitoring in Real Time Intracellular Epigenetic Protein Complexes

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The combinatorial complexity of DNA and histone modifications coupled with the multiple classes of proteins modifying or recognizing modifications creates a necessity for methodologies which can efficiently discern and study these events both *in vitro* and *in vivo*. Here we present examples of recent research on two classes of epigenetic proteins, the TET family members and the bromodomain readers, with use of HaloTag technology alone or in combination with other approaches for each. To determine interacting partners and further understanding of human TET2 and TET3 proteins, HaloTag complex isolations were performed, uncovering *O*-GlcNAc Transferase (OGT) as the primary binding partner. Further studies confirmed this interaction and genome-wide mapping revealed co-localization of TET and OGT at CpG-rich promoter regions. In additional efforts to measure the *in vivo* binding of proteins to chromatin, we focused on bromodomain interactions with histones. To this means a bioluminescent resonance energy transfer (BRET) assay was designed utilizing a NanoLuc-BRD4 protein and fluorescently labeled nucleosomes that have incorporated H3.3 or H4-HaloTag in HCT116 cells. Treatment with the BET family inhibitor, JQ1, shows specific disruption of BRD4-histone interaction with expected IC50 values, and as control, not with other non-BET family members. Taken all together, the multiple capabilities of these approaches create a powerful means to study multi-protein complexes, discover new interactions, and characterize chromatin binding events in live cells.

Keywords: protein complex, HaloTag, TET, OGT

POS-02-204 Molecular Dynamics Approach to Dynamical Protein Interaction

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We apply the random matrix theory in the mathematical physics to analyze the dynamical inter and intra protein interactions. The method is an advanced version of the well-known principle component analysis which have been used to analyze the dynamical motion of macromolecules. The random matrix theory enables us to extract the dynamical correlation among atoms and we can identify the correlation sectors and classify them as protein domains. The time series data of protein motion are produced by the all-atom molecular dynamics simulations with solvent. The variance-covariance matrices are constructed from the time series data for the different time intervals, 0.01fs, 0.1fs, 1fs, 10fs, 100fs, 1ps, and 10ps with the maximum duration of 1000ns to capture the characteristic dynamical motion of protein in the different time scales. We calculate the probability density of the matrices, eigenvalue distribution, unfolded eigenvalue distribution of the nearest-neighbor and the next nearest-neighbor level spacings, etc. They are the fundamental quantities which characterize the universality class in the random matrix theory. Throughout the results of different time scales, we find that the unfolded level spacings agree well with a universality class in the random matrix theory. On the other hand, the raw eigenvalue distribution has a crossover behavior between the universal and non-universal classes. Following the random matrix theory, we study the inverse participation ratio and classify the correlation sectors of proteins as a "dynamical domains" of proteins.

Keywords: molecular dynamics, protein interaction, protein domain

POS-02-205 Identification of a Novel Binding Partner of the Cell Polarity-Regulating Kinase, PAR-1

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Proteomic analysis of binding partners of the interested protein provides a powerful tool to decipher physiological functions of the protein. Here, we report the successful identification of a novel binding partner of the cell polarity regulating kinase, PAR-1b/MARK2 through this approach. To identify molecules that cooperate with PAR-1 for epithelial polarity, we established a stable MDCK epithelial cell line in which endogenous PAR-1b/MARK2 was replaced by streptavidin-binding peptide (SBP)-tagged human PAR-1b/MARK2 expressed at an almost endogenous level. This strategy enabled us to identify several proteins that were specifically copurified with PAR-1b. Mass spectrometric analysis of the possible PAR-1-binding proteins revealed an uncharacterized protein with central coiled-coil motif, which we named MARKAP1 (Mkp1). The subsequent biochemical and cell biological analyses demonstrated that Mkp1 is a novel microtubule (MT) crosslinking protein with two MT-binding regions at its N- and C-terminus. Interestingly, siRNA-mediated depletion of Mkp1 demonstrated that it is indispensablel for the development of non-centrosomal MTs essential for cell polarity, such as the Golgi-nucleated MTs in undifferentiated cells and the lateral MT bundles running along apico-basal axis of epithelial cells. Since PAR-1 is a kinase essential for maintaining dynamic state of MTs, the present results suggest the cooperative roles of Mkp1 with PAR-1 to control the dynamic stability of the non-centrosomal MT bundles. We will also report aberrant phenotypes of the Mkp1 gene-trap mice, which indicate a possibility that these mice will be model mice of a human disease.

Keywords: cell polarity, noncentrosomal, microtubule

POS-02-206 System-Wide Characterization of Temporal Dynamics of Macromolecular Protein Complexes during Oncogene-Induced Cell Transformation

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The majority of human cancers are caused by the malignant transformation of epithelial cells, one of the most frequently occurring malignancies being colorectal carcinoma (CRC). Activating mutations of the oncogene *BRAF* contribute decisively to the development of carcinomas. Thus, *BRAF* represents the most frequently mutated kinase gene in human tumors and is consequently exploited for therapeutic interventions. We hypothesized that oncogenic cell transformation leads to global alterations in protein-protein interactions driving tumor development. To address this we used a CRC model system and studied direct BRAF-dependent as well as global alterations in protein interactions upon oncogene expression. (1) Combining affinity purification with quantitative proteomics differences between BRAF interaction partners of normal and a constitutive active mutant were identified. (2) To further expand our search we applied a newly developed approach for high-throughput screening of temporal changes in the interactome, providing a platform for identification of altered complex formation of proteins downstream of BRAF. (3) Finally, we performed also experiments to identify regulation of phosphotyrosine dependent interactions. Using this panel of different approaches we were able to identify several thousand protein-protein interactions, including many that demonstrate differences between the wild type and oncogenic form of BRAF. This global, temporal investigation of the BRAF interactome elucidates new players in malignant cell transformation that represent potential new therapeutic targets.

Keywords: CRC, braf, interactome

POS-02-208 Charting Non-Sequence Specific Nucleic Acid-Protein Interactions in Human Cells

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Interactions between proteins and nucleic acids (NAs) play a pivotal role in a wide variety of essential biological processes. Transcription factors that recognize specific DNA motifs only constitute part of the NA-binding proteins (NABPs). In this study, we present the first large-scale effort to systematically map human NABPs with generic classes of nucleic acids. Using 25 carefully designed synthetic DNA and RNA oligonucleotides as baits and affinity purification mass spectrometry (AP-MS), we performed pulldowns in three cell lines that yielded 10,000+ protein-NA interactions and involved 900+ proteins. We found 139 novel NABPs and provided first experimental evidence for another 98. We determined significant affinity preferences for NA subtypes for 219 distinct proteins.

Successful validation of 10/11 chosen new affinity preferences confirmed among others the affinity of YB-1 for methylated cytosine. YB-1 is over-expressed in tumors and is associated with multiple drug resistance. Network analysis of YB-1 ChIP-seq peak nearest genes identified a subnetwork of 73 genes strongly associated with cancer pathways, thereby suggesting a potential epigenetic role of YB-1 in resistant tumors.

We could also show that non sequence specific proteins binding DNA do interact with nucleic acid chains through an interface that is more constraint in its geometry than proteins binding mRNA, which are known to contain more disordered regions.

To extend the experimental data we undertook a machine learning approach to derive a method of automatically inferring nucleic acid binding. We employed a family of support vector machines (SVMs) to predict NA binding *de novo*.

Keywords: nucleic acid binding protein, bioinformatics, systems biology

POS-02-209 Obtaining Interactome Map by Sifting the Collection of MS-Data

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Size of the human interactome is estimated at 650 000 interactions [Stumpf et al, 2008], but the proportion of known interactions is relatively small [Shoemaker et al., 2007]. Thus there is a continued need for the development and improving of methods for detecting and analysis protein-protein interactions (PPIs). Processing of large-scale collections of mass-spectrometry data can be used to obtain the additional supportive evidences in analysis of PPIs. We used the public MS repository The Global Proteome Machine Database to screen the PPIs by virtual co-immunoprecipitation method [Zhang et al, 2011]. The putative partner proteins were selected by frequency of co-occurrence with baits within the same experimental dataset. As baits were used proteins, coded by human chormosome 18. The results of analysis were represented as Cytoscape graph, where nodes were assigned to partner proteins and interconnections were established according to the number chromosome 18 encoded proteins shared between the nodes. For each prey protein of chromosome 18 the frequency of occurrence was calculated using the sampled MS-experiments. At the threshold rate above 1% of experiments, there were 130 "baits" (out of 277 genes of chromosome 18) having at least on interactor. The clusters on the Cytoscape graph corresponded to the 5 sets of chromosome 18 genes, which were annotated by the Gene Ontology.

Keywords: protein-protein interaction, MS-experiments, virtual co-immunoprecipitation

POS-02-210 A High-Resolution Quantitative BN-MS Approach for Comprehensive Analysis of Protein Complexes and Stoichiometries

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Blue-native gel electrophoresis (BN) is a powerful method for size-based separation of solubilized protein complexes. Combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) and, more recently, clustering algorithms, it allows for unbiased identification of protein complexes and new subunits as demonstrated for a number of model systems. However, the current BN-MS approaches do not make use of the full resolution nor the quantification potential offered by BN separation and mass spectrometry. Here, we present a new method combining cryo-microtome slicing of BN gels to obtain large series of well-defined gel slices (adjustable step size from 0.1-0.5 mm) and high-resolution LC-MS/MS analysis. To extract reliable protein profiles for clustering of protein populations from large amounts of highly complex MS datasets, we developed a stringent workflow involving m/z, retention time and intensity calibration, elimination of MS assignment errors as well as identification of marker complexes. Application of this approach to a mitochondrial reference sample demonstrated a high degree of comprehensiveness and accuracy, effectively resolving complexes differing in size by less than 10%. We finally analyzed solubilized membranes from total rat brain in the range of 0.3 to 1.5 MDa and identified more than 50 protein complexes associated with ion channels and receptors. Using concatenated peptide standards (QConCAT) we determined the core complex stoichiometries of several class III G protein-coupled receptors. In summary, the combination of native gel separation, high resolution sampling, advanced data processing and abundance calibration is a powerful approach for comprehensive identification and quantification of protein complexes.

Keywords: Blue native PAGE, liquid chromatography coupled mass spectrometry, protein complex stoichiometry

POS-02-211 Structural Study of Proteins C3 and C3b by Time Resolved Hydroxyl Radical Footprinting

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The complement system is the major component of innate immunity. The central complement protein, C3, and its activated opsonin fragment, C3b, play a major role in all three pathways of complement activation, and are crucial for an efficient immune response. Inappropriate activation and regulation of complement may lead to several autoimmune diseases; thus knowing the structure of C3/C3b will allow us to understand complement activity.
Hydroxyl radical protein footprinting followed by high-resolution mass spectrometry is an emerging method for identifying the dynamics of ligand dependent conformational changes, mapping interfaces and evaluating protein structure. Hydroxyl radicals (OH°), generated from Fenton's reagent, radiolysis or photolysis of water, provide high resolution structural information as they have solvent properties similar to water. We have evaluated two light sources to obtain OH° by radiolysis: linear accelerator ALIENOR (CEA Saclay) delivering high-energy X-rays, and synchrotron beamline Metrology (Synchrotron SOLEIL, Gif sur Yvette). Hydroxyl radicals react with solvent-accessible side chains of proteins, leading to mass shifts useful to study the accessibility of that site. Identification of modified peptides and evaluation of mass increases in the millisecond timescale by liquid chromatography coupled with mass spectrometry allow tracking of the regions implicated in conformational changes during protein complex formation.
Hydroxyl radical footprinting is an alternative to conventional bio-structural methods such as X-ray crystallography, hydrogen-deuterium exchange or electron microscopy, which do not allow the study of dynamic conformational changes. Furthermore, it is a robust and powerful technique for identification of protein interfaces and rearrangements, regardless of protein size.

Keywords: protein footprinting, C3b, mass spectrometry

POS-02-212 Human Cell Growth Regulator LYAR is Highly Expressed in Many Tumors and Accelerates Ribosome Biogenesis

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Mouse Ly-1 antibody reactive clone (Lyar) is a nucleolar protein, and its elevated expression level contributes to tumor formation in nude mice and maintains undifferentiated state of embryonic stem cells. However, cellular function of Lyar remains undetermined. In this study, we show that human homolog of Lyar (LYAR) plays an important role in ribosome biogenesis. We show that LYAR is present in the nucleolus, pre-ribosome fraction prepared by cell fractionation, and in pre-40S/pre-60S/pre-90S particles separated by sucrose gradient ultracentrifugation. We also show that LYAR has roles in both processing and transcription of 47S pre-rRNA. Proteomic analysis of the LYAR-associated proteins isolated from the nuclear extract of human cells identifies no less than 20 factors, which are involved in transcription. In addition, we show that the knockdown of LYAR slows 47S pre-rRNA transcription and cell proliferation, whereas overexpression of LYAR accelerates those. Finally, we show that the mRNA of LYAR is expressed at high levels in many tumors including those of colon, and lung cancers. Based on these results, we propose that some tumors and embryonic stem cells accelerate ribosome biogenesis by up-regulating the expression of LYAR and promote their cell growth and proliferation

Keywords: ribosome biogenesis, transcription, RNA processing

POS-02-213 Defining the Structure of Mitotic Chromosomes Using Multi-Classifier Combinatorial Proteomics Together with DT40 Genetics

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Packaging of DNA into condensed chromosomes during mitosis is essential for the faithful segregation of the genome into daughter nuclei. Although studied for over 100 years, mitotic chromosome structure and composition is yet to be fully elucidated. A novel approach called multi-classifier combinatorial proteomics (MCCP) has revealed the protein composition of mitotic chromosomes purified from chicken DT40 cells. One of the main advantages of MCCP is that it can be combined with SILAC to quantitatively compare chromosomal proteomes from different genetic knock-out cell lines. Here, we apply this method to compare chromosomal proteomes in the presence or absence of individual SMC complexes; key components that have been shown to play a crucial role in mitotic chromosome structure. While SMC1/3 and SMC2/4 form part of Cohesin and Condensin, respectively, the SMC5/6 complex is thought to be involved in DNA repair. Mitotic chromosomes were isolated from conditional genetic knockouts of Scc1, SMC2 or SMC5 cultured under SILAC conditions. Using quantitative Mass Spectrometry we were then able to rank >3,000 proteins based on whether their chromosomal association was dependent on individual SMC complex components. MCCP with using machine learning, Random Forest, will enable the first attempt at mapping the entire proteome regulation networks associated with SMC complexes, which in turn will explain how these complexes are coordinating chromosome structure.

Keywords: mitosis, chromosome, SMC

POS-02-214 Trypanosome Proteome

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The single celled protozoan parasite *Trypanosoma brucei* that causes African sleeping sickness has unique mechanisms for survival in the mammalian host and tsetse fly vector. In mammalian bloodstream trypanosomes evade the immune system by antigenic variation of their dense variant surface glycoprotein coat, whereas efficient infection of the tsetse vector depends on the expression of two distinct forms of another glycoprotein procyclin. The bloodstream form (BF) cells rely on glycolysis alone for ATP production, whereas insect-stage procyclic forms (PF) possess elaborate mitochondria for energy metabolism. In this study, *T. brucei* BF cells were analyzed by mass spectrometry, and approximately 70% of the predicted proteome was identified. Furthermore, using iTRAQ labeling and LC-MS/MS analysis we determined the relative abundance of a large sub-set of proteins between BF and PF *T. brucei* cells. The overall trends of these proteins based on predicted functional roles correlate well with biological processes in this organism, e.g. highly abundant surface glycoproteins in BF, and higher abundance of many mitochondrial proteins including those associated with respiratory pathway in PF, and glycosomal proteins in BF. Additionally, numerous proteins unique to trypanosomes were identified that were preferentially expressed in one of the life cycle stages. Sub-cellular location of selected candidate proteins was determined by tagging and immunofluorescence, and their functional association by affinity purification and LC-MS/MS analyses, with a focus in RNA processing pathways. Three putative RNA binding complexes were identified and characterized, including a novel complex that is required for cell growth. The results add to the knowledge base towards understanding Trypanosoma life-cycle regulations.

Keywords: infectious disease

POS-02-215 The Role of Nucleolar Protein Nop52 in the pre-rRNA Processing during Human Ribosome Biogenesis by Proteomic Approach

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Ribosome biogenesis, which takes place in the nucleolus, starts with the transcription of a large ribosomal RNA precursor (47S pre-rRNA in human cells), which soon combines with numerous factors to form 90S pre-ribosomal particle (pre-90S). After splitting into 40S and 60S pre-ribosomal particles (pre-40S and pre-60S, respectively), each pre-ribosomal particle undergoes maturation steps in nucleoplasm to produce small (40S) and large (60S) ribosome subunits. We recently reported that novel ribosome biogenesis factor p32 is cooperated with fibrillarin (a component of pre-90S) and Nop52 (a component of pre-60S) during the events that split pre-90S into pre-40S and pre-60S (*Mol Cell Proteomics, 2011, M110.006148*). In this study, we attempted a proteomic analysis of Nop52-associated pre-ribosomal particles to examine a detailed role of Nop52 in ribosome biogenesis.
We first purified Nop52-associated pre-ribosomal particles in the nuclear extract prepared from inducible FLAG-Nop52 expression 293 cells, characterized those using mass-based proteomic technologies, and showed that Nop52 was associated with a number of proteins involved in pre-rRNA processing. We also showed that the knockdown of Nop52 with a siRNA caused an accumulation of 47S pre-rRNA in pre-90S and 32S pre-rRNA in pre-60S. In addition, we showed that depletion of Nop52 inhibited cell growth of both HeLa and MCF7 cells. These results suggest that unlike Rrp1p which is a yeast homolog of Nop52 and is involved only in late steps of yeast ribosome biogenesis, Nop52 is involved in both early and late steps of human ribosome biogenesis.

Keywords: human ribosome biogenesis, pre-ribosomal particles

POS-02-216 A New Approach to Identify RNA-Protein Interactions Using a Conditional CRISPR Nuclease

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RNA-binding proteins control the fate and function of the transcriptome in all cells. Here we present technology for isolating RNA-protein partners efficiently and accurately using an engineered CRISPR endoribonuclease. An inactive version of the Csy4 nuclease binds irreversibly to transcripts engineered with a 16-nt hairpin sequence at their 5' ends. Once immobilized by Csy4 on a solid support contaminating proteins and other molecules can be removed by extensive washing. Upon addition of imidazole, Csy4 is activated to cleave the RNA, removing the hairpin tag and releasing the native transcript along with its specifically bound protein partners. This conditional Csy4 enzyme enables recovery of specific RNA-binding partners with minimal false-positive contamination. Following isolation of the RNA Binding Proteins, a modified Filter Aided Sample Preparation (FASP) methodology is used that utilized trifluorethanol (TFE) for efficient protein denaturation and solublization, as well as dual LysC, Trypsin digestion. This method has been adapted to a 96 well plate format for high throughput automation. The benefit of the FASP approach in this workflow is that it allows efficient clearance of buffers, detergents and small RNA fragments that might otherwise interfere with the subsequent MS analysis. The LC-MS/MS analysis was carried out with a Agilent HPLC-Chip QTOF System. We use this method, coupled with quantitative MS, to identify RNA-specific and cell type-specific human pre-microRNA-binding proteins. We also show that this technology is suitable for analyzing diverse size transcripts, and that it is suitable for adaptation to a high-throughput discovery format.

Keywords: RNA, interactions, mass spectrometry

POS-02-217 Mapping of Cross-Links in Complex Protein Samples Introduced by Bis (Succinimidyl) -3-Azidomethyl Glutarate

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Knowledge about the folding, mutual interactions and dynamics of composing subunits is required to comprehend structures and functions of protein complexes in living cells. Chemical cross-linking of proximate amino residues coupled with mass spectrometric analysis to identify the protease-generated cross-linked peptides is a promising approach in this endeavor. Here we explore the use of bis (succinimidyl) -3-azidomethyl glutarate (BAMG) for determining the maximal distances of proximate lysine residues. TCEP-induced reduction of the azido group in the spacer enables effective isolation of cross-linked peptides by diagonal strong cation chromatography and renders the two amide bonds formed during cross-linking labile in the gas phase, allowing determination of the masses of connected peptides. These features form the basis of a method for efficient mapping of cross-links in complex samples by peptide fragment mass fingerprinting from large protein databases. Reduction of the azido group renders the two cross-link amide bonds also cleavable by collision-induced dissociation, enabling cross-link identification by a second approach. Our analytical strategy is demonstrated with a HeLa cell nuclear extract by identification of > 250 cross-linked peptides from the human protein database at a false discovery rate < 0.4%. Novel interactions have been discovered giving rise to new hypothesis about biological regulation worth to be explored further.

Keywords: chemical-cross-linking, protein-protein interaction, BAMG

POS-02-219 Structural Analysis of Histone Multimers and NCPs by Ion Mobility Mass Spectrometry

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The minimum structural unit of chromatin is the nucleosome core particle (NCP) composed of 146 bp DNA wrapped around a histone octamer containing two H2A/H2B dimers and one $(H3/H4)_2$ tetramer, which possess functionally important tail regions that are intrinsically disordered. In order to unveil the mechanism of NCP assembly and disassembly processes, which are highly related to gene expression, structural characterization of the histone multimers should be of importance. In the present study, human NCP (200 kDa), histone H2A/H2B dimer (28 kDa) and $(H3/H4)_2$ tetramer (55 kDa) were prepared with recombinant histone proteins and characterized by electrospray ionization ion mobility mass spectrometry (ESI-IM-MS). To obtain the structural evidence for the observed collision cross section values in IM-MS, MD simulation was carried out for the histone multimers. Furthermore, synthetic peptides with sequences of the flexible tail regions were analyzed by IM-MS and MD simulation. These experiments showed that two levels of structural collapse of the histone tails in the gas phase were identified for the histone multimers by IM-MS and MD simulation while the reconstituted NCP demonstrated a single collision cross section value. Discussion focusing on the behavior of the disordered tail regions will be carried out in the paper.

Keywords: histone, ion mobility mass spectrometry

POS-02-218 Top-Down Proteomic Analysis of Enzymes and Complexes in *Trichoderma* Fungal Secretomes

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Biomass degrading enzymes produced by fungi must be understood in relation to their structural and functional properties in order to become fully usable for biotechnological applications, including biofuel production. Recently, we showed that *Trichoderma harzianum* and *Trichoderma reesei* secretomes contain cellulases and hemicellulases assembled as multienzymatic complexes as revealed by blue native-PAGE and LC-MS/MS bottom-up techniques. Herein, we report on a novel use of top-down proteomics for the identification of intact proteins and native complexes in the *Trichoderma reesei* secretome. The sample was collected after nine days of growth on sugarcane waste, and separated using SDS-PAGE and BN-PAGE followed by electroelution. Fractions were processed using methanol/chloroform/water precipitation prior to nano-LC analysis. Top-down mass spectra were collected on an Orbitrap Elite mass spectrometer with fragmentation performed by ETD and HCD. Proteins were identified using ProSightPC 3.0. Data from SDS-PAGE coupled to denatured electroelution yielded top-down MS identification of 531 proteoforms within 95 different protein accession numbers so far. Current work is aimed at applying blue native PAGE coupled to native electroelution, followed by top down LC-MS/MS to identify the protein subunits of high molecular weight complexes present in the *T. reesei* secretome.

Keywords: top-down proteomics, trichoderma secretome, complexes

POS-02-220 Genome-Wide Mass Spectrometry-Based RNA Analysis Reveals a Novel snRNA Metabolic Pathway in Human Cell

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RNA works together with proteins to form ribonucleoprotein (RNP) complexes in many cellular processes. The elucidation of the cooperative actions between protein and RNA is important to understand biological systems. Post-transcriptional or metabolic modifications of RNA play vital roles in those cooperative actions with proteins. We have developed a technology for direct analysis of RNA using mass spectrometry that allows unbiased identification of RNA and has great advantage in high throughput analysis to acquire information of post-transcriptional RNA modification. In this study, we applied this technology to a ribonucleoproteomic analysis of snRNP biogenesis driven by SMN1-complexes. SMN1 is a product of the SMN1 gene that is the causative gene of spinal muscular atrophy (SMA) characterized by the loss of lower motor neurons and atrophy of muscle, and is an incurable autosomal recessive disease. It is involved in the biogenesis of small nuclear ribonucleoprotein (snRNP) complexes, building blocks of splicing machinery (spliceosome), and in the biogenesis of mRNA transport complexes. Our present analyses reveal the presence of a short form of U1 snRNA (short U1) as a novel component of SMN1 complexes. Short U1 lacks Sm protein-binding region and stem loop 4 and has the mono-methylated cap structure at the 5'-end of the U1 snRNA molecule. We also show evidence that short U1 is formed dependently on transcription of the U1 snRNA genes, and is eliminated with SMN1 complexes from the regular pathway of U1 snRNP biogenesis through P bodies, in which RNA surveillance and decay take place. We will discuss a possibility that the deficiency of SMN1 causes aberrant metabolism of short U1 in SMA. In many diseases, especially neurological diseases, RNA metabolisms are disturbed, thus the technology for direct RNA analysis used in this study may be very useful for understanding the pathogenesis of those diseases.

Keywords: ribonucleoproteomics, snRNP biogenesis, quality control

POS-02-221 Proteomic Identification of Profilin1 as a Co-Repressor of Estrogen Receptor Alpha in MCF7 Breast Cancer Cells

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Nuclear receptor coregulators play an important role in the transcriptional regulation of nuclear receptors. Here, we aimed to identify Estrogen Receptor α (ERα) interacting proteins in Tamoxifen treated MCF7 cells. Using *in vitro* GST-Pull down assay with ERα ligand binding domain (ERα-LBD) and mass spectrometry based proteomics approach we identified Profilin1 as a novel ERα interacting protein. Profilin1 contains I/LXX/L/H/I amino acid signature motif required for co-repressor interaction with ERα. We show that these two proteins physically interact with each other both *in vitro* as well as *in vivo* by GST-pull down and co-immunoprecipitation respectively. We further show that these two proteins also co-localize together in the nucleus. Previous studies have reported reduced expression of Profilin1 in breast cancer; here we found that Tamoxifen enhances Profilin1 expression in MCF7 cells. Our data demonstrates that over expression of Profilin1 inhibits ERα mediated transcriptional activation as well as its downstream target genes in ERα positive breast cancer cells MCF7. In addition, Profilin1 over expression in MCF7 cells leads to inhibition of cell proliferation which apparently is due to enhanced apoptosis. In nutshell, these data indicate that mass spectrometry based proteomics approach identifies a novel ERα interacting protein Profilin1 which serves as a putative co-repressor of ERα functions.

Keywords: breast cancer, profilin1, estrogen receptor alpha

POS-02-222 Analysis of Plk1-Bora Interaction Using the ProteoChip System

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We investigated the interaction between Polo-Box Domain (PBD) of polo-like kinase 1 (Plk1) and Bora peptide based on protein-protein interaction using the ProteoChip (Proteogen, Korea) technology. Plk1 protein conjugated with glutathione-S-transferase (GST) was bound to anti-GST antibody immobilized on the surface of the Proteochip, after which Cy5-labeled Bora peptide was interacted with the Plk1 protein on the chip in a dose-dependent manner. A chemical compound E8 was screened as a novel inhibitor against the Plk1-Bora interaction using the ProteoChip. The compound significantly inhibited NCI-H460 (lung cancer) cell proliferation *in vitro* in a dose-dependent manner. The ProteoChip -based Plk1-Bora binding assay system will serve a novel promising tool which is applicable for screening of novel inhibitors against Plk1, regardless of the enzyme activity.

Keywords: Plk1, protein-protein interaction, ProteoChip

POS-02-223 Current Status of the Human DREAM/ Calsenilin/KChIP3 Interactome: Focus on ProtoArrays for the Screening of Novel Protein Interactions

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The human calsenilin, is a neuronal protein originally identified as a Ca^{2+}-binding protein. Shortly thereafter, this protein was found to be identical to the Ca^{2+}-dependent gene silencer DREAM and, later, to one of the interacting proteins (KChIPs)- or ß-subunits- of the voltage-gated Kv channels, KChIP3. The three descriptions correspond to the same protein, which localizes to the three different cellular compartments (membrane, cytoplasm, and nucleus). In these three cellular locations DREAM plays different roles (K^+ channel, Ca^{2+} binding protein and transcriptional repressor, respectively). Thus, it is foreseeable that the DREAM interactome significally varies depending on sub-cellular localization or the presence /absecen of Ca^{2+}. In this report, we reviewed published information on DREAM interactors, retrieved information using three different web-based platforms (PSICQUIC, DASMI, and BIPS) and screened for novel interactions using proto-array technology. As a result, previously published and novel information on DREAM interactome is tabulated in this report, which constitutes the deepest DREAM interactome currently reported. This working set of protein screened served as a "bona-fide" "training-set" for future improvements of protein-protein prediction algorithms.

Keywords: protein interactions, protein arrays

POS-02-224 Mapping the Human Methyltransferasome Reveals the Existence of a Posttranslational Modification Code That Targets Molecular Chaperones to Regulate Functional Organization of the Human Proteome

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In efforts aimed at identifying and characterizing protein substrates and regulators of all known and putative human methyltransferases (the methyltransferasome) using multiple cell compartment affinity purification coupled with mass spectrometry (MCC-AP-MS), our laboratory has discovered a novel family of lysine methyltransferases that preferentially target and regulate molecular chaperones. Our results indicate that methylation of molecular chaperones VCP by METTL21D and Hsp70 by METTL21A regulates the activity of both chaperones. In the case of VCP, trimethylation of lysine 315 by METTL21D is stimulated by the addition of the UBX cofactor ASPSCR1, which we show directly interacts with the methyltransferase. This stimulatory effect was lost when we used VCP mutants (R155H, R159G and R191Q) known to cause Inclusion Body Myopathy with Paget's disease of bone and Fronto-temporal Dementia (IBMPFD) and/or some familial forms of Amyotrophic Lateral Sclerosis (ALS). Lysine 315 falls in proximity to the Walker B motif of VCP's first ATPase/D1 domain. Our results indicate that methylation of this site negatively impacts the ATPase activity of this molecular chaperone. In the case of Hsp70, methylation of lysine 561 by METTL21A was shown to decrease formation of stress granules in response to arsenite treatment, a model that is widely used to study improper protein folding in degenerative disorders. In addition to having implications for the development of therapeutics for degenerative neuromuscular disorders such as ALS and IBMPFD, the discovery of a family of chaperone-targeting methyltransferases led us to propose the existence of a chaperone posttranslational modification code, that we termed the chaperone code, which is at play to orchestrate the proper folding and assembly of protein complexes that make up the human proteome. We will report on our most recent results relating to decryption of the chaperone code and its role in diseases.

Keywords: protein-protein interactions, methylation, molecular chaperones

POS-02-225 Function Through Interaction: Identification and Characterisation of SOCS4 and 5 SH2 Domain Targets

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The Suppressor of Cytokine Signaling (SOCS) family of proteins play critical roles in the regulation of cytokine and growth factor signaling, typified by their involvement in the JAK/STAT pathway. Growing evidence now points towards a role for SOCS5 as a tumor suppressor in the context of epidermal growth factor (EGF) driven malignancies, and SOCS36E, the *Drosophila* homologue of SOCS4 and 5, negatively regulates EGF receptor signaling *in vivo*. The SOCS proteins contain a central Src-homology 2 (SH2) domain, which binds to specific phosphotyrosyl motifs on target proteins, thus determining the signaling cascades negatively regulated by individual SOCS proteins. SOCS4 and SOCS5 share over 90% sequence homology across their SH2 domain, highlighting the potential for functional redundancy and they remain the least well-characterized SOCS family members. To identify candidate SOCS4 and SOCS5 SH2 targets, recombinant GST-tagged SOCS4-SH2 domain was used to probe T cell lysates and affinity-purified samples were eluted and subjected to tryptic digestion on a solid phase SCX resin, prior to analysis on an LTQ-Orbitrap mass spectrometer. The adapter protein Shc1 was identified as a specific target, and we have characterized the functionally important Y317 as a high affinity phosphotyrosine-binding motif for SOCS4 and SOCS5 (~0.16 μM K_D). Shc1 recruits the Grb2-Sos complex to activate MAPK downstream from a number of receptor complexes, including the epidermal growth factor receptor (EGFR). Interaction with Shc1pY317 places SOCS5 in the EGFR signaling cascade and the physiological relevance is currently being investigated in *Socs5*$^{-/-}$ mice.

Keywords: SOCS, SH2, EGF

POS-02-226 Fill-in-the-Blanks on the List of Players in the Histone Code: Limitations of Conventional Pull-Down Proteomics and Efforts to Overcome the Difficulties

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Histone PTMs have crucial roles in various cellular processes, epigenomic inheritance, and diseases. Today, huge numbers of novel histone modifications are being discovered by mass spectrometry, while their writer/eraser/reader proteins remain largely still elusive. We have been analysing histone H4 PTM dynamics in cell cycle by LC/MS/MS, wherein we observed some modifications which were written/erased around M phase. Currently we are focusing on identification of the partner proteins of those PTMs. Our strategy to find them is basically a pull-down approach. There are some problems with pull-down experiments: i) non-specific binding and ii) difficulty in distinguishing direct binders from indirect ones. To eliminate non-specific binders and indirect ones, crosslink-MS is emerging as a promising technique. Here we present our efforts to establish a label-free crosslink-MS method to capture novel epigenetic players, and discuss what parameters are important in optimizing the experiments.

Keywords: epigenome, label-Free, crosslink-MS

POS-02-227 Proteomic Profiling of the IL-3 Receptor Complex and Signal Transduction in Leukemia

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A component of the Interleukin-3 (IL-3) receptor, CD123, is highly over-expressed in leukemic stem cells, a population of cells capable of self-renewal, that are resistant to drug treatment and are the principal cause of disease relapse in Acute Myeloid Leukemia (AML). IL-3 is a member of a family of cytokines critical for the regulation of cell survival, proliferation and differentiation in normal hematopoiesis and inflammation. In our mouse models of AML, constitutive IL-3 signalling together with the expression of oncogenes that block differentiation have been demonstrated to be essential for leukemogenesis. Our data makes a compelling case that previously unrecognised features of the IL-3 receptor are critical for initiation and specificity of signal transduction. We have also recently identified that the Ikappa B kinase (IKK) complex is activated downstream of the IL-3 receptor, however, the mechanism by which IKK is activated is not clear. To identify novel signalling components we have employed a novel Tandem Affinity Purification approach by generating a tagged IL-3 ligand which is used to specifically purify the endogenous, activated IL-3 receptor complex for analysis by mass spectrometry. Using this unbiased approach we have identified novel proteins recruited to, and post-translational modifications of the receptor, including phosphorylation and ubiquitination. We are now establishing the role of these novel IL-3 receptor regulatory elements using *in vitro* and *in vivo* models, which will provide crucial insights into the pathogenesis and potential future treatment strategies of leukemia.

Keywords: Interleukin-3, immunoprecipitation, ubiquitin, signalling

POS-02-228 Cell-Type Specific Stoichiometries of the Human Nuclear Pore Complex

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To understand the structure and function of large molecular machines, accurate knowledge of their stoichiometry is essential. We developed an integrated targeted proteomics and super-resolution microscopy approach to determine the absolute stoichiometry of the human nuclear pore complex (NPC), possibly the largest eukaryotic protein complex. We show that the human NPC has a previously unanticipated stoichiometry that varies across cancer cell types, tissues and in disease. Using large-scale proteomics, we provide evidence that more than one third of the known, well-defined nuclear protein complexes display a similar cell type-specific variation of their subunit stoichiometry. Our data point to compositional rearrangement as a widespread mechanism for adapting the functions of molecular machines toward cell type-specific constraints and context-dependent needs, and highlight the need of deeper investigation of such structural variants.

Keywords: targeted proteomics, super-resolution microscopy, protein complex-based analysis

POS-02-229 Role of CYLD Deubiquitinase in EGF Signaling Pathway

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The tumor suppressor for human cylindromatosis, CYLD, is an ubiquitiously expressed deubiquitinase that specifically hydrolyzes K63-linked polyubiquitin chains. CYLD was initially described as an inhibitor of the TNF-activated NFkB pathway through deubiquitination and subsequent inactivation of key adaptor molecules such as NEMO, TRAF2, TRAF6 or Bcl-3. Accordingly, CYLD down-regulation has been reported in several types of tumors including lung, liver or colon cancer, whereas cyld-/- mice show increased susceptibility to develop colon or skin tumors. Nevertheless, recent evidences suggest its spectrum of biological activities is not limited to the NFkB pathways. Our earlier studies revealed that CYLD could be involved in the signaling cascade initiated by the epidermal growth factor (EGF), as it was found to be strongly tyrosine phosphorylated upon EGF stimulation. The engagement of CYLD in EGF signaling has also been documented in several very recent studies. However, the functional meaning and impact of the novel role attributed to CYLD still needs to be elucidated. In order to decipher the implication of CYLD in EGF signaling, we applied SILAC-based quantitative proteomics to compare the phosphoproteome as well as the CYLD-interacting protein network in wild type- and CYLD- silenced cells. These unbiased analyses may provide better understanding of the role of the CYLD deubiquitinase in the signal transduction initiated by EGF.

Keywords: EGF signaling, CYLD deubiquitinase, quantitative proteomics

POS-02-230 Interactome-Wide Analysis Identifies End-Binding Protein 1 as a Crucial Component for the Speck-Like Particle Formation of Activated AIM2 Inflammasomes

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Inflammasomes are cytoplasmic receptors that can recognize intracellular pathogens or danger signals and are critical for IL-1β production. Although several key components of inflammasome activation have been identified, there has not been a systematic analysis of the protein components found in the stimulated complex. In this study, we used the isobaric tags for relative and absolute quantification (iTRAQ) approach to systemically analyze the interactomes of the NLRP3, AIM2 and RIG-I inflammasomes in nasopharyngeal carcinoma (NPC) cells treated with specific stimuli of these interactomes (H_2O_2, poly (dA:dT) and EBER, respectively). We identified a number of proteins that appeared to be involved in the interactomes and also could be precipitated with anti-ASC antibodies after stimulation. Among them, EB1 was an interacting component in all three interactomes. Silencing of EB1 expression by small interfering RNA inhibited the activation of the three inflammasomes, as indicated by reduced levels of IL-1β secretion. We confirmed that EB1 directly interacted with AIM2 and ASC *in vitro* and *in vivo*. Most importantly, fluorescence confocal microscopy showed that EB1 was required for formation of the speck-like particles that represent activation of the AIM2 inflammasome. In NPC tissues, IHC staining showed that EB1 expression was elevated and significantly correlated with AIM2 and ASC expression in NPC tumor cells. In sum, we profiled the interactome components of three inflammasomes and show for the first time that EB1 is crucial for the speck-like particle formation that represents activated inflammasomes.

Keywords: inflammasome, interactome, nasopharyngeal carcinoma

POS-02-231 Investigation of Time Dependent Competitive Protein Adsorption to Surfaces Using Mass Spectrometry

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Introduction
Surfaces in a complex protein solution will adsorb proteins. This event is fast and dynamic and triggers a biological response against the inserted/implanted biomaterial that eventually will lead to biofouling and encapsulation. This affects the properties of the inserted devices, such as hampered membrane functions of microdialysis (MD) probes or distortion in response of biosensors.

Methods
Untreated and coated filtration membranes were used as adsorption templates for human ventricular cerebrospinal fluid (vCSF). After adsorption in an incubation chamber, the membranes were washed, dried and the proteins were reduced, alkylated and digested. The sample preparation procedure was conducted according to an on-surface enzymatic digestion (oSED) protocol previously described by our group. The oSED digests were analyzed by nanoLC ESI-MS/MS using a 7T hybrid LTQ FT and Velos pro orbitrap mass spectrometer.

Preliminary Data
In this study, we present a time resolved map of protein adsorption. Non-coated and tri-block polymer coated, polycarbonate membranes was used as templates. As expected, a time and surface property dependent protein adsorption relationship was observed. It is not surprising that the degree of protein binding onto modified and non-modified surfaces was dependent on the properties of the protein as well as the properties of the surface. The process of biofouling for *in vivo* inserted materials can be postponed and thereby increasing the lifetime and use of e.g. microdialysis probes for patient monitoring. The preliminary data are very promising making it possible to identify a spectra of adsorbed proteins on different surfaces in a time dependent way.

Keywords: competitive, surface, adsorption

POS-02-232 An Efficient Affinity Purification Method for Interaction Proteomics in Mammalian Cells

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Macromolecualr protein complex assemblies are critical to exercise their cellular functions. Therefore, the identification of protein-complex components are an important step in the resolving the molecular process of the analyzing proteins. Recent mass spectrometry-based proteomic analysis have been proven to be successful in identification of multi -protein complex components. Target protein specific antibodies have been used to isolate protein complex. In addition, affinity tags technologies also adapted to purify protein complex. Small antigen polypeptides which have specific antibodies and polypeptides with high affinity target molecules are widely used. Here we describe a strategy in which the SBP (streptavidin binding peptide) -tag based protein complex purification is combined RNA interference (RNAi) to exclude the competition from corresponding endogenous proteins. We identified that SBP is a best affinity tag among Flag-epitope-tag, HA-epitope-tag, CBP (Calmodulin-binding peptide), GST (glutathione S-transferase) and SBP in our analysis in mammalian cells. In addition, we found that reducing the bait protein expression to less than endogenous protein increased the efficiency of the identification of macromolecular complex. Since transient over production of the affinity-tagged-bait protein gave us the degradation products of bait protein and chaperon proteins, such as HSP70, after SDS-PAGE. Furthermore, the knockdown of corresponding endogenous protein also improved the efficiency of the affinity purified macromolecular protein complex.

Keywords: protein complex, SBP, immuno-depletion

POS-02-233 Quantifying the Dynamics of a 14-3-3 Protein Interaction Network by Affinity Purification and SWATH Mass Spectrometry

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Protein complexes and protein interaction networks are essential mediators of most biological functions. Complexes supporting transient functions such as signal transduction processes are frequently subject to dynamic remodeling. Currently, the majority of studies into the composition of protein complexes are carried out by affinity purification and mass spectrometry and present a static view of the system. To move toward a better understanding of inherently dynamic biological processes, methods which can reliably quantify temporal changes of protein interaction networks are essential. In this study we determined the capability of affinity purification combined with SWATH mass spectrometry (AP-SWATH) to quantify the reorganization of protein-protein interactions in time resolved perturbation experiments. We chose to study the dynamics of the 14-3-3β scaffold protein interactome after stimulation of the insulin/PI3K/AKT pathway. The analysis, performed using the open source software OpenSWATH, provided a complete quantitative data matrix highlighting patterns of dynamic regulation in 567 14-3-3β interacting proteins with respect to IGF1 stimulation. Quantitative changes in 14-3-3β interacting proteins clustered in to 5 distinct time profiles, 2 of which were strongly related to the activity of basophilic kinases such as AKT. To our knowledge this study represents the largest reported interactome for a single bait indicating that at least 2.8 % of the proteome is engaged by 14-3-3β containing scaffold dimers, and that a substantial portion of these are regulated after IGF1 stimulation. We therefore establish AP-SWATH as a tool to sensitively quantify dynamic changes in protein complexes and interaction networks in perturbed systems.

Keywords: quantitative interaction proteomics, data independent analysis, systems biology

POS-02-234 Proteomic Analysis of Alpha Synuclein-Containing Inclusions in Neurodegenerative Disorders

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Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB) and Multiple System Atrophy (MSA) are known as α-synucleinopathies and characterised by the hallmark α-synuclein-containing protein inclusions known as Lewy bodies (LBs) in affected neurons in PD and DLB, and Glial Cytoplasmic Inclusions (GCIs) in affected oligodendrocytes in MSA. Detailed curations of their protein components, crucial for understanding their pathogenesis, are yet to be achieved due to difficulties in obtaining pure inclusions in sufficient quantities. One hypothesis for the inclusion pathogenesis is that damaged proteins, unable to be degraded due to defects in protein degradation systems, are selectively assembled into inclusions via vesicle-mediated transport and serves as a protective mechanism. This study developed a more robust method for LB and GCI purifications which increased yield by 28-fold compared to previous methods. 2D-DIGE showed a 3.8-fold increase in α-synuclein (the major LB/GCI protein) and a corresponding 5.2-fold reduction in tubulin contamination. Purified GCIs (5 MSA cases examined individually) or LBs (2 DLB cases examined individually) were separated by 1D and 2D electrophoresis and analysed by nanospray Orbitrap mass spectrometry. We identified 160 proteins in GCIs in at least 4 out of the 5 MSA cases, 21% of them were vesicle-related proteins, and 112 proteins in LBs from both DLB cases with 25% of proteins being vesicle-related. Most the identified proteins are shared in both LBs and GCIs. The relative predominance of vesicle trafficking proteins in the inclusions supports the hypothesis that toxic α-synuclein is targeted to LBs and GCIs via vesicle trafficking mechanism.

Keywords: neurodegeneration, Parkinson's disease, lewy body

POS-02-235 Serum Peptide Profiles as a Candidate Biomarker for Dementia with Lewy Bodies

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Dementia with Lewy bodies (DLB) is a progressive neuropsychiatric disease, which is clinically characterized by parkinsonism and dementia in the senile stage. For early and accurate diagnosis of DLB, especially for differentiation of DLB from Alzheimer's disease (AD), it is needed to find a convenient biomarker for DLB. To achieve this, we comprehensively detected serum peptides from 30 DLB patients, 30 AD patients, and 30 healthy control (HC) subjects by mass spectrometry. Intensity of the detected peptides was subjected to orthogonal partial least square-discriminant analysis (OPLS-DA, Simca-P+) for the discriminations between the DLB and AD groups and between the DLB and HC groups. As a result, a total of 127 peptides were detected. In the DLB group compared to the AD group, ion intensity of 12 peptides was significantly increased to more than 1.2 folds, whereas ion intensity of 5 peptides was significantly decreased to less than 1/1.2 folds ($p<0.05$). In the DLB group compared to the HC group, ion intensity of 19 peptides was significantly increased to more than 1.2 folds, whereas ion intensity of 5 peptides was significantly decreased to less than 1/1.2 folds ($p<0.05$). The OPLS-DA results completely discriminated the DLB group from the AD group (R^2, 0.891; Q^2, 0.537), and also from the HC group (R^2, 0.816; Q^2, 0.522). Taken together, serum peptide profiles may be a candidate biomarker for the diagnosis of DLB, which facilitate the differential diagnosis between DLB and AD.

Keywords: dementia with Lewy bodies, biomarkers, serum peptides

POS-02-236 Proteomic Changes of Rat Lens during Cataractogenesis: Therapeutic Effect of Antioxidant SkQ1

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Cataract is one of the leading causes of impairment and loss of vision of elderly people all over the world. The process of cataract development includes PTMs of crystallins - major lens proteins. PTMs accumulate during aging and may cause protein aggregation which results in the loss of protein functions. Another possible reason of cataractogenesis is the decreased exchange of small molecules between inner and outer region of the lens. At present it is not completely clear which PTMs are cataract-specific and which are just the result of normal aging process. Moreover, there is no evident information about the changes in levels of important amino acids and antioxidants during cataract formation. Investigation of cataract development on human lenses is complicated by the lack of experimental material - normal human lenses are difficult to obtain. The most appropriate way is to use the biological models. Recent studies have shown that the OXYS rat strain meets the main requirements for the model of senile cataract. The senescence-accelerated OXYS strain of rats was developed from Wistar stock by selection for their susceptibility to the cataractogenic effect of galactose.

This report provides the data on the age and cataract-dependent proteomic analysis of two selected rat strains. All proteins were identified by TOF/TOF-MS analysis and the relative abundances of water-soluble and water-insoluble proteins were measured. The main crystallin PTMs were identified for the 12-week-old OXYS rat. The therapeutic effect of antioxidant SkQ1 and its derivatives was analyzed on OXYS rats by measuring the α-crystallin chaperone activity with the relevance to the age and the stage of cataract of the selected rats.

We thank: RFBR projects 11-04-00143, 11-03-00296 and 12-04-31244, Government of the Russia (grants 11.G34.31.0045 and 8094), and the President of the Russia (grant NSh-2429.2012.3).

Keywords: crystallins, PTMs, aging

POS-02-237 Proteomic and Functional Knockdown Study of the APP Binding Protein FE65 Shows a Down-Regulation of Proteins Involved in DNA Replication (Potential Relevance to Alzheimer's Disease)

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Alzheimer's disease (AD) research gives conclusive hints that cell cycle re-entry might contribute to a central and causative hallmark in AD. Neuronal cell re-entry into the cell cycle and DNA damage are described to result in apoptosis - a central event in neurodegenerative diseases. Our work provides evidence for the underlying mechanism including two prominent proteins. Initially, we identified both proteins as differentially abundant in a proteomic study comparing a stable FE65-knockdown cell line with respective controls. However, a set of proliferation assays in this work revealed that FE65-knockdown cells demonstrate significantly a reduced DNA replication and less cell growth. Derived from these experiments we hypothesize that elevated nuclear FE65 levels cause a cell cycle re-entry mediated by the interaction and abundance of our protein candidates. Various confirmation experiments, co-immunofluorescence and a FE65 interaction study using human brain lysates and human cell culture revealed that elevated nuclear levels of FE65, which have been shown by others to be present in AD brain neurons, result in a stabilization of one of our identified candidates in nuclear mobile spheres. These spheres are able to grow and fuse, and potentially correspond to the nuclear domain 10. The findings from this knockdown study result in a putative pathway which might be highly relevant for AD.

Keywords: Alzheimer disease, APP binding protein FE65

POS-02-238 Glycoproteomics Study of Plasma Glycoproteins in Japanese Semisuper Centenarians

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Aging can be considered as a result of interactions among genetic, environmental and lifestyle factors, which influence longevity. Proteins, such as enzymes, structural components, mediating and modulating cell adhesion and signaling components, etc., play important roles in many biological events, and also influence longevity through their functions. The functions of proteins are regulated by various post-translational modifications such as glycosylation. Thus, we performed glycoproteomic analyses to elucidate the difference in the plasma glycoprotein profiles of Japanese semisuper centenarians (SSCs). Such a study will lead to the discovery of glycoproteins and the change of glycosylation related to healthy aging. We prepared the plasma samples from SSCs (106-107 years), aged controls (70-74 years), and young controls (20-38 years), and performed glycoproteomics analyses using lectin microarray (GP Bioscience). Binding profiles to several lectins were altered in the plasma glycoproteins of SSCs compared with other control groups. Furthermore, lectin-binding profiles of plasma glycoproteins were compared between three groups using lectin blot of 2D-PAGE (Auto 2D, Sharp). The binding to ECA (*Erythrina cristagalli* agglutinin), PHA-L (*Phaseolus vulgaris* leucoagglutinin), and BPL (*Bauhinia purpurea* lectin) were increased in the plasma glycoproteins of SSCs. These results suggest that the complex *N*-glycans of plasma glycoproteins may be increased in SSCs and that the change of *N*-glycosylation processing may associate with aging.

Keywords: semisuper centenarians, glycoprotein, lectin microarray

POS-02-239 Proteomic Analysis of Glycation and Glycosylation in a Diabetic Model Otsuka Long-Evans Tokushima Fatty (OLETF) Rat Using a Novel Boronate Affinity 2-DE

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Methacrylamide phenylboronic acid (MPBA) acts as a glycoprotein binding tag compound. Recently, a novel proteomic tool: MPBA-SDS-PAGE method was developed by Morais et al (Proteomics 2010). We applied this method for the second dimension of the agarose 2-DE especially for high-molecular-mass proteins. The MPBA 2-DE patterns showed different profiles from those of the agarose 2-DE without MPBA. Many protein spots, including actin and mitochondrial ATPase beta chain, in kidney of the diabetic OLETF rats, had vertically elongated shapes and stained with Pro-Q Emerald, indicating most of these proteins were glycated or glycosylated. Chronological studies of carbohydrate modifications in kidney proteins of the diabetic OLETF and its control LETO rats were simultaneously done in a MPBA-2DE gel. Surprisingly, a new actin spot, with molecular mass lower than that of the original actin spot, appeared in 20-week-old OLETF kidney, whereas not in the 20-week-old LETO kidney. Moreover, the protein amount of the new spot increased in the 40-week-old OLETF rat. The results indicate that kidney actin changed its affinity to MPBA with ageing in the OLETF rat. The MPBA 2-DE would provide a means toward clarifying a comprehensive view of carbohydrate modifications in proteins during a long progression of ageing and age-related chronic diseases such as diabetes.

Keywords: diabetes, affinity electrophoresis, glycation

POS-02-240 Label Free Quantitative Proteomic Analysis of Phosphoprotein and Glycoprotein of Aged Mouse Muscle Tissue

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Sarcopenia can be defined as the age-related loss of muscle mass, strength and function. The disease causes serious problems of health. The onset of sarcopeina related to old age is untill now traced to unclear cause. In this study, it focused on differentially expressed PTM proteins. This PTM is thus involved in many diseases, interpreting localization and assessment of extent of phosphorylation and glycosylation of major scientific interest. The animal model are six groups of C57BL/6J female mice, control and drug treated 8months (young control), 23months (normal aging and sarcopenia model), 30months (complete sarcopenia model). Proteome were extracted from three kinds of muscle, type 1 muscle of soleus (SOL), type 2 muscle of extensor digitorum longus (EDL) and type 3 muscle of gastrocnemius (GA). Phosphopeptides were enriched by TiO_2 cartridge. Glycopeptides were captured by hydrazide beads. Same sample were run by triplicated MS/MS analysis for label free quantification. And then, using IDEAL-Q of label free quantification, results were cut off by two-fold changed expression level. Based on aging related muscle proteins, these differentially expressed proteins were analyzed by Ingeunity of interactive pathway analysis software. In EDL of old mice, it was decrease of glycolysis. In process network analysis, aging related with SOL and EDL were commonly changed muscle of contraction, development and actin filaments process. Specific protein related with glycolysis is enolase beta (Eno3). The specific protein was decreased in 23/30 months mice of EDL but, it was increased in SOL group. Tropomyosin alpha-1(Tpm1) is important protein of muscle contraction. In contrast, the protein was increased in 23/30 months mice of EDL, but it was decreased in SOL group. Increase of Eno3 is related with muscle tissue of damage. Decrease of Tmp1 is related with cause of muscle disease. Therefore, this study was obtained important of Eno3 and Tmp1 in aging related with sarcopeina.

Keywords: aging, PTM , pathway analysis

POS-02-241 Absolute Quantitation of Beta-Amyloid Peptide and Alpha-Synuclein Protein in Animal Models of Neurodegenerative Diseases Using Selected Reaction Monitoring

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Beta-amyloid (abeta) oligomers have been reported to account for the cognitive decline in Alzheimer's disease (AD) and alpha-synuclein (asyn) oligomers are believed to be the primary toxic species in Parkinson's disease (PD). Here we report the new observation that abeta aggregation is associated with the generation of a typical peptide fragment signature as a result of spontaneous peptide hydrolysis in the N-terminal region. The biological significance of our finding is highlighted by the fact that identical peptide fragment fingerprints were identified in transgenic mice and human post-mortem AD brain extracts using mass spectrometry. This information is crucial for monitoring and targeting protein aggregation per se, as well as sets a new benchmark for the absolute quantitation of abeta as an indicator of AD pathology. To elucidate the mechanisms of protein aggregation in AD and PD pathogenesis it is important to develop sensitive assays to monitor key regulatory triggers and biomarkers before the development of pathological symptoms. In clinical diagnostics, analytical specificity and inter-laboratory reproducibility requires the development of robust analytical methods for biomarker validation. Selected Reaction Monitoring (SRM) has evolved as an indispensable analytical tool allowing for unambiguous quantitation of proteins in a complex matrix. In this work we present a SRM assay for the absolute quantification of abeta and asyn, while focusing on relevant post-translational modifications associated with disease pathogenesis.

Keywords: Alzheimer's disease, Parkinson's disease, SRM

POS-02-242 Acetylated Proteins Involved in Resistance to EGFR Tyrosine Kinase Inhibitors in Non-Small Cells Lung Cancer

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Current targeted therapies prescribed in non-small cell lung cancer (NSCLC) such as gefitinib, are inhibitors of the epidermal growth factor receptor tyrosine kinase activity (EGFR-TKI). As most patients developed resistances, many efforts were made to understand the resistance mechanisms and were focused mainly on EGFR signalling, especially on EGFR gene mutations. In addition, some studies showed that these resistances could be related to post-translational modifications (PTMs) such as acetylation. Thus, this study aimed to identify the acetylated proteins involved in the mechanism of resistance to apoptosis in NSCLC cell line resistant to EGFR-TKI, such as H358. To achieve this goal, we used iTRAQ quantitative proteomics approach to determine the acetylated proteins, which were over- or under-expressed depending on the apoptotic response observed after EGFR-TKI treatment. The effect of histone deacetylase inhibitors, such as Trichostatin A (TSA), will be also investigated. Practically, H358 cells, in presence or not of TSA, were treated or not with gefitinib for 96h. Apoptosis assays were performed by a flow cytometry analysis of active caspase-3 (immunostaining). Proteomics approach was carried out by performing trypsin digestion on extracted proteins before labelling the resulting peptides by iTRAQ reagent. Then, acetylated peptides were enriched by immuno-precipitation against acetylated peptides before fractionating them by nano-HPLC and analyzing them by MALDI TOF/TOF in MS & MS/MS modes. The data were analyzed by Protein Pilot software in an iTRAQ relative quantitation approach. By this way, some acetylated proteins were identified to be subjected to variations depending on the treatment with HDAC inhibitors and gefitinib. These proteins could be involved in the resistance mechanism to gefinitib in NSCLC, and therefore, could represent new targets to overcome this resistance.

Keywords: EGFR-TKI resistance, proteomics, acetylation

POS-02-243 Quantitative Analysis of the Phosphorylation State of a Hybrid Histidine Kinase in Bacterial Two-Component System by Using Phosphate-Affinity SDS-PAGE

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Protein phosphorylation at the residue of His or Asp plays an important role in the signal pathway of bacteria. The post-translational modification has been developed as a means of the adaptation to external stimuli, which is referred to as "two-component system". Each two-component system consists of the histidine kinase (HK) and response regulator (RR). When a certain external environment changes, the signal pathway progresses as follows: i) an HK on the outer membrane senses the change, ii) the HK autophosphorylates at the His residue, iii) the HK transfers the phosphate group to the Asp residue of the cognate RR, iv) the phosphorylated RR binds to the genome because of regulating the gene expression required for the adaptation. Some hybrid-type HKs show intramolecular phosphotransfer of His-Asp-His as the priming event for the activation of RR. However, characterization of the phosphospecies involved in the system has been technically challenging. The quantitative analysis of His- or Asp-phosphoprotein is limited due to the chemical instability.
We have reported a novel type of phosphate-affinity SDS-PAGE (Phos-tag SDS-PAGE) for the mobility shift detection of phosphoproteins. The technique, which uses a polyacrylamide-bound Phos-tag, has been widely used in determining the phosphorylation state of many proteins. By using Phos-tag SDS-PAGE, we tried to visualize the intramolecular phosphotransfer for the characterization of His- and Asp-phosphoproteins, which are unstable in nature, in *Escherichia coli*. In this presentation, we discuss the kinetics of hybrid HKs based on the quantitative measurement of phosphorylation state in bacterial two-component system.

Keywords: phos-tag, protein phosphorylation, two-component system

POS-02-244 Functional Proteomics Defines the Molecular Switch Underlying FGF Receptor Trafficking and Cellular Outputs

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Stimulation of Fibroblast Growth Factor Receptors (FGFRs) with distinct FGF ligands generates specific cellular responses. For instance, we show that FGF-7 stimulation leads to FGFR2b degradation and ultimately, cell proliferation, whereas FGF-10 promotes receptor recycling and cell migration. However, the mechanisms underlying this paradigm have remained elusive. By combining mass spectrometry-based quantitative temporal phosphoproteomics, interaction proteomics based on affinity pulldown screens, using either a receptor-derived phosphopeptide or SH2 domains as baits, and a targeted approach with fluorescent microscopy and biochemical methods, we find that FGF-10 specifically induces rapid phosphorylation of tyrosine (Y) 734 on FGFR2b, which leads to PI3K and SH3BP4 recruitment. This complex is crucial for FGFR2b recycling and responses as FGF-10 stimulation of either FGFR2b_Y734F mutant- or SH3BP4-depleted cells switches receptor endocytic route to degradation resulting in decreased breast cancer cell migration and inhibition of epithelial branching in mouse lung explants. Together, these results identify an intriguing ligand-dependent mechanism for the control of receptor fate and cellular outputs that may explain the pathogenic role of deregulated FGFR2b, offering novel opportunities for therapeutic intervention. In addition, our findings point to functional proteomics as a valuable multi-disciplinary approach to characterize in-depth RTK signaling.

Keywords: RTK signaling, phosphoproteomics, multi-disciplinary approach

POS-02-245 Phos-Tag Biotin as an On-Demand Tool for Study on Protein Phosphorylome

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Protein phosphorylation is a well-characterized post-translational modification that plays a role in the regulation of a variety of cellular events. Abnormal phosphorylation has been implicated in a wide range of human diseases, including cancers and neurodegenerative disorders. Therefore, effective analytical strategies for quantitative monitoring of alterations in the levels of phosphorylation of certain proteins are essential tools for studies on the proteome, particularly in relation to the elucidation of the molecular origins of diseases and the rational molecular designs of drugs.

We have recently developed an improved phosphate-affinity probe that is suitable for the detection of protein phosphorylation. This probe is known as monobiotinylated Phos-tag (Phos-tag Biotin BTL-111), and it contains a dodeca(ethylene glycol) spacer group. The BTL-111 has been used for Western blotting analysis of phosphoproteins on a protein-blotting membrane, for a quartz-crystal microbalance analysis of a phosphoprotein, and for high-throughput assays of cellular protein kinase activities on a peptide microarray chip. Here, we further describe two procedures for performing sandwich assays of the phosphorylation of protein multiplexes in which BTL-111 is used in conjunction with several antibodies. The first procedure is based on an antibody microarray technique with an enhanced chemiluminescence system, and it permits the simultaneous and highly sensitive detection of multiple phosphoproteins in a cell lysate. The second is based on the Bio-Plex suspension array technique with a flow-based microplate fluorescence reader system. By using this procedure, we demonstrated the quantitative detection of the entire level of phosphorylation in a target protein involved in intracellular signaling. In conclusion, these procedures would be useful for resolving the nature of intracellular signaling networks, possibly leading to improvements in disease diagnosis and drug discovery.

Keywords: Phos-tag, Protein phosphorylation, Biotin

POS-02-246 Phosphorylation Profiling of MAPK Signaling Cascade Proteins by Using Neutral Phos-Tag SDS-PAGE

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Phos-tag acts as a phosphate-binding tag molecule in an aqueous solution under neutral pH conditions. We have reported a novel type of phosphate-affinity SDS-PAGE (Phos-tag SDS-PAGE) for the mobility shift detection of phosphoproteins. The original Phos-tag SDS-PAGE, which uses a polyacrylamide-bound Phos-tag and the Laemmli's buffer system under alkaline pH conditions, has been widely used in determining the phosphorylation state of many proteins. However, it has some limitations for the separation analysis of certain phosphoproteins because of the low affinity to the phosphate group of Phos-tag under alkaline pH conditions. Recently, to overcome these limitations, we developed an improved Phos-tag SDS-PAGE method that uses a neutral-pH gel buffer, Bis-Tris-HCl (pH6.8). The Bis-Tris-buffered Phos-tag gel showed dramatic improvements in the resolving power of various phosphoproteins including endogenous cellular proteins.

By using the improved neutral Phos-tag SDS-PAGE, in this study, we characterized the status of phosphorylation of MAPK signaling cascade proteins, MEK and ERK. Regarding the MEK protein, multiple phosphorylation sites have been identified by various phosphoproteomic analyses. The hyper-phosphorylation profiling of MEK was demonstrated by our affinity-elctrophoretic strategy in conjunction with the site-specific mutagenesis based on the phosphoproteomic information. With regard to the phosphorylation profiling of ERK, an overview of the kinase/phosphatase-dependent dynamics of various phosphorylation states of ERK in the EGF-signaling pathway was demonstrated. In this presentation, we discuss the resolving power of the current Phos-tag SDS-PAGE methodology as a means for research on signal transduction in the coming generation.

Keywords: Phos-tag SDS-PAGE, protein phosphorylation, signal tranduction

POS-02-247 A New Signaling Cascade in Breast Cancers with p53 Inducible Protein Phosphatase PPM1D Overexpression

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The nucleolus, which is a highly dynamic nuclear compartment, plays a key role in ribosome biogenesis. Even though the nucleolar number and size are used for cytological examination in tumors, the molecular mechanism of collapsing the nucleolar integrity has poorly understood. PPM1D (Wip1, PP2C δ) is a p53-inducible Ser/Thr phosphatase and is localized in the nucleolus and the nucleoplasm. One of the functions of PPM1D in normal cells is a negative feedback regulation of p38-p53 pathway by dephosphorylating cell-cycle checkpoint kinases such as CHK1 and tumor suppressor p53. On the other hand, gene amplification and protein overexpression of PPM1D were reported in many human tumors including breast cancers. We have identified nucleolar protein Nucleophosmin as a novel binding partner of PPM1D by mass spectrometry analysis. We found that the high expression level of PPM1D was strongly correlated with the number of nucleoli in a p53-independent manner. We also showed that PPM1D overexpression induced the phosphorylation of NPM at Ser4 and Thr199 and that these phosphorylations increased the nucleolar number. *In vitro* kinase assay showed the sequential phosphorylation of NPM at Thr199 by CDK1 and Ser4 by PLK1. We also showed that PPM1D overexpression induced an activation of CDC25C independently of p53 status. Taken together, we concluded that PPM1D overexpression induced an increase of the nucleolar number through phosphorylation of NPM by CDC25C-CDK1-PLK1 cascade in tumors.

Keywords: breast cancer, phosphorylation, nucleolus

POS-02-248 Old Tools in a New Jacket: Phosphopeptide Enrichment by *TiO_2* and IMAC Columns

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Advances in phosphopeptide enrichment methods enable the identification of thousands of phosphopeptides from complex samples. Current offline methods using TiO_2 and IMAC material in batch mode or microtip column format are widely used, but clearly lack reproducibility in part owing to a strong dependence on the ratio of the quantity of the proteome digest and the enrichment material. Here we used TiO_2 and IMACHPLC guard columns to enrich phosphopeptides from lysates. First we evaluated reproducibility and loadability of both columns. In addition we investigated the nature of phosphopeptides enriched by both methods. Finally, we examinedwhether these column enrichments can be performed sequentially, to yield the maximum number of phosphopeptides from a single biological sample. We found our columns to be largely competitive with the current standards in the field, however clearly benefiting from increased reproducibility and selectivity. Surprisingly, we did not see a clear separation effect of mono and multiple phosphorylated peptides on both columns. To increase the coverage of the phosphoproteome and to evaluate the most suited secondary separation dimension, we compared SCX, hSAXand HILIC chromatography prior to identification by LC-MS/MS.We found that hSAX and HILIC in combination with sequential IMAC and TiO_2 pre-enrichment outperforms SCX due to the increased orthogonalityto reversed phase separation. In conclusion, this works shows that TiO_2 and IMAC guard columns provide an easy, efficient and more reproducible alternative to the current batch enrichment methods.

Keywords: phosphopeptide pre-enrichment

POS-02-249 Global Survey of Mouse Liver Protein Expression Using Off-Gel Isoelectric Focusing of Tryptic Peptides and LTQ-Orbitrap

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The sample fractionation steps conducted prior to mass detection are critically important for the comprehensive analysis of complex protein mixtures. In terms of analyzing real samples, OFFGEL isoelectric focusing makes less samples loss than conventional gels dose, because the OFFGEL electrophoresis differs from conventional gel electrophoresis in that the sample components do not remain in the gel. Based on characteristics, pre-separated peptides solution is more suitable for doing downstream LC-MS/MS. To comprehensively and quickly identify proteins of mouse liver, the tryptic peptides were pre-fractioned and enriched by isoelectric focusing using Agilent 3100 OFFGEL Fractionator into 24 fractions, followed by RPLC and MSMS analysis. Each MS/MS spectrum was searched for mouse specie against the Swissprot database. The resulrs were analyzed which resulted in the identification of about 5871 proteins in mouse liver. About 90% of the identified individual peptides were found in only one or two fractions. All identified proteins were bioinformatically annotated in detail. The combined use of OFFGEL fractionation and LC-MS allows a considerable increase in proteome coverage of very complex samples prepared from the tissues extracts and supports the low-concentrated protein identification.

Keywords: proteomics, OFFGEL electrophoresis, LC-MS/MS

POS-02-250 Enhanced Phosphopeptide Identification in *Escherichia coli* by Stepwise Hydroxy Acid-Modified Metal Oxide Chromatography with Elevated Sample Loading Capacity

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Protein phosphorylation in bacteria is as important as in other living organisms to regulate physiological processes. However, the much lower phosphoprotein stoichiometry severely hampered the progress in phosphoproteomic field in microorganisms. Here, the stepwise enrichment with large-scale hydroxy acid-modified metal oxide chromatography (HAMMOC) was integrated into phosphoproteomic analysis in gram-negative model organism, *Escherichia coli*. The large-scale HAMMOC (200-μL C8 StageTip) showed the comparable enrichment efficiency with the conventional one (10-μL C8 StageTip). From 100 μg protein lysates, 31 and 38 phosphopeptides accompanied with 137 and 122 non-phosphopeptides were identified in conventional and large-scale HAMMOC, respectively. Also, these two methods had similar enrichment specificity with an average of 48.8 % and 44.6 % in conventional and large-scale HAMMOCs. As increasing the lysates from 500 to 1000 μg with 100 μg interval, 998 unique phosphopeptides accompanied with 4,186 non-phosphopeptides were identified in total by large-scale HAMMOC. 268 serine, 104 threonine, and 38 tyrosine phosphorylation sites were found from 318 phosphoproteins yielding a Ser/Thr/Tyr phosphorylation ratio of 65.4/25.4/9.2%. Since the large number of non-phosphopeptides would suppress the phosphopeptide ionization and thus hindering the identification of phosphopeptides, further development on the stepwise strategy was adopted to reduce the non-phosphopeptides. Successfully, the enrichment specificity was significantly improved to higher than 95 %. This approach exhibited its advantages in higher enrichment efficiency in aspect of less sample amount and LC-MS/Ms analysis time and simpler manipulation with higher number of identified phosphopeptides and phosphoproteins. Therefore, this method displayed its feasibility in bacterial phosphoproteomic analysis.

Keywords: bacterial phosphoproteomics, phosphopeptide enrichment

POS-02-251 Mapping Global Histone Acetylation Patterns Using MALDI-TOF Mass Spectrometry

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Keeping the balance between histone acetylation and deacetylation is essential event for the epigenetic control of gene expression, DNA repair and developmental processes. Perturbation of normal histone acetylation status have been implicated in abnormal cell function, including carcinogenesis or progression of cardiovascular and neurodegenerative diseases. Thus, agens modulating epigenetic marks have emerged as a promising strategy in disease therapy. Here, we present an MS-based approach for mapping global changes in the acetylation of histones. The workflow consisted of histone extract fractionation by RP-HPLC, Glu-C digestion of fractions and MALDI-TOF MS analysis of N-terminal peptides enables monitoring overall acetylation pattern of histones H2B, H4, H2A and H3 in context with basic molecular responses.

Acknowledgement: This work was supported by the Program of "Employment of Newly Graduated Doctors of Science for Scientific Excellence" (grant number CZ.1.07/2.3.00/30.0009) co-financed from European Social Fund and the state budget of the Czech Republic. This work was realised in CEITEC - Central European Institute of Technology with research infrastructure supported by the project CZ.1.05/1.1.00/02.0068 financed from European Regional Development Fund.

Keywords: histones, acetylation, MALDI-TOM MS

POS-02-252 A Large Synthetic Phosphopeptide Library for Mass Spectrometry Based Proteomics

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The rate at which mass spectrometry based proteomic data is generated is continuing to increase rapidly and challenges the fields' ability to analyze such data quickly and reliably. Owing to the absence of suitable synthetic reference standards, software solutions for large scale peptide sequence identification and post-translational modification site localization can generally not be validated rigorously using experimental data derived from biological sources. To improve on this situation, we have synthesized 96 tryptic peptide libraries containing >100,000 unmodified peptides plus their corresponding >100,000 phosphorylated counterparts with precisely known sequences and modification sites.

Analysis of the library by mass spectrometry yielded a data set that can be used to develop, evaluate and improve experimental and computational proteomic strategies. We evaluated the merits of different search engines (Mascot and Andromeda) and fragmentation methods for peptide identification. We also compared the sensitivity and accuracy of phosphorylation-site localization tools (MDscore, PTMscore and phosphoRS) and characterized the chromatographic behavior of peptides in the library. We found that HCD identified more (phospho-)peptides than did ETD, that phosphopeptides generally eluted later from reversed-phase columns and were easier to identify than unmodified peptides and that current computational tools for proteomics can still be substantially improved. The physical library and the generated mass spectrometric data can be used in numerous ways by the communiy to develop, evaluate and improve experimental and computational proteomic strategies.

Keywords: synthetic library

POS-02-253 *In Vivo* Screening of Kinase-Specific Substrates by Phosphatase Inhibitor and Kinase Inhibitor Substrate Screening (PIKISS)

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Protein kinases are major components of signal transduction pathways in multiple cellular processes. More than 500 protein kinases exist in human genome, but efficient methods to search for their substrates are poorly developed despite the importance of their identification to fully understand the signaling network of respective kinases. We developed *in vivo* screening method for the substrates of the specific kinase by treating cells with protein kinase inhibitor and phosphatase inhibitors. Cell lysates were subjected to affinity chromatography using beads coated with 14-3-3 protein, which interacts with proteins containing phosphorylated serine or threonine residues, to enrich the phosphorylated proteins. The identities of the molecules and phosphorylation sites were determined by liquid chromatography tandem mass spectrometry (LC/MS/MS) after tryptic digestion and phosphopeptide enrichment with TiO column. The phosphorylated proteins whose phosphopeptide ion peaks were suppressed by treatment with the kinase-specific inhibitor were regarded as candidate substrates of the specific protein kinase. We developed this method for several protein kinases such as rho-kinase and ERK.

Keywords: phosphorylation, protein kinase inhibitor

POS-02-254 Phosphoproteomic Analysis of Gamma-Irradiated Human Leukemic Cells

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Protein phosphorylation is a transient, reversible PTM, which is involved in many cellular processes including DNA-damage response (DDR). DDR is triggered by the presence of gamma-radiation-induced DNA double strand breaks (DSBs), which are considered to be the most lethal class of DNA damage. DSB induce phosphorylation-mediated signalling pathways leading to cell cycle arrest and DNA repair or apoptosis. Since radiation is one of the treatment modalities in cancer therapy, we used human leukemic cells and phosphoproteomic approach in order to study DDR pathways on molecular level. In our present work, we employed SILAC-based high resolution quantitative TiO_2-phosphoproteomics. Based on data set analysis we identified several hundreds of phosphorylated proteins, whose activity has been modified. The key enzymes regulating DNA repair, cell cycle progress, and pro-/anti-apoptotic signalling were observed among the identified proteins. Our results also indicate that a significant amount of phosphorylation sites was dephosphorylated suggesting that not only phosphorylation plays an important role in regulation of DDR upon DNA damage. Detailed overview and discussion of the obtained data will be given.

Keywords: phosphoproteomics, DNA damage response, leukemia

POS-02-255 Quantitative Analysis of Effects of pH on the Stability of His- and Asp-Phosphoproteins in Bacterial Two-Component System by Using Phosphate-Affinity SDS-PAGE

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Two-component systems are prevalent signaling pathways in bacteria and have been developed as means of the adaptation to multiple and variable environments throughout their evolution. The systems typically comprise a membrane-spanning sensor histidine kinase protein (HK), the activity of which is responsive to relevant environmental or physiological signals, together with a cognate target response regulator (RR) protein. The degree of Asp-phosphorylation of a given RR is generally an indicator of its activity as a transcriptional activator of relevant genes. Two-component systems are found in virtually every species of bacteria and have been found to regulate virtually almost all the cellular processes. These processes include essential metabolic and physiological activities but also secondary characteristics that confer highly adaptive traits for specialized environments, such as virulence factors of bacterial pathogens. It is thus very important to monitor the level of phosphorylation reaction involved in the systems, but characterization of the His- or Asp-phosphoprotein has been technically challenging due to the chemical instability.

We have reported a novel type of phosphate-affinity SDS-PAGE (Phos-tag SDS-PAGE) for the mobility shift detection of phosphoproteins. The technique, which uses a polyacrylamide-bound Phos-tag, has been widely used in determining the phosphorylation state of many proteins. By using Phos-tag SDS-PAGE, we attempted to examine effects of pH on the stability of His- and Asp-phosphoproteins, which are unstable in nature, in *Escherichia coli*. In this presentation, we discuss the pH stability of HK and RR based on the quantitative measurement of phosphorylation state in bacterial two-component system.

Keywords: Phos-tag, protein phosphorylation, two-component system

POS-02-256 Sequential Phosphoproteomic Enrichment by Complementary Metal-Directed Immobilized Metal Ion Affinity Chromatography: Case Study on Kinase Substrate Mapping in Human Lung Cancer Tissue

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Despite recent advances in instrumentation and analytical strategies for identification and quantitation of protein phosphorylation, methodologies to enrich the heterogeneous phosphopeptides is critical towards comprehensive mapping of the under-explored phosphoproteome. Herein, we designed a metal-directed immobilized metal ion affinity (MD-IMAC) chromatography to fractionate heterogeneous types of phosphopeptides. First, taking advantage of the distinctive binding affinity between transition metal ions and phosphopeptides, the Ga^{3+} and Fe^{3+}-based IMAC purified 4554 and 3107 phosphopeptides (FDR<1%) with only 29% overlapping phosphopeptides in both two methods. The low degree of overlap demonstrated the complementary nature by different (MD-IMAC). On the pilot study of Raji B cell line, the complementary property of Ga^{3+} and Fe^{3+}, not only mono phosphopeptides and multiply phosphopeptides but also basic and acidic phosphopeptides can be sequential fractionated by Ga^{3+}-IMAC and Fe^{3+}-IMAC. Among 6283 phosphopeptides, only 8% phosphopeptides identified in both methods which revealing superior fractionation ability. Finally, this approach has been applied to perform human lung cancer tissue phosphoproteomic analysis. Among 2569 identified phosphopeptides, different property of kinase substrate can be successfully separated by Ga^{3+}-IMAC (70% were acidic substrate) and Fe^{3+}-IMAC (46% were Pro-directed substrate). Up to 30 sequences Motif from over-activated kinase can be obtained. The improved sequential by different metal ions of IMAC allow more detailed characterization of phosphoproteins in functional phosphoproteomics research projects.

Keywords: IMAC, phosphoproteomics, lung cancer

POS-02-257 Rapid and Selective Separation of Phosphorylated Biomolecules by Using a Phos-Tag-Based Magnetic-Bead Method

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Phosphorylated biomolecules with low molecular weights, such as nucleotides and phosphopeptides, have attracted much interest in the fields of metabolomics and phosphoproteomics. Therefore, effective analytical strategies for separation and enrichment of them are essential.
Here, we describe a simple and efficient method based on magnetic-bead technology for the separation of phosphorylated and nonphosphorylated low-molecular-weight biomolecules, such as nucleotides, phosphorylated amino acids, or phosphopeptides. The phosphate-binding site on the bead is an alkoxide-bridged dinuclear zinc (II) complex with 1,3-bis (pyridin-2-ylmethylamino)propan-2-olate (Phos-tag), which is linked to a hydrophilic cross-linked agarose coating on a magnetic core particle. All steps for the phosphate-affinity separation are conducted in buffers of neutral pH with 50 μL of the magnetic beads in a 1.5-mL microtube. The entire separation protocol for phosphomonoester-type compounds, from addition to elution, requires less than 12 minutes per sample if the buffers and the zinc (II) -bound Phos-tag magnetic beads have been prepared in advance. The phosphate-affinity magnetic beads are reusable at least 15 times without a decrease in their phosphate-binding ability and they are stable for three months in propan-2-ol. The Phos-tag-based magnetic bead method can be successfully used for the purification and isolation of various phosphorylated biomolecules with high recovery yields and high purities, and it should provide the means to perform a variety of experiments in metabolomics and phosphoproteomics by coupling with various down-stream applications including advanced mass spectrometry-based technology.

Keywords: Phos-tag, phosphorylated biomolecule, magnetic bead

POS-02-258 Proteomic Identification of the Posttranslational Modifications in TrkA-Mediated Tyrosine Phosphorylation Signaling Network

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The receptor-type protein tyrosine kinase TrkA plays an important role in cell survival, death and differentiation via NGF-dependent or -independent mechanisms in various cell types. We have previously showed that TrkA overexpression caused its tyrosine-490 phosphorylation in SK-N-MC neuroblastoma cells, leading to the activation of apoptotic signaling pathways. Here, we have shown that the inhibition of TrkA tyrosine-490 phosphorylation by GW441756 resulted in the suppression of tyrosine phosphorylation of cellular proteins including ERK and JNK. To study novel TrkA-mediated tyrosine phosphorylation signaling network, we investigated the inhibitory effects of GW441756 on TrkA-dependent targets in SK-N-MC cells by proteomic analysis. The results showed that hnRNP C1/C2, α-tubulin, β-tubulin, β-tubulin homologue, actin homologue and eIF-5A-1 protein spots were upregulated by TrkA, whereas α-enolase, peroxiredoxin-6, PROS-27, PP1-gamma and PDH E1-alpha were downregulated by TrkA, and these TrkA-dependent upregulation and downregulation were significantly suppressed by GW441756. Moreover, we found that most of the targets underwent certain modifications in a normal condition, and this phenomenon was either upregulated or downregulated by TrkA. Our results suggest that TrkA could play an important role in the cytoskeleton, cell death, cellular processing and glucose metabolism by regulation of the posttranslational modifications in TrkA-mediated tyrosine phosphorylation signaling network.

Keywords: signaling network, posttranslational modification, tyrosine phosphorylation

POS-02-259 Identification of Dysregulated Kinase-Mediated Pathways in Hepatocellular Carcinoma by a Quantitative Phosphoproteome Approach

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Phosphorylation is one of most common post-translational modifications in mammalian cells. It regulates protein activity, cellular localization, conformation, and binding affinity and further mediates diverse key physiological functions. Numerous literates have established the close association between aberrant phosphorylation and many diseases, including cancer.
Hepatocellular carcinoma (HCC) is a common and poor prognostic cancer worldwide. Surgical resection is the most effective modality, but there are many limitations, and unfortunately, the recurrence rate is about 50% even after resection. In previous studies, various kinases and phosphorylation-mediated signaling pathways such as HGF/c-Met signaling pathway, ERK/MAPK pathway and Wnt/β-catenin signaling pathway have been found to be significantly altered in HCC patients. Conceivably, phosphorylation plays an important role in these molecular mechanisms that can affect tumorigenesis. Therefore, exploring the tissue phosphoproteome profiles will facilitate the identification of critical factors involved in HCC.
In this study, we established a technology platform for quantitative phosphoproteome analysis via combining stable isotope dimethylation labeling and online SCX-TiO_2/RP-LTQ-Orbitrap, and then compared tissue proteome and phosphoproteome between tumor tissues and paired adjacent non-tumor counterparts in three HCC patients. The results yielded 3100-4700 quantifiable phosphopeptides corresponding to over 2600 proteins with high confidence. In order to check the accuracy of our platform, we proceeded to confirm the quantitative results by Western blotting using phospho-specific antibodies.
Furthermore, key upstream protein kinase(s) responsible for the phosphorylation of those phosphosites dysregulated in tumor tissues were predicted, enabling us to unravel aberrant active kinases as biomarkers, as well as discover potential therapeutic targets for HCC patients.

Keywords: phosphoproteome, hepatocellular carcinoma, dimethylation labeling

POS-02-260 A Phospho-Peptide Spectrum Library for Improved Targeted Assays

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The profound and diverse effects of protein phosphorylation have created a keen interest in their characterization and quantitation in biologically relevant samples. However, they present unique challenges to the mass spectrometrist. The fragmentation pattern of phosphorylated peptides is less predictable than that for unmodified peptides, increasing the uncertainty of MS/MS spectra identifications and making it more difficult to predict reliable fragment ions to monitor in targeted assays. Further, the presences of multiple isoforms that are not chromatographically resolved present an additional complication. The intact peptide mass can be used to distinguish between modified and unmodified forms, but one must rely on the specific fragmentation patterns to distinguish between different modified forms when a peptide contains multiple sites for phosphorylation.
Generating targeted SRM assays requires choosing pairs of precursor/fragment ion masses for each peptide of interest. Knowing which fragment ions will produce a strong signal requires either a good prediction model of fragmentation or prior measurement of the fragmentation pattern. Because fragmentation of phospho-peptides is difficult to predict, we turned to empirical observation.
We collected spectra from synthetic phospho-peptides into a spectrum library. By acquiring HCD spectra at several activation energies from carefully selected mixtures synthetic peptides, we have an unbiased measure of relative intensities of fragment ions under targeted assay conditions. In particular, we know which of the few fragment ions distinguishing one isoform from another are readily seen. Using the relative intensities stored in the library, we generate instrument methods that out-perform methods constructed based on heuristics alone. Knowing the expected relative retention times also helps build scheduled SRM assays and improves confidence in specificity.

Keywords: spectrum library, phosphorylation

POS-02-261 Phosphoproteomics Approach Coupled with *In Vitro* Kinase Reaction to Construct Kinase-Substrate Network

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Cellular signal transduction regulates various biological functions. Phosphorylation, caused by protein kinases, is one of the most ubiquitous post-translational modifications. All kinases are known to catalyze essentially the same phosphate transfer reaction, nevertheless, they display remarkable diversity in their substrate specificity. Recent development of liquid chromatography-tandem mass spectrometry (LC-MS/MS) with effective enrichment methods for phosphopeptides has enabled to identify thousands of phospho-sites on substrates at one analysis, but this method never reveal upstream kinases, which actually work on those substrates. The whole picture of signaling network essentially consisting of cascades of phosphorylation reactions cannot be revealed without kinase-substrate information. In order to address this problem, we have developed an experimental procedure which is LC-MS/MS-based phosphoproteomics coupled with *in vitro* kinase assay.

In our experimental workflow, proteins are extracted from cells and intrinsic phosphates are removed by phosphatase treatment. Then proteins are phosphorylated *in vitro* by spiked kinases followed by phosphopeptide enrichment. The resultant substrates are identified by LC-MS/MS on a proteome scale. We have applied this method to three representative protein kinases: PKA, ERK1 and AKT1. As a result of extensive LC-MS/MS analyses, we have obtained about 3,300, 3,800 and 1,600 phospho-sites from reactions with PKA, ERK1 and AKT1, respectively. They showed partial overlap with phospho-sites, which have been confirmed *in vivo*. Moreover, the motifs extracted from the sequences around those sites have the similar features with those have previously reported. Taken together, this large number of *in vitro* substrates will be useful information to predict upstream kinases based on phosphorylated peptides identified by LC-MS/MS, leading to unveiling signaling network.

Keywords: phosphorylation, kinase/substrate, LC-MS/MS

POS-02-262 Targeted Phosphoproteomics Analysis of Immunoaffinity Enriched Tyrosine Phosphorylation in Mouse Tissues

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Tyrosine kinases play a prominent role in regulating various cell signaling pathways and dysregulation of this key PTM drives inappropriate proliferation and cell survival. MS-based global phosphoproteomics studies have significantly evolved in recent years, but cellular proteome complexity and sub- stoichiometry levels of tyrosine phosphorylation sites, when compared to phosphoserine and phosphothreonine residues, limit extensive mapping of the phosphotyrosine repertoire. In our current study we employed coupling of peptide-level anti-pTyr immunoaffinity purification (IP) approach to evaluate the phosphotyrosine component from mouse tissues including liver, brain and embryo. Tissue samples were lysed in denaturing conditions and the soluble proteins were tryspin digested, resulting tryptic peptide mixture was immunoprecipitated for phosphotyrosine target peptides using pY1000 antibody coupled to protein A agarose beads. The enriched peptide mixtures from mouse tissues were analyzed on LC-MS/MS, using a microfluidic-based nanoflow LC coupled to Q-TOF MS. Acquired spectra were searched with Mascot against the Mus musculus protein database. Higher sensitivity levels of peptide detection with dual-stage ion funnel technology have resulted in the identification of hundreds of target phosphotyrosine peptides from the mouse tissues. Our preliminary results demonstrate the ability of the immunoaffinity, LC-MS enrichment strategy, to identify the phosphotyrosine repertoire. Identified peptides include moderately abundant cellular protein tyrosine kinases e.g. tyrosine-protein kinase BTK, mitogen-activated protein kinase 3, A-kinase anchor protein SPHKAP and low abundant transcriptional regulators including transcriptional activator GLI3, histone-lysine N-methyltransferase, ETS-related transcription factor El and catenin alpha. Pathway-level enrichment of the data using automated software is currently underway.

Keywords: LC-MS: liquid chromatography coupled mass spectrometry, IP: immunoaffinity purifcation, QTOF: quadrupole time of flight

POS-02-263 A Novel Titanium Dioxide Plate (TiO_2 Plate) for Phosphopeptide Enrichment and On-Target MALDI-TOF Analysis

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Protein phosphorylation is a major protein post-translational modification that regulates many cellular processes and activities. Because mass spectrometer (MS) signals of low abundance of phosphorylated peptides are commonly suppressed by the presence of abundant non-phosphorylated peptides, one of the major challenges in the detection of phosphopepdies is the enrichment of low-abundant phosphopeptides from complex peptide mixtures. Titanium dioxide (TiO_2) has been proven to be a highly efficient approach and is widely applied for phopshopeptide enrichment. In this study, a novel TiO_2 plate was proposed by coating TiO_2 particles onto MALDI plates, glass or plastic substrates with a simple and rapid approach. The TiO_2 plate can be used for on-target MALDI-TOF analysis or as a purification plate, on which phosphopeptides were eluted out and subjected to MALDI-TOF or nanoLC-MS/MS analysis. The detection limit of the TiO_2 plate is 10 folds lower than TiO_2-packed tips approach. The capacity of the 2.5 mm TiO_2 spot was estimated to be about 10 μg of β-casein. With TiO_2 plate enrichment of SCC4 cell lysate digests and nanoLC-MS/MS analysis, 82% of the detected proteins were phosphorylated, illustrating the TiO_2 plate is practical and effective to enrichment phosphopeptides from complex samples.

Keywords: phosphorylation, on-plate, MALDI-TOF

POS-02-264 Novel Strategy for Lysine Acetylation Discovery Without Using Immunoprecipitation

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Recent development of new technologies such as state-of-the-art mass spectrometry together with antibodies to enrich acetylated peptides has enabled the identification of lysine ε-acetylation on over 2,000 human proteins. The revealed nature of the ubiquitous and conserved acetylome suggests the important roles to regulate various cellular functions, and efforts to decode the acetylome associated with diseases have been an exciting frontier in this field. However, the current strategy for acetylomics based on immunoprecipitation has disadvantages such as the high cost for antibodies, the bias of antibody to substrates and the contamination of non-acetylated peptides.

In this study, we designed a new method to enrich lysine acetylated peptides without using antibody, in which chemically protected lysine acetylated peptides are deacetylated by lysine deacetylase (KDAC), followed by biotinylation to enrich the target peptides. In order to validate this method, we applied it to a test mixture of non-acetylated and acetylated peptides. As a result, acetylated peptides were exclusively enriched without contamination of non-acetylated peptides. Then, we tried this method to HeLa cell lysate and successfully identified peptides with lysine acetylation sites. In addition, we revealed the deacetylation profiles of six different KDACs since this approach is based on the enzymatic reaction by KDAC.

Keywords: lysine acetylation, lysine deacetylase, biotin

POS-02-265 LC-MALDI-TOF-TOF Mass Spectrometry Distinguishing between Symmertric/Asymmetric Dimethylated Arginine3 of Histone H4 Peptide

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Histone proteins are subject to multiple modifications containing acetylation, methylation, phosphorylation, ubiquitination and so on. Their combinatorial pattern of modifications consists key regulatory machineries in the epigenome. We are analyzing dynamics in combinatorial modification patterns of histones to discover disease-specific modifications using LC-MS. The discovered modifications include dimethylation at arginine 3 in histone H4 (H4R3Me2). Arginine dimethylation is reported to take two forms, namely symmetric and asymmetric, which are differently regulated in the epigenome. Indeed, symmetric and asymmetric H4R3Me2 are methylated by different subtypes of protein arginine methyltransferases and differentially regulated during biological processes such as mRNA splicing, transcription, and DNA repair. Although the two forms are partly distinguishable in MS/MS/MS of LC-MS, it is difficult to distinguish them in a low amount of H4R3Me2 in a complex sample.
MALDI provides simpler MS/MS spectrum than ESI. We have previously succeeded in distinguishing between symmetric and asymmetric synthetic peptides by MALDI tandem mass spectrometry, where symmetric and asymmetric arginine had peaks corresponding to losses of 70/31 and 45, respectively. The newly developed axial spatial distribution focusing had improved the identification. Here, we tried to analyze samples spotted on MALDI target after LC-MS separation off line. Toward the long-term goal to reveal the pathological function of symmetric and asymmetric H4R3Me2, we are developing the system.
Keywords: epigenetics, chromatin, histone

POS-02-266 Deep Profiling of Molecular-Targeted Drugs by One-Shot Quantitative Phosphoproteomics

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Protein phosphorylation is well known to regulate various cell functions, and many diseases including cancer are closely related to abnormal protein phosphorylation. Therefore, many small compounds or antibodies which inhibit specific kinases have been developed, and some of these drugs are used for molecular-targeted therapy of a cancer in widespread clinical use. However, it is still poorly understood how whole signaling pathways are influenced by these kinase inhibitors. In this study, we developed a high-throughput method for profiling of the kinase inhibitors based on quantitative phosphoproteomics using meter-long monolithic silica capillary columns, and profiled various types of kinase inhibitors using the profiling method. On average more than 10,000 phosphopeptides were identified by single LC-MS run without any other fractionation, and phosphorylation-state of the peptides were quantified using stable-isotope dimethyl labeling. Some of the drug decreased phosphorylation of the known target kinases and/or their downstream molecules. Moreover, some phosphorylation sites were commonly affected by all of the drugs. We also performed cluster analysis using the quantitative phosphoproteome data to characterize the drugs independent of the known signaling pathways, and successfully classified the drugs based on the target specificity. This phosphoproteomic approach is expected to be useful for deep and high-throughput characterization of molecular-targeted drugs.
Keywords: phosphoproteomic profiling, molecular-targeted drug, monolithic columns

POS-02-267 mTOR Complex 2 Phosphorylates Filamin A to Regulate Cell Migration

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Mammalian target of rapamycin (mTOR) is a protein kinase that plays a central role in a variety of cellular events, such as cell growth, proliferation, autophagy and actin cytoskeleton. To regulate them, mTOR forms two complexes with different functions. mTORC1, composed of raptor and mLST8 and mTOR, phosphorylates S6 kinase 1 (S6K1) and eIF4E-binding protein 1 (4E-BP1) to enhance protein translation, while mTORC2, consisting of mTOR, rictor, mLST8 and Sin1, regulates cell migration and actin cytoskeleton. However, little is known about how mTORC2 regulates these events. In this study, we analyzed mTORC1/2-associated proteins by co-immunoprecipitation assays and revealed that filamin A binds rictor but not mTOR or raptor. Immunostaining of HeLa cells showed that rictor, mTOR and filamin A were colocalized at membrane ruffles and this colocalization was dependent on insulin-PI3 kinase-Rac signaling pathway. In addition, filamin A was phosphorylated by mTORC2 *in vivo* and *in vitro*. Stable expression of filamin A in M2 filamin-deficient melanoma cells showed enhanced focal adhesion formation and cell migration, but these effects were negated by the addition of mTOR kinase inhibitor. Ectopic expression of a non-phosphorylatable filamin A mutant failed to promote focal adhesion formation. Taken together, our results suggest that mTORC2 regulates cell migration through the phosphorylation of filamin A.
Keywords: mTOR, filamin, cell migration

POS-02-268 Functional Analysis of Targeted Phosphorylation on Signaling Proteins

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Tightly controlled by protein kinases and phosphatases, reversible phosphorylation on serine, threonine, and tyrosine residues, plays a central role in mediating most if not all cellular signaling pathways. Now, MS-based phosphoproteomics has allowed the detection and quantification of tens of thousands of phosphorylation sites from a typical biological sample in a single experiment, which has posed new challenges in functional analysis of each and every phosphorylation site on specific signaling phosphoproteins of interest. Most recently, in an effort to explore whether and how site-specific phosphorylation on coactivators like p300 and CBP would affect their interactions with the common binding partner beta-catenin in the Wnt/beta-catenin pathway, we integrated multidisciplinary approaches including phosphoproteomics, site-directed mutagenesis, mammalian two-hybrid assay, and specific kinase inhibitors aimed to develop a general workflow. In this context, We firstly identified two novel phosphorylation sites, Ser12 of p300 and Ser92 of CBP using phosphoproteomic approach, which critically mediated the interaction of beta-catenin with p300 and CBP respectively; we further demonstrated that the p38MAPK is the upstream kinase required for the phosphorylation at Ser12 of p300, and that MAPK kinase (MEK/ERK) signal pathway may be responsible for phosphorylation at Ser92 of CBP by combining biochemical methods with kinase specific inhibitors. Based on our success in using the integrative strategy for the functional analysis of targeted phosphorylation, it is expected that this strategy will find more applications in the functional annotations of site-specific phosphorylation in any biomedical events.
Keywords: phosphorylation, p300, beta-catenin

POS-02-269 Impact of High Glucose Concentration on ASA-Induced Acetylation of Human Serum Albumin: an *In Vitro* Study

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Aspirin (ASA) plays a key role in the prevention of ischemic events in high risk cardiovascular patients through the inhibition of COX-1. ASA mediates its effect via trans-acetylation with amino groups of lysine and N-terminal residues. However, the effect of ASA was also demonstrated on several blood proteins including hemoglobin, fibrinogen and serum albumin (HSA). HSA is a multifunctional protein that exerts relevant roles in metabolism. Of note, the beneficial effect of ASA seems to be reduced in patients with diabetes mellitus, suggesting that protein glycation may impair the acetylation process of ASA. The aim of this study was 1) to characterize the acetylation degree on HSA after incubation with an increasing ASA concentration, and 2) to evaluate whether high glucose concentration interferes with ASA-acetylation. HSA was incubated with an increasing ASA concentration and the acetylation level was measured by immunoblotting and high-resolution tandem mass spectrometry (MS). In a second analysis, HSA was first incubated with glucose followed by ASA and the acetylation and glycation levels were also measured in this experiment. Incubation of HSA with ASA resulted in a dose-dependent increase of acetylated sites. A significant decrease in the acetylation level was observed by Western blot and MS when HSA was previously incubated with glucose, suggesting that glycation has a major impact in decreasing the aspirin-induced acetylation process on HSA. MS allowed identifying five preferential identical amino acid sites for both post-translational modifications. The present workflow will be applied to analyze the interplay between glycation and acetylation on different blood compartments (plasma, leukocytes, platelets and erythrocytes) from diabetic patients after ASA therapy.

Keywords: aspirin, glycation, mass spectromety

POS-02-270 Discovery of Novel Inhibitors for Human Sirtuins

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Posttranslational modifications of proteins modulate protein activity and stability regulating metabolism and aging processes. The (de)acetylation of lysine residues is one of the most abundant modifications and originally discovered for histone proteins. The functional role of acetylations in non-histone proteins is mostly unknown, although more than 6500 acetylation sites are known in the human proteome. The level of this modification is regulated in cells through lysine-acetyltransferases and lysine-deacetylases. The NAD^+-dependent lysine-deacetylases (Sirtuins) are acting as sensors in metabolic pathways and stress response. In mammalia there are seven Sirtuin isoforms known which emerged as potential therapeutic targets due to molecular links between cell metabolism and human disorders. To date, only a few pharmacological inhibitors have been reported for Sirtuin 5 (Sirt5). We identified several CPS1 derived peptide inhibitors for Sirt5 with different inhibition mechanisms. To elucidate the molecular basis of inhibitor interactions we determine x-ray crystal structures of Sirt5 in complex with various inhibitors.

Keywords: Sirtuin, Sirt5, inhibitor

POS-02-271 Quantitative Proteomic Analysis of Post Translational Modifications in Alzheimer's Disease Brain

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Introduction: Post-translational modifications (PTMs) are crucial for the regulation of protein structure and function. It is now widely accepted that protein expression alterations are mainly a consequence of dysregulated PTMs resulting in protein dysfunction. Targeting proteins to specific modifications is a complex process that requires sophisticated proteomics technology. Proteomic analysis represents a new aspect to re-evaluate clinical trials and therapeutics for Alzheimer's disease.
Methods: The present study analyzed the proteome-wide changes in the phosphorylation and S-nitrosylation associated with the activation of signaling pathways and biochemical links. A combinatorial effect of these PTMs on several metabolic and signaling cascades was assessed using computational tools like STRING 8.3 and MINT while the functional enrichment of the altered PTMed proteins was searched through KEGG and REACTOME databases.
Results: A number of proteins involved in several cellular and molecular pathways were identified with aberrant phosphorylation and S-nitrosylation levels in the AD brain regions**.** The identified altered proteins can be helpful for biomarker discovery and can provide a broader understanding of the brain dysfunction. A close interaction pattern was also observed among the identified proteins in AD brain.
Conclusions: The current findings focused on several significant proteins that accounts for biological and morphological alterations in AD that will be helpful to establish a broad database of potential protein targets of aberrant PTMs in AD brain.

Keywords: quantitative proteomics, phosphorylation

POS-02-272 Quantitative Phosphoproteome Analysis for Identification of Novel GSK-3 Substrates in HEK293 Cells

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Glycogen synthase kinase 3 (GSK-3) is a serine/threonine kinase which is involved in diverse biological functions including embryogenesis, cell motility, cell proliferation and differentiation. Although many substrates of GSK-3 had been identified by traditional biochemical and molecular biology methods, few efforts have been focused on the systemic approach for identifying new substrates of GSK-3. Recent advancement in mass spectrometry-based proteomic analysis has allowed high-throughput protein identification (including various modifications) and quantification (comparison with stable isotope standard) in tiny amount of biological samples. To explore the regulatory role of GSK-3 in a global view, especially in post-translational modification events, we herein used quantitative phosphoproteome analysis to discover novel substrates of GSK-3 in human HEK293 cells. Starting from 0.5 mg total extracts of SILAC-labeled HEK293 cells, in which GSK-3 was pharmacologically inhibited or not, we have identified/quantified ~4900 proteins and ~3700 phosphopeptides using both basic RP-HPLC/on-line TiO_2/RP-LTQ Orbitrap and on-line SCX/RP-LTQ Orbitrap platforms. We identified 22 potential GSK-3 substrates with high confidence, whose phosphorylation levels were reduced in cells when GSK-3 activity was pharmacologically inhibited. In addition to known substrate of GSK-3 such as CRPM4 (DPYSL3), we have also validated some novel substrates involving in regulating diverse cell functions. Our results have revealed many novel candidate substrates and their phosphorylation sites for GSK-3, which will provide valuable clues to facilitate further exploration of the GSK-3-mediated physiological functions and their mechanisms.

Keywords: GSK-3, phosphoproteome

POS-02-273 Global In-Depth Quantitative Proteomic Analysis of HIV Infected Cells Using a Novel Q-OT-qIT Mass Spectrometer

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A complete understanding of the molecular events following infection by HIV is key for developing therapeutic strategies. A comprehensive view of how the virus re-wires the host during infection is far from complete. For the first time, we present an in-depth, quantitative proteomic analysis of cells infected by HIV by dynamically monitoring the changes in protein levels, as well as the phosphorylation and ubiquitination status of host proteins. This unbiased systems approach has enabled the identification of exciting new contributors involved in the pathogenesis of HIV infection, which could ultimately serve as therapeutic targets. Jurkat T cells were labeled in light/heavy SILAC medium and either infected with HIV or mock-infected. Harvested cells were lysed, digested and fractionated for analysis of protein abundance and/or enriched for phosphorylation and ubiquitination. Data were acquired on a novel hybrid instrument, based on mass resolving quadrupole, Orbitrap, collision cell, linear ion trap (Q-OT-qIT) architecture using CID/ETD fragmentation.

Jurkat T cells infected with HIV were used for proteomic experiments to establish changes in protein, phosphorylation, and ubiquitination abundance. The novel mass spectrometer, with its unique architecture, was engineered to optimize both speed and efficiency enabling sensitive MS/MS analysis at a high rate. Due to the quadrupole isolation, parallelization of Orbitrap and ion trap detection, and pipelining of ion injection and mass analysis, the extremely high resolution full scan data necessary for accurate SILAC quantitation can be acquired with simultaneous acquisition of sensitive MS/MS spectra. This massive time-dependent data set enabled the determination of proteins with altered abundance, those targeted for degradation, and those whose modification may play a role in the signaling cascade leading to the severe and life threatening pathogenesis of HIV infection.

Keywords: phosphorylation, ubiquitination, orbitrap

POS-02-274 Phsphoproteomic Analysis of the Model *Cyanobacterium Synechococcus* sp. Strain PCC 7002

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Increasing evidence shows that protein phosphorylation on serine (Ser), threonine (Thr) and tyrosine (Tyr) residues is one of the major post-translational modifications in the bacteria, involved in regulating a myriad of physiological processes. Cyanobacteria are one of the largest groups of bacteria, and are the only prokaryotes capable of oxygenic photosynthesis. Many cyanobacteria strains contain unusually high number of protein kinases and phosphatases with specificity on Ser, Thr and Tyr residues. However, only a few dozen phosphorylation sites in cyanobacteria are known, presenting a major obstacle for further understanding the regulatory roles of reversible phosphorylation in this group of bacteria. In this study, we carried out a global and site-specific phosphoproteomic analysis on the model cyanobacterium *Synechococcus* sp. PCC 7002. In total, 280 phosphopeptides and 410 phosphorylation sites from 245 *Synechococcus* sp. PCC 7002 proteins were identified through the combined use of protein/peptide prefractionation, TiO_2 enrichment and LC-MS/MS analysis. The identified phosphoproteins were functionally categorized into an interaction map and found to be involved in various biological processes such as two-component signaling pathway and photosynthesis. Our data provides the first global survey of phosphorylation in cyanobacteria by using a phosphoproteomic approach, and suggests a wide-ranging regulatory scope of this modification. The provided dataset may help reveal the physiological functions underlying Ser/Thr/Tyr phosphorylation and facilitate the elucidation of the entire signaling networks in cyanobacteria.

Keywords: cyanobacteria, phosphoproteomics, photosynthesis

POS-02-275 Quantitative Phosphoproteomic Analysis of Calmodulin-Dependent Calcium Signaling

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Calcium signaling affects multiple biological processes including differentiation, neurotransmission and immunity. In this signaling, calcium/calmodulin (CaM)-dependent enzymes, such as CaM-dependent protein kinase (CaMK) and calcineurin (CN), function as key modulators of transcriptional activation. However, it remains unclear whether these enzymes have any roles other than transcriptional activation in response to calcium signaling. To explore new roles of these enzymes, we undertook a quantitative phosphoproteomic analysis of calcium signaling. To focus on CaM-dependent enzymes, we stimulated normal mouse thymocytes in the presence of 1) DMSO (vehicle control), 2) cyclosporin A (a CN inhibitor), 3). ionomycin (a calcium ionophore), or 4) cyclosporin A and ionomycin, and compared their phosphoproteomic profiles. We used IMAC to enrich phosphopeptides. The enriched phosphopeptides were subsequently labeled with iTRAQ reagent. These peptides were finally separated with a use of strong cation exchange chromatography, and were identified and quantified by LC-MS/MS. As a result, we identified 8586 phosphopeptides. Of these peptides, 50 phosphopeptides showed a decrease by the treatment of cells with ionomycin, and this decrease was canceled by cyclosporin A treatment. We thus defined 38 proteins that include these phosphopeptides as candidates for CN substrates. These candidates include NFATc2, a well-known target of CN, and were classified into functionally diverse groups. Sequence comparison analysis revealed that CN preferentially dephosphorylates proline-directed serine phosphorylation (pSP). In addition, motif-based classification among all identified phosphopeptides showed an increase in CaMK-dependent phosphorylation by treatment with ionomycin, whereas not in pSP. On the basis of these data, we concluded that CaM-dependent enzymes would have multiple roles other than transcriptional activation via dephosphorylation of pSP during calcium signaling.

Keywords: phosphoproteome, calcineurin

POS-02-276 Quantitative Variation of Protein Components and Their Phosphorylations in the SWI/SNF Chromatin Remodeling Complex Associated with High Malignancy of Ovarian Clear Cell Adenocarcinoma

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Ovarian clear cell adenocarcinoma (CCA) is a highly malignant type of ovarian cancer. Somatic mutations and down-regulations of *ARID1A* (Baf250a), a key component of SWI/SNF chromatin remodeling complex, are frequently found in CCAs but not in the other tissue-types of ovarian carcinomas (non-CCAs). Recently, down-regulations of the other SWI/SNF components have been also reported in various tumor types, indicating their roles in tumor suppression. In this study, phosphoproteomic analyses were performed both in comprehensive and targeted manners using cell lines derived from CCAs and non-CCAs. For the comprehensive analysis, phosphopeptide mixtures TiO_2-enriched from the tryptic digests of cell lysate were subjected to the shotgun LC-MS/MS analysis. A total of 194 phosphopeptides showed CCA-specific up/down-regulation (ANOVA, $p < 0.01$), including 15 phosphorylation sites of three SWI/SNF components including Baf250a. Immunoblot assay using specific antibodies indicated that the three components were down-regulated in some CCA cell lines but not in non-CCA cell lines. Using CCA cell lines with positive expression of each SWI/SNF component, the phosphorylation levels were analyzed for 38 phosphorylation sites of these components, including those deposited in Uniprot database. Proteins immunoprecipitated from cell lysate were used for LC-selected reaction monitoring (SRM) assay. Pairs of phospho/nonphospho peptides including each phosphorylation site were quantified simultaneously, showing both up and down-regulation of phosphorylation levels occurred independently in each site of SWI/SNF component in CCAs.

These findings may elucidate that both down-regulations and aberrant phosphorylation patterns of SWI/SNF components are involved in high malignancy of CCA.

Keywords: phosphorylation, SRM, ovarian cancer

POS-02-277 Improved Phosphotau SRM Assay Sensitivity Enables Multi-Site Tau Phosphorylation Quantitation in a Preclinical Model of AD Treated with Novel Small Molecule Inhibitors of Casein Kinase 1 Delta

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Neurofibrillary tangles, comprised of paired helical filaments (PHFs) of hyper-phosphorylated tau, are a pathological characteristic of Alzheimer's disease (AD). We previously characterised PHF tau isolated from post-mortem AD brain tissue by mass spectrometry. Subsequently, we developed the Phospho-Tau SRM assays to measure tau phosphorylation levels in preclinical AD models. The Phospho-Tau SRM 6plex assay enables the quantitation of total tau plus five phosphorylation sites; distinct human and mouse pThr181 measurements, pSer199, pThr231, pSer262 and pSer396 (human 2N4R numbering). The Phospho-Tau SRM 7plex assay quantifies six phosphorylation sites, pSer46, pThr50, pSer113, pSer396, pSer404, pSer433, as well as distinct measurements covering the R406W mutation, present in the TMHT tau transgenic mouse model. Prior to SRM analysis, phosphopeptides were resolved by microflow (100 μL/min) reversed phase chromatography (XBridge C18 3.5 μM, 1.0 x 100mm, Waters). The linear working range of the microflow-PhosphoTau SRM 6plex assay was 5-1000fmol on column (o/c), with CVs ranging from 5-20%. Per analysis up to 10 μg total protein per sample was consumed. Herein we describe the miniaturisation of the PhosphoTau SRM assays to nanoflow (200nl/min; Easy C18 3 μM ID 75 μM x 100mm, ThermoFisher). Preliminary data demonstrates a 50 fold improvement in linear working range, down to 100 atmol o/c, and detection of endogenous Tau phosphorylation levels in preclinical AD models from as little as 1 μg of material. The utility of the nanflow-PhosphoTau SRM assays to quantify multiple site specific phosphorylation events will be demonstrated in a tau transgenic mouse model treated with novel small molecule inhibitors of Casein Kinase 1 delta.

Keywords: selective reaction monitoring, targeted quantitative phosphoproteomics, Alzheimers disease

POS-02-279 Potent Lipolytic Action of Lactoferrin: Elucidation of the Lipolytic Action Mechanism in Mature Adipocytes, Using Proteomic Approach

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Background: Lactoferrin (LF) is a multi-functional glycoprotein. We have proved that enteric-coated LF significantly decreased visceral fat accumulation in the human clinical trial. As one of the LF's action mechanism, we reported that LF increased the cellular cAMP in mature adipocytes and caused lipolysis.
Objective: To clarify the underlying mechanism of lipolysis induced by LF.
Methods: Pre-adipocytes derived from rat mesenteric fat were differentiated into mature form, and cells were collected at 1, 3, 9 hour after LF treatment. Total proteins were extracted and digested by trypsin. Purified peptides were subjected to the analysis by LC-MS/MS. For the validation of proteomics analysis, some focused proteins were analyzed by western blotting.
Results: Proteomic analysis revealed that the expression levels of adenylate cyclase (AC), which increased cellular cAMP concentration, and hormone sensitive lipase (HSL) were up-regulated and that of perilipin (PLIN), which protected lipid storage droplets from the lipase, was down-regulated. These results seemed to be suggesting that LF promoted the lipolysis by regulations of protein expression levels. Furthermore, LF treatment had the tendency to increase phosphorylated HSL and PLIN. Time-course analysis by western blotting revealed that the phosphorylation levels of these proteins were significantly increased at 15 min. The enzymatic activity of PKA known as the inducer of phosphorylation of these proteins was also activated. These results suggested that PKA activated by cAMP transduced phosphorylation signals in adipocytes, and promoted fat breakdown event.
Conclusion: LF may promote the lipolysis in mature adipocytes by both regulations of protein phosphorylation and expression levels.

Keywords: lactoferrin, lipolysis, adipocyte

POS-02-280 SILAC-Based Quantitative Phosphoproteome Analysis of Glioblastoma Stem Cell Differentiation by High-Resolution nanoLC-MS/MS

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Glioblastoma is one of the most malignant brain tumors with the median survival of twelve months after diagnosis. Glioblastoma stem cells (GSCs) have been considered as a cause of glioblastoma's aggressive malignancy and possess the properties of cancer stemness such as self-renewal, pluripotency, high tumorigenicity and resistance to chemotherapy. In order to develop new therapies against GSCs, their differentiation-inducing mechanisms are intensively studied because the characteristics of tumorigenicity and drug resistance are known to be lost in differentiated states. Several cell signaling pathways including Notch, Sonic hedgehog and Wnt have been found to maintain stemness of GSCs, in which post-translational modifications, especially phosphorylation, have critical roles. In this study, we applied a combination of Stable Isotope Labeling by Amino acids in Cell culture (SILAC), TiO_2 phosphopeptide enrichment and nanoLC-MS/MS to quantitative phosphoproteome analysis of serum-induced differentiation in GSCs.
Here, we tried to perform SILAC-based comparative quantification of the phosphoproteome in the differentiation of GSCs isolated from the human glioblastoma patients. First, we observed the alteration of cellular morphology and confirmed the change of stemness and differentiation markers between serum-containing and serum-free conditions. Next, we recently obtained the phosphoproteome data by high-resolution nanoLC-MS/MS and analyzed the signaling network status with some pathway analysis software. We will report system-wide characterization of phosphorylation-dependent networks related to stemness conservation and differentiation in GSCs.

Keywords: phosphoproteomics, glioblastoma, cancer stem cells

POS-02-281 Phosphoproteomics of the Cellular Response to MAPK Blockade in V600EBRAF Mutant Cells

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Activating mutations in components of the MAPK pathway (e.g. NRAS, BRAF and RET) are prevalent drivers of many cancers (thyroid, melanoma, colon). The chief consequence of mutation is a hyperactive ERK1/2 MAP kinase able to promote cell proliferation, producing critical hallmarks of metastatic disease. Biochemically, the ERK pathway is well characterised, however how the pathway achieves different outcomes in the face of genetic aberrations of cancer and subsequent treatment with small molecule based inhibition is not clear. To investigate regulation of oncogenic MAPK pathway and clinical inhibition we have completed a phosphoproteomic analysis of a ^{V600E}BRAF cell-line after treatment with the mutation selective inhibitor Vemurafenib (PLX-4032) and MEK inhibitor (AZD-6244). From approximately ~2300 (~2000 site localised, ~1900 quantified) identified phosphopeptides, 97 showed significant regulation by treatments. Kinase landscape analysis revealed the involvement of 100 possible kinases, with 16 directly implicated by MAPK inhibition. As expected p38 family and CDK2/3 substrates were enriched amongst down regulated peptides, but remarkably the abundance of peptides containing consensus motifs for CK2 increases after inhibition. To validate our data and determine the genetic sensitivity to inhibition several sites have been measured by MRM-HR in distinct cell-lines; quantifying both phosphorylation and total protein abundance. Our investigation not only enabled the discovery of novel processes involved in aberrant MAPK signalling, but also highlights the response to drug treatments that sets a paradigm for designing effective combined therapeutic treatments.

Keywords: cancer, phosphoproteomics, MAPK

POS-02-282 Acetyl-Phosphate Links Metabolism to Global Acetylation Dynamics in *E. coli*

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Recent proteomic studies identified thousands of lysine acetylation sites in diverse organisms from bacteria to humans. However, little is known about their origin and regulation. We used a quantitative mass spectrometry-based quantitative proteomics approach to study acetylation dynamics at thousands of sites in the model bacterium *Escherichia coli* (*E. coli*). We identified more than 8,000 unique acetylation sites and found that acetylation occurred at a low level and accumulated in growth-arrested cells in a manner that depended on the formation of acetyl-phosphate (AcP) through glycolysis. Mutant cells unable to produce AcP had significantly reduced acetylation levels while mutant cells unable to convert AcP to acetate had significantly elevated acetylation levels. We showed that AcP can chemically acetylate lysine residues *in vitro* and that AcP levels are correlated with acetylation levels *in vivo*, suggesting that AcP acetylates proteins nonenzymatically in cells. Most acetylation occurred independently of the YfiQ acetyltransferase and the CobB deacetylase suppressed acetylation at~10% of sites. CobB-regulated acetylation sites were more sensitive to increased acetylation in growth-arrested cells and to AcP *in vitro*, suggesting that CobB suppresses acetylation at these sites to maintain very low levels of acetylation. These results uncover a critical role for AcP in bacterial acetylation and indicate that most acetylation in *E. coli* occurs at a low-level and is dynamically affected by metabolism and cell proliferation in a global, uniform manner.

Keywords: acetylation

POS-02-283 MRM-Based Absolute Quantitation of Metabolic Enzymes Acetylation

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Recent studies have revealed that acetylation is a well conserved metabolic regulatory mechanism that plays critical roles in regulating/coordinating cell metabolism. Zhao *et al* . have identified thousands of acetylation proteins during glycolysis, gluconeogenesis, the tricarboxylic acid (TCA) cycle, the urea cycle, fatty acid metabolism, and glycogen metabolism in human liver tissue. Protein acetylation is a transient occurrence, and typically presents in sub-stoichiometric amounts, therefore extracting biologically meaningful information from these acetylation sites requires an adaptable, sensitive, specific and robust method for their quantification. MRM-MS is a targeted quantitative technology commonly performed on QQQ-MS that generates unique fragment ions associated with their corresponding precursor ions that can be quantified in a very complex matrix, so our research focus on determining acetylation stoichiometry of some important metabolism enzymes using MRM-MS. Firstly, we choose appropriate peptides that can be used to absolutely quantify acetylation by MRM based on discovery experiments. Secondly, we synthesize peptides with incorporated stable isotopes (^{13}C, ^{15}N, etc.) as internal standards to mimic native peptides (AQUA) derived from proteolysis and develop method. And furthermore, we can use MRM-MS to quantify the acetylation dynamics of these metabolism enzymes under different physiology and pathophysiology conditions. The development of this quantitative workflow is a pivotal step for advancing our knowledge and understanding of the regulatory effects of protein acetylation in physiology and pathophysiology.

Keywords: MRM, absolute quantitation, metabolic enzymes acetylation

POS-02-284 Quantitative Phosphoproteome Analysis in Differentiation of Human Promyelocytic Leukemia HL-60 Cells

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Human promyelocytic leukemia HL-60 cells are differentiated into the neutrophilic cells by treatment with dimethylsulfoxide (DMSO). After the 5 to 7 days of differentiation process induced by DMSO treatment, HL-60 cells acquire the ability of phagocytosis, and their nuclei get lobulated. In order to investigate the molecular mechanisms of the changes of cellular function and morphology in the differentiation of promyelocytic leukemia cells, we analyzed the change of phosphorylation states of the cellular proteins during the differentiation of HL-60 cells.

HL-60 cells were cultured with and without DMSO treatment. Cells were harvested just before DMSO treatment and after the cultivation for 1, 3, and 5 days. The proteins were extracted from each cell sample, and digested with lysylendopeptidase and trypsin. Phosphopeptides were enriched by the immobilized metal affinity chromatography, and obtained phosphopeptides were labeled with the iTRAQ 4plex reagent. After fractionation by the strong cation exchange chromatography, phosphopeptides were analyzed by a nano flow LC-MS/MS system. Similarly, the changes of whole proteome of HL-60 cells during differentiation were also analyzed by iTRAQ-based quantitative proteomic approach.

As a result, the phosphopeptides derived from more than 2,000 proteins were identified and relatively quantified. Among them, more than 2,800 peptides were detected in both differentiated and undifferentiated samples, and some of the peptides were newly discovered. We could measure the relative amounts of the proteins from which about half of the detected peptides were derived, and revealed the relation between the changes of these phosphopeptides and those of the proteins during differentiation.

Keywords: HL-60 cells, phosphoproteome

POS-02-285 Optimization of Enrichment Conditions on TiO_2-Affinity Chromatography for Phosphoproteomic Analysis

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Protein phosphorylation is an important posttranslational modification that plays significant roles in cell growth, differentiation, and adhesion *via* a variety of signaling pathways. To identify this modification by proteomic strategies, titanium dioxide (TiO_2) columns have been widely used for phosphopeptide enrichment prior to mass spectrometry (MS) analysis. However, ionization of phosphopeptides was dominantly decreased in the presence of non-phosphorylated peptides. Thus, one of the key success factors is considered to be improvement in the enrichment efficiency of phosphopeptides. In the present study, we investigated the effects of various reagents on TiO_2 column chromatography, and found that polyhydric alcohols such as glycerol markedly improved phosphopeptide selectivity. In addition, the two-step elution method with ammonium hydroxide and bis-Tris propane solution also contributed to effective phosphopeptide recovery, which made it possible to perform more comprehensive analysis.

To verify the practical applicability of our improved method of phosphopeptide enrichment, we carried out discovery of target molecules of a multi-kinase inhibitor, Dasatinib, in PC3 prostate cancer cells. Over 7400 phosphopeptides and 2600 phosphoproteins were identified by LC-MS/MS analysis. Phosphorylation sites of target kinase domains, such as Y419-SRC and Y772-EPHA2, were detected as strongly suppressed phosphorylation sites under conditions of Dasatinib treatment. These results indicated that our method is useful for identification of the targets of kinase inhibitors, which will be useful for drug discovery and development.

Keywords: phosphoproteomics, posttranslational modification, phosphopeptide enrichment

POS-02-286 Identification of Novel p38 MAP Kinase Substrates Using *In Vitro* Phosphorylation

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Protein phosphorylation plays a pivotal role in regulating many basic cellular processes, including growth, differentiation, cell death and signal transduction. To clarify complex signaling networks in which protein kinases participate, it is important to identify and characterize substrates for protein kinases. However, it still remains difficult to globally identify direct substrates for a given protein kinase. Here we propose a novel proteomic approach using *in vitro* kinase assay to discover substrates for protein kinase. We first dephosphorylated cell extracts as a source of substrates and then phosphorylated them with recombinant p38 mitogen-activated protein kinase (p38 MAP kinase) as a model protein kinase. Phosphorylated proteins were enriched with IMAC and subjected to 2-D DIGE to quantitatively compare the profile of phosphoproteins between the samples undergone kinase reaction in the presence or absence of p38 MAP kinase. We identified 23 proteins as candidate substrates for p38 MAP kinase and 3 known substrates. We showed that seven selected candidates (DPYSL3, ALDH5, TIF1β, alpha-taxillin, unr, hnRNP H1 and SH3GL1) among them were phosphorylated in response to the activation of p38 MAP kinase in HEK293T cells by two-dimensional immunoblot and *in vitro* kinase assay. This proteomic approach could be applied to any protein kinase to globally identify kinase substrates.

Keywords: phosphoproteomics, IMAC, 2-D DIGE

POS-02-287 Multidimensional Phosphoproteomic Characterization of Human Embryonic Stem Cells SILAC Labelled Using a Chemically Defined Medium

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Stable isotope labeling with amino acids in cell culture (SILAC) is a prominent technique for comparative quantitative proteomics. SILAC is, however, traditionally limited to simple tissue culture regimens. Classic human embryonic stem cells (hESCs) culture is based on the use of mouse embryonic fibroblasts (MEFs) as a feeder layer. As a result, possible xenogeneic contamination, is a concern in conjunction with SILAC. In the context of proteomic characterization of post-translational modifications (PTMs) using enrichment strategies, the complexity of the cell culture regime may be even more harmful, as often abundant xenogeneic contaminants may compete with molecules of interest. We have previously demonstrated a feeder-free SILAC culture system based on a customized version of a commonly used chemically defined hESC medium developed by Ludwig et al. (2006) and commercially available as mTeSR1 which facilitated quantitative global proteomics of hESCs. Beyond the existing cell biology and proteome level characterization of cells grown under those conditions, we herein examine the impact of the culturing strategy in signaling pathways in labeled hESCs as compared to those grown under default conditions. Due to low abundance of phospho-proteins relative to unmodified proteins, their identification and characterization requires specialized enrichment protocols. For phosphoproteomics profiling of hESCs, we use a multidimensional phosphopeptide enrichment strategy employing titanium dioxide combined with sequential elution immobilized metal ion affinity chromatography (SIMAC) and hydrophilic interaction liquid chromatography (HILIC). We demonstrate, that mTeSR1-based SILAC labeling under adjusted culturing conditions, is fully compatible with advanced phospho-peptide enrichment strategies and greatly enhances the usability of quantitative proteomics by SILAC as a tool for the study of mechanisms underlying hESCs differentiation and self-renewal.

Keywords: SILAC, human embryonic stem cells (hESCs), phosphoproteomics

POS-02-288 Unravelling Cell Signaling Events with Sub-Minute Temporal Resolution

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Large-scale phosphoproteomics studies enable the profiling of more than 15000 phosphosites from mg size of cell extracts. Investigation of dynamic changes in protein phosphorylation is a promising approach to dissect signaling events and correlate interactions between kinases, phosphatases and their substrates. Many protein phosphorylation/dephosphorylation events take place rapidly in response to environmental perturbation and thus require special sample handling techniques. Here, we present a novel sample collection protocol tailored for phosphoproteomic analysis of fast signaling events. We measured changes in phosphorylation within the first minute following osmotic shock in *Saccharomyces cerevisiae* with a temporal resolution of 5 sec. Our approach provided an unprecedented temporal resolution enabling the collection of high quality phosphorylation profiles for ~5,500 phosphosites on ~1,600 proteins. Evolutionary analysis showed that our dynamic phosphosites are more conserved than static ones, and are more functionally significant. We also found high enrichment of these sites on regulatory proteins such as kinases and phosphatases. Particularly we detected dynamic changes in phosphorylation on more than 25 % of the 128 kinases from *S. Cerevisiae* proteome. Temporal resolution achieved in our experiments enabled the dissection of dynamic events within a MAPK pathway responsible for high osmolarity including dual phosphorylation of MAPK Hog1. We also for a first time identified dynamic changes in phosphorylation on the majority of key players such as Sla1 (S785, S996), Abp1 (S357), Akl1 (S12, S985), and Syp1 (S347, S405) involved in clathrin mediated endocytosis. Subsequent site mutagenesis experiments confirmed our findings and allowed us to get deeper understanding of interplay between osmotic shock and endocytosis events.

Keywords: phosphoproteomics, dynamics, osmotic shock

POS-03-001 Abrogated Expression and Identification of O-GlcNAcylated Proteins in Colorectal Cancer Cells

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O-GlcNAcylation is a post translational modification of proteins which are found in nucleus, cytoplasm, and mitochondria. Two enzymes, regulating the addition and removal of O-GlcNAc moiety on proteins, are O-GlcNAc transterase (OGT) and O-GlcNAcase (OGA), respectively. Although, a number of researches reveal that O-GlcNAcylation plays important roles in malignancies of cancers, the associations to colorectal cancer were not well explained. In this study, the expression levels of O-GlcNAcylation as well as OGT and OGA in colorectal cancer tissues (Grade II) and their adjacencies were examined by immunoblotting (n=7). The results showed that the expression levels of O-GlcNAcylation and OGT were increased while OGA level was unchanged in most colon cancer samples. OGT knockdown using siRNA approach was performed to study the biological effects of reduction of O-GlcNAcylation on colorectal cancer cell lines (HT29 and SW480). OGT knockdown cells led to the enhancement of invasion and migration of HT29 cells but decreased the anchorage independent growth when compared to scrambled control cells. The total proteins and O-GlcNAc immunoblotted proteins comparing between scrambled and OGT knockdown cells were identified using two-dimensional gel electrophoresis (2-DE). The significant differences of protein expression were not observed in 2-DE. However, using cell fractionation techniques, the O-GlcNAcylated proteins had different patterns among four fractions (nucleus, cytoskeleton, membrane, and cytoplasm) and the differences of O-GlcNAcylated proteins was observed between OGT knockdown cells and scrambled cells. These data indicate that O-GlcNAcylation may be a novel fine tune regulator which plays distinct roles in biological effects of colorectal cancer.

Keywords: O-GlcNAcylation, colorectal cancer

POS-03-002 Alcoholic Liver Disease Alters the Glycosylation of Membrane Proteins

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Glycosylation is one of the most important post-translational modifications of proteins. The global glycan profile of the cell membrane proteins from *ex-vivo* liver tissue was analysed to determine if changes in membrane glycosylation are associated with the liver damage caused by alcoholic liver disease (ALD). Liver tissue samples were obtained from ALD patients with end-stage cirrhosis developed as a result of excessive alcohol consumption and from non-diseased donor tissue. Membrane proteins were enriched by ultra-centrifugation and Triton X-114 phase partitioning, *N*- and *O*- glycans were subsequently released by PNGaseF treatment and reductive beta-elimination and the released glycans were analysed by graphitised carbon LC-ESI-MS/MS and the relative abundance of the structures determined. Microarray analyses of glycosylation associated enzymes were carried out on the same tissue samples. The global glycosylation profiles of membrane proteins of liver samples from six individuals were compared from each category of ALD and non-diseased tissue. We show that the glycosylation profile of tissue from patients with ALD exhibit significant changes in the abundance of specific glycan structures compared to the non-diseased tissues. On a global scale it was observed that fucosylated and sialylated glycan structures were up- and down-regulated respectively. These changes were supported by microarray data which showed up-regulation of the associated fucosyltransferases (FUT4 and FUT8) and down-regulation of sialyltransferases (ST3GAL6 and ST3GAL2) genes, suggesting that alcohol-induced damage to the liver causes specific alterations in the protein glycosylation pathway.

Keywords: glycosylation, alcoholic liver disease, glycosyltransferases

POS-03-003 Large-Scale Identification of Mouse and Human N-Glycoproteins and Data Sharing Through An Experimental-Based Glycoprotein Database, GlycoProtDB

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Protein glycosylation is a common post-translational modification that plays important roles in protein functions. However, functional studies of glycans remain technically demanding because the glycans show diverse and heterogeneous structures. We believe that the first step to elucidate the glycan functions is to determine the status of protein glycosylation systematically under physiological conditions. Therefore, we analyzed a series of glycoproteomes in mouse tissues and serum to identify glycosylated proteins, glycosylation sites and glycan structural motifs at each site utilizing "lectin-IGOT-LC/MS method". In total, over 2,500 glycoproteins were identified and about 5,600 sites were mapped on the sequences and then the dataset was registered to our experimental-based glycoprotein database, GlycoProtDB [http://jcggdb.jp/rcmg/gpdb/index.action]. The knowledge on the DB also serves to uncover the status of protein glycosylation by coupling with systematic lectin array analyses of mouse tissues performed as a part of the B/D-Glycoproteomics Project (Please refer to the poster of Kuno A. *et al.*). Meanwhile it is widely recognized that the structure of glycans produced by cancerous cells is altered and different from that of original/healthy cells. The aberrant glycans are good candidates of the disease biomarkers. Thus the human glycoproteins identified for the biomarker development have been also registered to the GlycoProtDB.

Keywords: glycoproteome, glycoprotein database, glycobiomarker

POS-03-004 Milk Protein Modifications Associated with Processing Treatments

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Milk is subjected to a number of processing steps prior to consumption. This includes treatments such as homogenisation, pasteurisation and filtration. These steps may induce chemical reactions which result in protein modifications such as glycation, carbonylation and cross linking. The aim of this work is to increase our understanding of the impact of such treatments, by investigating the specific proteins that undergo modifications; the type and location of these modifications; when the modifications occur; and to what extent.

For this study, samples of bovine milk were treated using three different levels of pasteurisation: 72°C for 15 sec; 115°C for 9 sec (extended shelf life (ESL)) and 140°C for 3 seconds (ultra heat temperature (UHT)). The samples were separated into casein and whey fractions prior to analysis and were compared pre and post pasteurisation to determine the location and type of proteomic modifications occurring. Taking into consideration the physical properties of casein and whey proteins and the requirement to identify changes in protein modifications, isoelectric focusing and ion-exchange separation methods were investigated prior to LC-MS/MS analysis for suitability to examine changes occurring during pasteurisation.

Differences in protein modifications between raw milk and the three different types of pasteurised milk were identified. An in-house developed algorithm and scoring system was used to evaluate and compare the levels of modification in the different samples. This study highlights how proteomics provides a valuable tool for profiling the complex chemical reactions which occur in foods and may lead to the development of new optimised processes for the food industry.

Keywords: LC-MS/MS, food processing, protein modifications

POS-03-005 Multi-Acquisition Ion Mobility Strategies Utilizing LC/MS and MALDI for the Characterization of Enriched Glycopeptides

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Glycoprotein samples originating from horseradish peroxidise (HRP) and fetal calf serum bovine fetuin were denatured using TFE before reduction and alkylation with DTT and IAM respectively, generating a mixture of peptides and glycopeptides. A HILIC spin column was used for glycopeptide enrichment. Comparative evaluation of enrichment using TiO_2 spin columns was also performed for fetuin. Following proof of principle, a more complex cell lysate sample was investigated. For LC/MS, glycopeptides were separated using a nanoscale LC system, configured with a trap column and a 75 μm analytical column. A 90 min gradient, 300 nL/min gradient was performed. Data was collected using a Synapt G2-S either in ESI or MALDI configuration using a combination of data independent (MS^E, $HDMS^E$) and dependent (DDA) acquisition strategies. Time Aligned Parallel (TAP) fragmentation was utilized for MALDI acquisitions. Raw data was processed using dedicated post processing software and searched against sequence specific database(s) to provide protein identifications and sites of glycosylation. A false discovery rate of 4% was implemented. Processed spectra were exported and searched using the GlycoPeptide Search tool to provide glycan composition. Data collected with $HDMS^E$ shows greater protein and glycan sequence coverage and is complimentary with the MALDI collected data. This method of analysis and data processing provides a means of generating protein identifications, site of glycosylation and glycan composition in an automated workflow without the need of deglycosylation and derivitization methods being implemented. The aim moving forward is to apply this methodology for high throughput glycopeptide analysis.

Keywords: glycosylation, ion mobility, HILIC

POS-03-006 Advanced nano-LC-MALDI Spotting System for the Detection of Low-Abundance Glycopeptides from Complex Samples

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Introduction: While online LC-ESI-MS is the mainstream of the mass analysis of biomolecules in the proteomics field, offline LC-MALDI-MS systems, which offer advantage to the detailed analysis of protein PTMs, have also been developed. Recently, we developed a prototype of MALDI spotter suitable for nano-LC-MALDI system to improve sensitivity of PTM peptides analysis.
Methods: The novel MALDI spotter instrument was designed for the highly precise and accurate collection of nanoliter fractions eluting from nano-LC system on MALDI targets to support 1536 well format. The probe was constructed from 0.075 mm I.D. 0.150 mm O.D. x 150 mm length fused silica capillary tubing. Unison™ UK-C18 resin (Imtakt, Japan) was self-packed into the capillary with sol-gel frit. The 2,5-dihydroxybenzoic acid (DHB) pre-coated targets were prepared using piezoelectric inkjet device with the spot size of 150 µm diameter. MALDI-QIT-TOF MS (AXIMA Resonance™; Shimadzu/Kratos, UK) measurement was performed in positive ion mode.
Results: The performance of this system has been evaluated in terms of a high selectivity and high sensitivity detection of the PTM-containing peptides. The peptides with PTMs (glycosylation) spiked in high-abundant complex mixtures were reproducibly detected using the nano-LC-MALDI (RP) system combined with multi-dimensional LC (SCX-HILIC). The improved MALDI spotter with nano-LC system is a powerful tool for the highly sensitive analysis of low-abundance peptides and therefore this new system represents a significant advancement in the sample complexity in the field of proteomics or PTM analysis.

Keywords: LC-MALDI spotter, column-integrated probe, glycopeptide

POS-03-007 In-Depth Characterization of Glycopeptides by Combination of CID and ETD Fragmentation After Charge State Enhancement

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For complete glycopeptide characterization, like in biomarker discovery and therapeutic glycoprotein QC, both the localization of the glycosylation sites and determination of protein sequences are required. CID of glycopeptides mainly yields glycan fragments but produces limited information about the peptide backbone. In contrast, Electron Transfer Dissociation (ETD) dissociates the N-Cα bonds of the peptide backbone, while the glycan remains as a whole attached to the amino acid residue. Therefore, the combination of CID and ETD fragmentation is ideal for the comprehensive characterization of glycopeptides. Several standards (fetuin, HCG and EPO) were reduced, carbamidomethylated and digested with trypsin, *argC* or GluC. The generated (glyco) peptides were separated on a Dionex nanoRSLC system (Acclaim PepMap C18) and analyzed with an amaZon speed ETD ion trap, equipped with a CaptiveSpray source (Bruker Daltonics). Solvent-enriched nitrogen was used as sheath gas to enhance glycopeptide ionization. CID and ETD were done in auto-MS/MS mode in enhanced resolution. Glycopeptide spectra were classified within ProteinScape 3.1 and searched against the GlycomeDB database by the GlycoQuest search engine.
The lower ionization efficiency and a high glycosylation micro-heterogeneity make the analysis of N- and O-glycopeptides still difficult. In particular ETD fragmentation can suffer from low charge states for high mass glycopeptides. Thus a new nano source set-up has been used to enhance glycopeptide charge states and to increase their overall signal intensities: solvent-enriched sheath gas is introduced into the CaptiveSpray source. The MS signal for glycopeptides is most enhanced for large glycans attached and/or high degrees of sialylation. Both N- and O-glycosylation are determined and similarities as well as differences are discussed in CID and ETD fragmentation.

Keywords: glycopeptides

POS-03-009 Identification of Ectonucleotide Pyrophosphatase/Phosphodiesterase 3 (ENPP3) as a New Modifier of Glycan Biosynthesis

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Our previous studies on a brain-specific β1,6-*N*-acetylglucosaminyltransferase, GnT-IX (GnT-Vb), a homolog of GnT-V, revealed that the enzyme has a broad transfer activity toward *N*-linked and *O*-mannosyl glycans and its gene expression is regulated by epigenetic histone modifications. Herein we demonstrated the existence of an endogenous proteinaceous inhibitor for GnT-IX that functions as a key regulator for GnT-IX enzymatic activity in Neuro2a (N2a) cells. We successfully purified this inhibitor from N2a cells and found that it is identical to ectonucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3) as evidenced by mass spectrometry and examinations on the knocking down and overexpression of ENPP3. The underlying mechanism of GnT-IX inhibition was found to be the ENPP3-catalyzed hydrolysis of the nucleotide-sugar donor substrate, UDP-GlcNAc. Indeed, the ENPP3-knockdown cells exhibited significantly increased levels of intracellular nucleotide sugars that suggests a biological function of ENPP3 in the regulation of the intracellular nucleotide sugar levels. In addition to chaperones or other known regulators of glycosyltransferases, the ENPP3-mediated hydrolysis of nucleotide sugars may have significant impacts on broad-spectrum glycosyltransferase activities and could be responsible for altering the total cellular glycosylation profile and modulating cellular functions.

Keywords: glycosylation, N-acetylglucosaminyltransferase, ENPP3

POS-03-010 Application of the Lectin Microarray System to Glycome Mapping of Mouse FFPE Tissue Sections

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Cell glycome is defined by the glycan synthesis machinery regulated by harmonized expression of more than 100 glycogenes. The machinery-dependent glycome is distinctive in each cell type and drastically shifts during cell differentiation, tumorigenesis, and tumor progression of the cells. Thus, it motivates us to find the biological functions of glyco-epitopes. Glycan-targeted histochemical analyses in mouse tissue sections using lectins and antibodies against glycogene products have provided some key information to characterize specific histological types of cells in biology and pathology. In this study, we have sophisticated this approach toward the comprehensive analysis targeting the cell glycome. We adopted a lectin microarray system for the rapid and systematic glycome shift analysis targeting formalin-fixed paraffin-embedded (FFPE) mouse tissue sections. For the initial trial, we used commercialized tissue array (5 serial sections of the same tissue block) comprising 11 organs (brain, lung, heart, thymus, spleen, pancreas, skin, kidney, small intestine, testis, and liver) originated from C57BL/6J mice (8w, male). As a result, we obtained a total of 93 glycan profiles from every 1 mm^2 of tissue sections. The hierarchical clustering could divide each organ into several groups corresponding to morphological difference of tissue by signal patterns of 45 lectins. Now our research has gained interest in the variability and distribution of cell glycome in the tissues, i.e., tissue glycome mapping toward the establishment of "glycoproteome atlas" handled by B/D-GPP initiative.

Keywords: glycome, lectin, FFPE tissue

POS-03-011 Off-Line Hydrophilic Interaction Liquid Chromatography Enrichment of Glycopeptides Coupled with LC/MS Analysis

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Glycoproteins are directly related to numerous biological processes and diseases because protein glycosylation is one of the most important post-translational modifications. However, the glycans attached to the protein are heterogeneous and the sites of glycopeptides are diverse. The structural analysis of glycoproteins is the most difficult and challenging task. In addition, it is well known that MS sensitivity of glycopeptides from tryptically digested glycoprotein is quite less than that of nonglycosylated peptides due to the suppression of the glycopeptides ionization during MS analysis and the proportions of the glycopeptides from total tryptic peptide mixture are minor. It is highly demanded to find out the best methods how to effectively enrich glycopeptides in the analysis of glycoproteomics to prevent the ion suppression effect from coeluted nonglycopeptides. Many kinds of HILIC materials (ZIC-HILIC, Sepharose and Click Maltose) are commonly used. We compared the glycopeptides enrichment efficiency of each HILIC material for tryptic glycopeptides digested from standard glytcoprotein by using UPLC/LTQ-Orbitrap mass spectrometer coupled with CID and HCD fragmentation techniques. GPA (Glycoprotein Analysis) platform which is a homemade software in our laboratory was automatically identified many different type of glycopeptides. We applied the best glycopeptides enrichment method (ZIC-HILIC) to human plasma sample. After enrichment, the number of glycopeptide spectra from human plasma highly increased.

Keywords: off-line hydrophilic interaction liquid chromatography, glycopeptide enrichment

POS-03-012 Comprehensive Characterization of the Phosphorylation and Glycosylation of OPN Using Integrated Mass Spectrometry-Based Approaches

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Osteopontin (OPN) is a multifunctional phosphorylated glycoprotein and it is involved in a broad range of functions in both physiological and pathological processes. A significant regulation of OPN function is mediated through PTM (post-translational modification). In this study, the detailed characterization of phosphorylation and glycosylation of recombinant OPN was conducted by employing an integrative proteomic and glycomic approach. A total of 31 phosphoresidue were identified, including two novel phosphoresidues (T190, Y225), by using two kind of protease digestion.Asn79 of human OPN was experimentally revealed as an N-glycosylation site for the first time. 14 neutral N-glycans were detected by MALDI QIT TOF-MS and the major N-glycans were assigned as complex type by MS-MS analyses. 22 O-glycopeptides of human OPN were also reported for the first time and monosaccharide compositions of these O-glycopeptides were deduced by RPLC-LTQ-Orbitrap MS. The presence of some important carbohydrate epitopes, such as Tn epitopes, (α-2, 3) sialylation, and (α-2, 6) sialylation was also revealed by lectin blots. Our results will help to understand the structure-function relationship of human OPN.

Keywords: OPN, phosphorylation, glycosylation

POS-03-013 LC-MS Ion Trap Workflows for Glycan and Glycopeptide Analysis Using CID and ETD Fragmentation

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Protein glycosylation is the most complex form of PTMs. It plays a crucial role in many biological processes e.g. enzymatic or hormone activity. The glycan moieties are involved in biological processes. Changes in glycosylation patterns are associated with a number of human diseases, e.g. cancer. Sophisticated ways for the interpretation of glycan and glycopeptide MS data are therefore crucial. For mass spectrometric analysis of therapeutic glycoproteins, *N*-glycans are generally cleaved with PNGase F, and analyzed separately yielding the overall glycan pattern. Biomarker analysis on the other hand focuses on the analysis of proteolytic glycopeptides. This allows distinguishing the glycosylation at different sites, since changes may influence the glycoprotein properties in different ways. The analysis of released glycans in positive mode by CID (giving Y- and B-ions) provides identification of their composition and the sequence of the monosaccharide residues. Measurements in negative mode provide additional information. E.g. diagnostic and cross-ring fragment ions give information about the position of fucoses [1] or linkages [2]. The identification of glycan compositions and the unambiguous assignment of glycosylation sites can be carried out by analyzing glycopeptides. Here, the combination of CID and ETD fragmentation is powerful for characterizing glycan structures (CID) and the glycopeptide sequence (ETD). An overview over the different analysis workflows performed on an ion trap MS (amaZon speed ETD) is demonstrated on different samples. [1] Harvey, D. J.; *J Am Soc Mass Spectrom* **2005***, 16,* 622-659.[2] Tang, H., Mechref, Y. and Novotny, M. V.; *Bioinformatics* **2005***; 21(Suppl 1),* i431-i439.

Keywords: glycosylation

POS-03-014 Proteomic Analysis of O-Glcnacylated Proteins Associated to Cancer Metastasis in Breast Cancer Cells

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O-GlcNAcylation is a post-translational modification of nuclear, cytoplasmic and mitochondrial proteins and regulated by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) which catalyze the formation and removal of β-D-N acetylglucosamine (O-GlcNAc), respectively. Emerging evidence reveals that aberrant O-GlcNAcylation is associated with malignant phenotypes of cancer; however, the functional roles of this modification remain unclear and O-GlcNAc proteomic is largely unexplored. The goals of this study were to (1) examine the levels of O-GlcNAcylation and enzymes controlling this modification in breast cancer cell lines (MDA231 and MCF-7) in comparison to a normal breast cell (HMEC); (2) study the functional roles of O-GlcNAcylation, and (3) identify O-GlcNAc modified proteins. Using O-GlcNAc and OGT immunoblotting, both cancerous cells had an increase of O-GlcNAcylation level when compared to normal breast cells and this augmentation was associated with increased OGT level. Transient knockdown of OGT was performed using si-RNA approach in breast cancer cells. Compared to scrambled control, OGT knockdown cells showed that decreasing O-GlcNAcylation was related to inhibition of cancer growth determined by soft agar colony formation assay and non-adherent condition (Poly-Hema coated plates). Using 2-D IEF/SDS-PAGE and LC-MS/MS analysis, OGT knockdown cells revealed up-regulated proteins including enzymes/proteins involved in RNA processing and modification, stress response, signal transduction and energy metabolism. These data suggest that O-GlcNAcylation and certain O-GlcNAc modified proteins may promote malignant characteristics of breast cancer through the mechanisms of anchorage independent growth and anoikis resistance.

Keywords: O-GlcNAcylation, breast cancer

POS-03-015 Comprehensive Glycome/Glycoproteome Analysis in α1,3-Fucosyltransferase-9 Knockout Mice

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α1,3-Fucosyltransferase 9 (Fut9) acts as a key enzyme to synthesize the Lewis x structure (Galβ1-4(Fucα1-3)GlcNAc-R) carried on glycolipids, proteins, and proteoglycans, which is known as an antigenic epitope of stage specific embryonic antigen-1 (SSEA-1) and also as a neural stem cell marker. We have generated α1,3-fucosyltransferase 9 deficient mice (*Fut9*$^{-/-}$ mice) and found atrophic gastritis, ulcerative colitis, in the mice. However, relation of these disease-related phenotypes with the glyco-epitope products has not been solved. In this study, we aim to perform comprehensive glycome/glycoproteome analysis between *Fut9*$^{-/-}$ mice and wild-type (wt) mice to acquire knowledge for phenotype-related glycosylation alteration on proteins *in vivo*. We initially collected the organs expressing Fut9 from wt mice, and the tissue samples were immunostained with antibodies/lectins to detect the glycan structures synthesized by Fut9. Through this study, we are trying to identify the proteins carrying the target glycans by glycoproteomic approach and to construct a tissue glycome map of wt mice using the highly-sensitive and high-throughput glycan profiler, lectin microarray system in comparison with that of the *Fut9*$^{-/-}$ mice.

Keywords: lectin microarray, lectin affinity capturing, glycoproteomics

POS-03-016 Site-Specific N-Linked Glycosylation Analysis by Nano-LC Tandem Mass Spectrometry Coupled with a Spectral Library Searching Approach

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Protein glycosylation is one of the most common forms of post-translational modification in eukaryotic proteins, playing critical roles in protein structures and functions. The most common approach of protein glycosylation analysis by mass spectrometry involves chemical or enzymatic release of the glycans from glycoproteins, followed by LC-MS analyses of the glycans and the deglycosylated peptides separately. However, the glycan heterogeneity on each glycosylation site is lost in this approach. In our work, protein N-linked glycosylation is characterized at the glycopeptide level to reveal the microheterogeneity on each glycosylation site. Glycopeptides were enriched by hydrophilic affinity interaction using cellulose microcrystalline, analyzed by nano-LC/MS, and fragmented by collision-induced dissociation in data-dependent acquisition mode. For automated identification, the resulting spectra are searched against a library of reference spectra of *N*-linked glycopeptide predicted by MassAnalyzer [Zhang et al, *Anal. Chem.* **2010,** *82*, 10194-10202], using SpectraST [Lam et al, *Nat. Methods* **2008**, *5*, 873-875]. Both the m/z and the intensity information of the possible fragmentation ions are taken into consideration in our search algorithm to achieve a more reliable glycopeptide identification. We validated our method using purified glycoprotein standards, and evaluated its potential to be used in automated profiling of complex samples.

Keywords: site-specific N-glycosylation analysis, automatic glycopeptide identification and profiling, spectral library searching

POS-03-017 Large-Scale Identification of Glycoproteins Having LDN Glycans for Mechanism Elucidation in Protein-Specific Glycosylation

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Protein specific glycosylation (PSG) has been reported to occur in a manner dependent on the carrier proteins. For instance, LDN (*N,N*'-diacetyllactosediamine, LacDiNAc: GalNAcβ1-4GlcNAc) is a unique glycan structure found on a certain kind of glycoproteins such as glycoprotein hormones secreted from the pituitary gland. On the other hand, LN (*N*-acetyllactosamine: Galβ1-4GlcNAc) is an abundant glycan structure widely observed on many kinds of glycoproteins. The LDN synthase (B4GALNT) and LN synthase (B4GALT) use uncovered GlcNAc residue in the non-reducing termini as a common glycan accepter to synthesize LDN-glycans and LN-glycans, respectively; however, the mechanism to recognize their specific carrier protein is still unclear. In order to understand the mechanism of PSG, we performed the large-scale identification of glycan carriers using differential glycoproteome technologies. The glycopeptide subsets having different glyco-form were captured from culture medium of neuroblastoma cells using affinity columns containing lectins with distinct specificity, *Wisteria floribunda* agglutinin (WFA) for LDN and *Ricinus communis* agglutinin (RCA) for LN, and peptide sequences of the glycoproteins were determined by LC/MS-based glycoproteomic method. Approximately 260 and 150 glycoproteins were identified as WFA(+) and RCA(+) glycoproteins, respectively. MALDI-MS analyses of glycans released from these glycopeptide subsets showed that both lectin columns could enrich glycopeptides according to specificity of each lectin immobilized. In this poster, we summarize the characteristic protein properties shown in the LDN and LN carrier subsets. This work was performed as a part of the Medical Glycomics (MG) Project supported by NEDO.

Keywords: glycoproteomics, lacdinac, glycosyltransferase

POS-03-018 Comprehensive Study of O-Linked Glycans of Erythropoietin

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Erythropoietin (EPO) controls the production of red blood cells in the bone marrow. Recombinant human EPO is produced on a large scale for treating anemia related to different diseases. However, it is also known as blood doping agent in endurance sports. Human EPO (~30 kDa) has one O- and three N-glycosylation sites. In pharmaceutical drug production, glycosylation heterogeneity of EPO is an important quality characteristic. Here we describe a mass spectrometric approach including dedicated software tools to automatically identify O-linked glycosylation patterns of EPO. EPO BRP (LGC Standards) and recombinant human EPO expressed in HEK 293 cells (Sigma-Aldrich) were reduced, alkylated and tryptically digested. All peptides were separated by nano-HPLC. Fractions were collected on a stainless steel target with a sheath flow of matrix solution (DHB in water/acetonitrile or HCCA in water/acetonitrile) providing co-crystallization of sample and matrix and supplied to MALDI-TOF/TOF-MS in linear and reflector mode. The ProteinScape software was used for protein (Mascot) and glycan database searches (GlycoQuest).The MS/MS spectra were used to evaluate a characteristic fragmentation pattern of O-linked glycopeptides. The pattern showed up the peptide m/z, one fragment peak -18 Da distance (loss of water) and peaks +203 Da (GalNAc) and +162 Da (Gal) and was applied to the complete dataset in an automatic way, which included the determination of the peptide mass. Subsequently, protein and glycan database searches were performed resulted in the peptide sequence and mainly core 1 structure with one or two neuraminic acids and 0-4 acetylations. Since not all acetylated EPO O-glycans are integrated in standard databases, an individual glycan database was created.

Keywords: EPO

POS-03-019 Qualitative and Quantitative Investigation of Glyco-Proteoforms from Prostate-Specific Antigen (PSA) in Healthy and Cancer Samples

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Qualitative and quantitative changes in glycosylation play a crucial role in medicine and in biopharma industry. In the current approach, *N*-glycosylation changes of PSA, an important cancer biomarker with a high variety of glycan structures on a single *N*-glycosylation site, were investigated by mass spectrometry. This was part of a study organized by the ABRF gPRG group in 2013. Glycan identification was performed on the glycopeptide level, whereas the intact proteoforms were used for relative quantitation. This combined bottom-up and intact protein approach provides a powerful tool for analyzing the glycosylation profile - the first for in-depth glycan structure elucidation and the second for elimination of digestion artifacts and reduction of glycan charge derived artifacts.
For the bottom-up approach, reduced, alkylated and tryptically digested PSA was separated by LC-MS/MS using ESI ion trap MS. Glycopeptide spectra were extracted from the LC-MS/MS dataset using a classifier algorithm and masses of the peptide moiety were automatically determined. Glycan database searches using the GlycoQuest search engine were performed. Glycan quantitation was carried out on intact PSA using an Ultrahigh Resolution (UHR)-ESI QTOF. Monoisotopic masses were derived from maximum entropy deconvoluted spectra. More than 50 glycans were identified.
Quantitation performed on intact PSA showed significant changes toward sialylated glycans in the cancer sample. For the proper quantitation, the mass areas for all previously identified glycans were compared: about 30 % up-regulation in the cancer sample, and about 30 % were down-regulation resulted. This result was in coincidence with quantitation results on released glycans of the identical PSA samples.

Keywords: glycosylation, intact protein, GlycoQuest

POS-03-020 Enhancement of the Search Function in JCGGDB

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As entering upon the final year of the Japan Science and Technology Agency (JST) / National Bioscience Database Center (NBDC) integrated database program in 2013, we are driving the development of JCGGDB (Japan Consortium for Glycobiology and Glycotechnology DataBase) to achieve the integration of Japanese databases as well as the international collaboration.
JCGGDB was selected as a promotive program in the project, aiming at the integration of all the glycan-related databases in Japan and establishment of user-friendly search systems. As a part of the project, we also intended the integration of DB within Asia and the construction of ACGG-DB (Asian Communications of Glycobiology and Glycotechnology DataBase) in cooperation with Asian countries.
So far we have consolidated the data from various Japanese institutes into JCGGDB and developed search functions such as cross-search by keyword entry and integrated search by glycan structures and Glycoproteins.
In addition, "Glyco"-Keywords have been collected using a text-mining technique to analyze the frequency of keywords as well as the relationship between them. Collected data are also used to organize the synonyms and similar terms of glycan structures. We are developing retrieval techniques which enable the system to display the co-occurring words to be displayed as "Related Words" in the DB and to exchange links with related databases individually. Our final goal is to create the contents and search functions which could be easily and intuitively understood by every researcher.
This work is supported by Integrated Database project in JST/NBDC in Japan.
JCGGDB web site: http://jcggdb.jp

Keywords: glycoinformatics, glycoscience, glycoproteins

POS-03-021 An LC/MS-Based Glycoproteomic Approach for Systematic Identification of *In Vivo* Target Proteins Specific for a Glycosyltransferase Isozyme

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Model organisms carrying deletion or mutation in the glycosyltransferase gene exhibit various physiological abnormalities, suggesting that the loss of specific glycan motifs on certain proteins disrupts functions of the proteins, and leads to physiological abnormalities. To determine proteins responsible for the abnormalities and also their underlying molecular mechanisms, it is highly desirable to identify the target proteins for the glycosyltransferase responsible for the biosynthesis of the glycan motif. However, co-expression of other isozymes interferes with the identification of the target proteins of the isozyme.
The aim of our study is the identification of the target proteins specific for a glycosyltransferase isozyme in a proteome-scale. Here, we attempted to identify the target proteins for ß1,4-galactosyltransferase-I (ß4GalT-I), the most characterized glycosyltransferase. We comprehensively identified proteins carrying Galß1,4-terminated glycans in wild-type and *ß4GalT-I*$^{-/-}$ mice by utilizing a lectin-mediated affinity capture of glycopeptides carrying Galß1,4-terminated glycans, isotope-coding glycosylated site specific tagging (IGOT) and shotgun LC/MS analysis. Glycoproteins that were present in the wild-type mice, but not in the *ß4GalT-I*$^{-/-}$ mice, were assigned as candidates for the ß4GalT-I-specific target proteins, because the ß4GalT-I-specific target proteins were assumed not to be ß1,4-galactosylated in the *ß4GalT-I*$^{-/-}$ mice. Among 1176 proteins identified in mouse liver, 181 proteins were identified as the attractive candidates. Our approach offers common features and trends in the target proteins while multiple ß4GalT isozymes are present, and, thus, facilitates understanding of the mechanism that controls assembly of a particular glycan motif on specific proteins.

Keywords: glycoproteomics, lectin-IGOT-LC/MS, functional glycomics

POS-03-022 Inhibition of N-Glycan Biosynthesis Reveals Site-Specific Glycan Types by a Glycoproteome Approach

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The factors affecting the heterogeneities of glycosylation and the role of different glycoforms in harmonizing the function of glycoproteins are important questions for glycobiology. To explore these questions, the information of large amount of site-specific glycosylation is needed. The development of high throughput glycoproteomics approaches helped to unravel the distribution of N-glycosylation sites in a large scale. However, the site-specific characterization of N-glycoforms in -omic range is still a great challenge. Here, we presented a strategy of N-glycoproteome research based on inhibitors of N-glycan biosynthesis which can discriminate the glycosites carrying complex type glycans from those carrying high-mannose and hybrid types. Two chemical molecules, swainsonine and 1-deoxymannojirimycin were used to interfere with the N-glycan biosynthesis process, and hybrid and/or high-mannose type N-glycans were accumulated as the results of inhibition. The ConA lectin was applied to enrich the glycopeptides before and after the inhibition. Comparing the glycosites identified before and after inhibition through label-free quantitation, glycosites carrying complex type N-glycan could be picked up and identified. In total, 2498 unique N-glycosites from 898 proteins have been identified from HepG2 cells and the N-glycosylation type on each site was analyzed, in which 813 sites were found bearing complex-type glycans. These site-specific information of glycosylation was used to analyze the structure character, location and function of different sites and glycoproteins.

Keywords: glycosylation, mass spectrometry, lectin enrichment

POS-03-023 Glycan Changes of GP73 in Hepatocellular Carcinoma Cell Lines

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Protein glycosylation plays an important role in many biological processes and the alteration of glycosylation patterns have been observed in many diseases. Thus, relevance between N-glycans of glycoprotein and human disease has been a major foucus of glycobiologists. The LCA-reactive fraction of AFP (AFP-L3) has become a more specific glyco-biomarker for hepatocellular carcinoma (HCC). Recently, fucosylated Golgi protein-73 (GP73) was reported to be a major specific marker for early HCC. The aim of this study was to investigate the glycan patterns of GP73 in various HCC cell lines with differential metastatic potential (L02, HepG2, Huh7, SMMC7721, MHCC97L, MHCC97H, and HCCLM3). The protein expression level of GP73 was found to be higher in the metastatic cell lines than that in the non-metastatic cell lines by Western blot. 8 different lectins (WGA, PHA-E, AAL, LCA, PHA-L, Con A, SNA, and DSA) were selected to analyze GP73 glycoforms. The blotting index was calculated as visible glycan intensity obtained using lectin blot, divided by reblotted band intensity in Western blot using anti-GP73 antibody. Blotting index analysis suggested that fucosylation and bisecting GlcNAc of GP73 were increased along with the metastatic potential of the four cell lines (Huh7, MHCC97L, MHCC97H, and HCCLM3) by LCA and PHA-E lectin blot. Further, LC-ESI-HCD-MS/MS will be used to assess the glycan variation in these cell lines and explore their biological significance.

Keywords: GP73, hepatocellular carcinoma(HCC) cell lines, glycosylation

POS-03-024 A Complete Workflow Solution for Monoclonal Antibody Glycoform Characterization Combining a Novel Glycan Column Technology and Bench-Top Orbitrap LC-MS/MS

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Because glycosylation is so critical to the efficacy of antibody therapeutics, the FDA requires that a consistent human-type glycosylation be maintained for recombinant monoclonal antibodies, irrespective of the system in which they are produced. We characterized the N-linked glycans released from a monoclonal antibody by LC-MS/MS methods using a new column technology and a bench-top quadrupole-orbitrap mass spectrometer. Previous intact mass measurement of a monoclonal antibody identified glycoforms derived from the combination of any two of the three N-glycans, G0F, G1F and G2F. However, the mass errors for some of the intact glycoforms of this antibody ranged from 20-60 ppm which is larger than the expected 10 ppm for Orbitrap FTMS. Furthermore, the intact mass error for the deglycosylated form of this antibody was within 10 ppm, suggesting that some minor glycosylation forms of this molecule that were not detected at intact level had interfered with the observed intact mass of the major glycoforms. To further characterize this protein, released glycans from this protein were separated using the GlycanPac AXH-1 column. The separation and elution of glycans from GlycanPac AXH-1 column are based on charge. Glycans of each charge state are further separated based on their size and polarity. Characterization of glycans in each peak was performed by Full MS and data dependent MS/MS usingHCD. Three different types of glycans were found from this monoclonal antibody, the majority of glycans identified were neutral, including G0F, G1F and G2F. Also identified were less abundant, non-fucosylated forms of G0, as well as minor amounts of mono-sialylated and di-sialylated species that were not identified at the intact protein level. Rapid and sensitive antibody glycan profiling can be achieved using GlycanPac AXH-1 column and HR/AM Orbitrap LC MS/MS.

Keywords: glycan, antibody,Q Exactive,orbitrap

POS-03-025 Aberrant Expression of *O*-GlcNAcylated Proteins Associated with Primary Breast Cancer

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O-GlcNAcylation is a single *N*-acetylglucosamine (GlcNAc) attachment on hydroxyl group of serine or theronine residues of various proteins in nucleus, cytoplasm, and mitochondria. It is dynamically regulated by *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA), which catalyze the formation and removal of *O*-GlcNAc, respectively. Growing evidence reveals that this single sugar modification is associated to many metabolic diseases and cancer and though to a nutrient sensor in the high proliferated cells via the hexosamine biosynthesis pathway, a minor branch of glycolysis. However, the global of *O*-GlcNAcylated proteins and its role in cancer is still largely unexplored. This study, therefore, aims to examine *O*-GlcNAcylation level and identify *O*-GlcNAc modified proteins in primary breast cancer tissues. Here, we demonstrate that *O*-GlcNAcylation and OGT levels were increased in primary breast malignant tumors, not benign tumors. Using 2-D *O*-GlcNAc immnoblotting and LC-MS/MS analysis, 29 proteins were successfully identified and 7 was uniquely *O*-GlcNAcylated or associated with *O*-GlcNAcylation in cancer. Of these identified proteins, some were related to metabolic enzymes, proteins associated in stress responses, RNA metabolism, gene expression, and cytoskeleton. In addition, OGT knockdown showed that decreasing *O*-GlcNAcylation in specific proteins was related to inhibition of cancer growth *in vitro*. Taken together, it indicates that aberrant protein *O*-GlcNAcylation is associated with breast cancer. Abnormal modification of these *O*-GlcNAc proteins might be one of the vital malignant characteristics of cancer.

Keywords: o-GlcNAcylation, tumor biomarker, breast cancer

POS-03-026 Biomarker Discovery of N-glycopeptide of Immunoprecipitated Vitronectin from Human Cancer Plasma by High Resolution Mass Spectrometry

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The protein glycosylation is one of the major post-translational modifications, which is related to protein fate, folding and stabilization. Specially, it is well known that N-glycoproteins are cancer biomarkers, which can be found in human plasma during the process of cancer metastasis. However, the analysis of N-glycoprotein is most challenging in mass spectrometry (MS) because of their micro-heterogeneity and low abundance in human plasma. As a model study for the discovery of cancer biomarker in N-glycoprotein formula, Vitronectin is selected to be breast, colorectal and liver cancer biomarker candidates in complex human plasma. Immunoprecipitation was performed to pull down the Vitronectin from plasma sample, following hydrophilic interaction liquid chromatography (HILIC) to enrich their tryptic N-glycopeptide. Here, we identified many different kinds of typical Vitronectin N-glycopeptide by CID and HCD fragmentation in tandem mass spectrometry. In order to discover a cancer biomarker from human plasma, we performed N-linked glycopeptide analysis of Vitronectin. As the result, we first report several fucose and sialic acids in N-glycopeptide forms from human cancer plasma.

Keywords: glycoproteome, glycopeptide analysis, Vitronectin

POS-03-027 GlycoProtein Analysis (GPA): High-Throughput MS Platform for Automated N-Linked Glycoproteome Analysis and Its Application to Human Plasma for Biomarker Discovery

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We have reported the development of a fully automated high-throughput method for N-linked glycoprotein analysis, named GlycoProtein Analysis (GPA), which can identify the N-linked glycopeptide including glycan compositions and amino acid sequences, and individually quantify the each glycopeptides. N-linked glycoproteins were digested with trypsin and analyzed by LC/MS to obtain MS and MS/MS spectra. In the GPA platform, we used the glycan oxonium ions from HCD or CID fragmentation for glycopeptide selection and MW from MS1 scan for glycopeptide match using home-made human glycopeptides database. Then, we identified glycopeptide by comparing MS/MS spectra to theoretical those of glycopeptide matched. Label-free quantification of the identified N-glycopeptides was performed by summation of peak area of three most intense isotope MS in LC/MS chromatogram. The GPA platform has been evaluated for different types of N-glycopeptides tryptically digested from a variety of glycoproteins including RNaseB, Alpha-1-glycoprotein, and human plasma. As an application for biomarker discovery, we performed N-linked glycoprotein analysis in human hepatocellular carcinoma (HCC) plasma. Tryptic digested N-linked glycopeptides were enriched by ZIC-HILIC resin and analyzed by LC/Orbitrap-MS to obtain MS/MS spectra. A various of N-linked glycopeptides with different glycans were identified and quantified. Specially, the levels of fucose and sialic acid in N-linked glycoproteins were highly increased in HCC plasma compared to normal.

Keywords: glycoprotein, biomarker, plasma

POS-03-028 Breast Cancer Tumour Transformation from Primary Tumour to Secondary Site

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Breast cancer is a very heterogeneous disease and some patients are cured simply by surgical removal of the primary tumour while other patients suffer from recurrence and spreading of the disease. A number of treatment predictive factors have been identified such as tumour size, estrogen (ER) and progesterone (PgR) receptor status and human epidermal growth factor receptor 2 (HER2) status. Lymph node involvement is also assessed during surgery to determine if the tumour has started spreading and thus determine if lymph node stripping is required. The predictive factors assessing the nature of the tumour are all based on the status of the primary tumour. However, it could be anticipated that the cancer cells undergo a molecular transformation allowing the spreading to a secondary site. If the lymph nodes are positive for cancer cells or if distant metastases are identified, this disease would likely be more successfully treated by assessing predictive markers characterizing the cells having undergone spreading. We are analysing a unique tumour material comparing a set of 18 primary breast cancer tumours with matched axillaries positive for breast cancer cells and a set of 20 primary tumours with matched distant metastases spread to different sites in the body to further understand the molecular changes during the spreading and identify novel predictive markers. We are analysing these tumours for glycoproteins. Protein glycosylation is predominant in both membrane proteins and secreted proteins. Importantly, changes in glycosylation of these proteins have been shown to correlate with cancer states. Glycopeptide capture was used in this study to selectively isolate and quantifies N-linked glycopeptides from mixtures of glycoproteins. The captured glycopeptides were subjected to mass spectrometry analysis. Glycopeptide capture gave the most satisfactory results with 1145 proteins identified in total, all samples combined.

Keywords: glycosylation, breast cancer, biomarker discovery

POS-03-029 Site-Specific Analyses of N-Glycans on Haptoglobin in Sera of Patients with Various Kinds of Cancers: A Possible Implication of Differential Diagnosis for Cancer

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Fucosylation is one of the most important glycosylation involved in cancer and fucosylated proteins could apply to cancer biomarkers. We previously reported that fucosylated *N*-glycans on haptoglobin in the sera of patients with pancreatic cancer were increased by lectin blotting using AAL as well as mass spectrometry analysis (Okuyama *et al*, Int. J. Cancer, 2006). However, it has been reported that an increase in fucosylated haptoglobin was observed in various cancers. To know whether or not characteristic fucosylation is observed in each cancer, we presently undertook site-specific analyses of *N*-glycans on haptoglobin in the sera of patients with five kinds of operable gastroenterological cancers (esophageal, gastric, colon, gallbladder and pancreatic cancers), a non-gastroenterological cancer (prostate cancer) and normal controls. Haptoglobin has four potential *N*-glycans binding sites (Asn184, Asn207, Asn211, Asn241). Difucosylated tri-antennary *N*-glycans were significantly increased at Asn207 in esophageal, gastric and gallbladder-cancer samples. Monofucosylated tetra-antennary *N*-glycans were significantly increased at Asn211 in gastric, gallbladder and pancreatic-cancer samples. Monofucosylated di-antennary *N*-glycans were significantly increased at Asn241 in esophageal and gallbladder-cancer samples. We analyzed *N*-glycan alditols released from haptoglobin using LC-EIS MS to identify the linkage of fucosylation. Both Lewis-type and core-type fucosylated *N*-glycans were increased in gastroenterological-cancer samples. However, only core-type fucosylated *N*-glycan was increased in prostate cancer samples. These data suggest that the original tissue/cell producing fucosylated haptoglobin is different in each cancer and site-specific analyses of haptoglobin might be a novel type of cancer biomarker, which can make a differential diagnosis.

Keywords: haptoglobin, glycan, cancer biomarker

POS-03-030 Comparison of Single, Serial and Multiple Lectin Affinity Chromatography Using Secreted Proteins from Colorectal Cancer Cell Lines

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Colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer related to death and claims more than 639,000 lives worldwide annually, according to WHO statistics. Altered glycosylation such as, increased β-1, 6 GlcNAc branching, fucosylation and sialylation, as well as elevated high mannose glycans has been shown to be associated with malignant cancers including, CRC and may serve as potential biomarkers. Since, altered glycoproteins are present in minute quantities, an enrichment strategy may be critical in biomarkers search. Lectin affinity chromatography is one such strategy, which is widely used for glycoprotein enrichment.

In this study we have first performed *N*-glycan profiling of secreted proteins from three CRC cell lines, LIM1215, 1899 and LIM2405, representing different CRC phenotypes. *N*-glycans from secretome were released enzymatically using PNGase F enzyme and analysed on PGC-LC-ESI-MS/MS. Our analysis revealed that complex glycans were the most abundant glycan types observed in CRC secretome. We have also observed presence of Lewis blood group antigens and tri-antennary branched complex structures.

Lectin chromatography method was subsequently employed to enrich *N*-glycans from CRC secretome. Based on our initial *N*-glycan profiling of the CRC secretome, four lectins, namely, SNA (*Sambucus nigra*), AAL (*Aleuria Aurantia*), ConA (Concanavalin A), and PHA-L(*Phaseolus vulgaris* leukoagglutinin) were selected and used in three formats, single, serial and multiple lectin chromatography. These methods were then compared for maximum recovery as well as *N*-glycans profiles of enriched fractions. We found significant differences in the recoveries of the three methods. In addition, the *N*-glycan profiles enriched by each method were also quite distinct

Keywords: colorectal cancer, lectin affinity chromatography, N-glycans

POS-03-031 Acquired Drug Resistance by Alteration of Glycan Structure on Leukemia Cell-Membrane Glycoproteins

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The main treatment of leukemia is chemotherapy using antitumor drugs, but the acquisition of drug resistance often makes treatment continuation impossible. The mechanism of acquired drug resistance must be elucidated so that clinical treatment can be optimized. We found that the number of α2-6 sialylated *N*-glycans decreased on the cell-membrane proteins of a desoxyepothilone B (dEpoB)-resistant leukemia cell line (CEM/dEpoB cell line); dEpoB is an antitumor drug targeting microtubules. Moreover, these changes occurred on all cell membrane glycoproteins. The amounts of mRNA of the sialyltransferase group in the CEM/dEpoB cell line were measured by qRT-PCR, and the expression of β-galactosamide α2-6 sialyltransferase 1 (ST6GAL1) gene was decreased significantly. To ascertain whether reduction in the number of α2-6 sialylated *N*-glycans on the cell-membrane proteins of the CEM/dEpoB cell line was a cause or a result of acquired drug resistance, we used CHO cells (which lack expression of α2-6 sialylated glycans). We undertook comparative experiments of cell-growth inhibition by addition of an antitumor drug to the CHO cells and the CHO cells-transfected ST6GAL1 gene (CHO/ST6GAL1 cells). CHO/ST6GAL1 cells showed significantly greater cell-growth inhibition than CHO cells. Therefore, reduction in the number of α2-6 sialylated *N*-glycans could be the cause of dEpoB-resistant acquisition. Currently, we are investigating the relationship between dEpoB-resistant acquisition and α2-6 sialylated *N*-glycans using the CEM/dEpoB and CEM cell lines by transfecting and knockdown of the ST6GAL1 gene to ascertain if their cell lines rescue and lose the drug sensitivity.

Keywords: glycan, drug resistance, sialyltransferase

POS-03-032 Improvement of Lectin Microarray-Based Tissue Glycan Profiling with Lectin-Assisted Fractionation for Glyco-Biomarker Discovery

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Lectin microarray is known as a highly sensitive and high throughput technique for differential glycan profiling. Using this system, we previously developed an ultra-sensitive method for glycan analysis targeting small lesions on formalin-fixed paraffin-embedded tissue sections (FFPT) (Matsuda A., *et al.*, *BBRC., 2008*). In this study, we improved this method to be useful in the uppermost stream of the pipeline of glyco-biomarker discovery, that is, selection of discriminator lectin. To achieve this, we employed "lectin-assisted fractionation" of glycoproteins prior to the lectin microarray analysis. The methodological concept was proved by the following steps using colorectal FFPT array. (1) Comparative analysis of tissue glycan profiles between the colorectal cancer patients with good (n=34) and poor (n=11) prognosis. (2) Statistical analysis for screening of the discriminator lectin(s). (3) Fractionation of tissue glycoproteins by the selected lectin (s). (4) Comparative analysis of glycan profiles between the pre- and post-fractionation samples. As a result, we selected two lectins (AAL and ABA) as potential discriminators. Subsequent lectin microarray analysis for tissue glycoproteins fractionated by the AAL-affinity capturing enabled us to identify AAL (-) /ABA (+) /SNA (+) glycoproteins (e.g., sialomucin) as the biomarker candidates for prognosis prediction, which was consistent well with previous reports described regulation of sialomucin production in colon carcinoma cells.

Keywords: lectin microarray, lectin affinity capturing, glyco-biomarker

POS-03-033 Extensive Characterization of Glycoconjugates in Nanobioparticles in Blood - Analysis of HDL and Other Lipoproteins

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We describe for the first time a comprehensive approach for analyzing the glycome of HDL and other lipoprotein particles, highlighting that HDL are highly sialylated particles. This approach is applied to better understand a number of important biological functions of HDL that are likely mediated by glycans. Many of the functional proteins and lipids in HDL are potentially glycosylated yet very little is known about the glycoconjugates of HDL. We developed a detailed and quantitative analytical strategy to examine the exact glycan structures on the glycoproteins and glycolipids of HDL. HDL was isolated from plasma of healthy human donors by sequential micro-ultracentrifugation, followed by glycoprotein and glycolipid analysis by MS. N-glycans, glycopeptides, and gangliosides were extracted and purified followed by analysis with nano-HPLC-Chip Q-TOF MS and MS/MS. HDL particles were found to be highly sialylated. Most of the N-glycans (90%) from HDL glycoproteins were sialylated with one or two neuraminic acids (Neu5Ac). The most abundant N-glycan was a biantennary complex type glycan with two sialic acids ($Hexose_5HexNAc_4Neu5Ac_2$), and was found in multiple glycoproteins based on site-specific analysis. Fucosylated, high-mannose and hybrid type N-glycans were also observed. The observed O-glycans were all sialylated and most contained a core 2 type with two Neu5Acs, including those that were associated with apolipoprotein C3 and fetuin A. GM3 (monosialoganglioside, NeuAc2-3Gal1-4Glc-Cer) and GD3 (disialoganglioside, NeuAc2-8NeuAc2-3Gal1-4Glc-Cer) were the major gangliosides in the HDL. A 60% GM3 and 40% GD3 distribution was observed. Both GM3 and GD3 were composed of heterogeneous ceramide lipid tails, including d18:1/16:0 and d18:1/23:0.

Keywords: glycomics, LC/MS, glycoproteomics

POS-03-034 Significance of Glycosylation-Focused Proteome Strategy for Discovery of the Biomarker of Heart Failure

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Development of a specific, accurate, and effective marker of heart failure (HF) is strongly required for early diagnosis and prognosis, in addition to the commonly-used marker, brain natriuretic peptide (BNP). Because the majority of BNP precursor is *O*-glycosylated and this post-translational modification inhibits the precursor processing, we evaluated whether if changes in glycosylation during development of HF would be useful for discovery of a novel biomarker of HF. We utilized Dahl salt-sensitive rats fed high salt (8% NaCl; HS) and low salt (0.3% NaCl; LS) diets for 6-10 weeks from 6-week old as a HF group and a control, respectively. The quantitative gene expression analyses revealed up-regulation of genes coding enzymes involving the initiation steps of *O*-glycan synthesis, as well as other glycosylation and deglycosylation processes, in the left ventricle (LV) of HS rats compared to that of LS rats. Up-regulation of protein expression of these enzymes was confirmed by western blot analyses and enzyme-linked immunosolvent assays. Comparison of glycan profiles of the LV lysates and plasmas between HS and LS rats using the lectin array indicated decreases in T-antigen/sialyl-T, the core fucose of *N*-glycans, and high-mannose-type *N*-glycans in HS rats. These changes in gene and protein expression were observed even in HS rats with preserved ejection fraction. These results indicate that characteristic glycan modifications occur during formation and progression of HF and thus glycosylation-focused differential analysis is useful for mining a novel biomarker of early-stage HF.

Keywords: glycosylation, gene expression analysis, lectin array analysis

POS-03-035 Direct Proteomics Analysis of Protein Oxidation in Blood Plasma of Alzheimer's Disease Patients

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Oxidative stress is a major source of protein damage and has been closely related with ageing and ageing-related neurodegenerative diseases like Alzheimer's disease (AD). Increased level of oxidized and carbonylated proteins have been found correlating with AD pathological features in brain. So far, these results were mainly obtained from immunological or chemical enrichment methods. In the present study, we aimed at identifying and quantifying protein carbonylation/oxidation in AD blood plasma samples with conventional proteomics approach, and tried to correlate the extent of oxidation in blood proteins with AD progress. 218 human blood plasma samples were pooled into eight age-matched groups based on sex and disease progression. Each of the pooled samples was independently digested three times by trypsin, and analyzed in duplicates by liquid chromatography online with Orbitrap Velos mass spectrometer (MS) using both HCD and ETD MS/MS. Lysine carbonylation/oxidation was selected for analysis as it is known to be one of the most common carbonylated residues *in vivo*. Besides accurate mass (10 ppm), we also evaluated peptide sequence coverage, presence of adjacent fragments from both N- and C-terminus of the modified Lys, and chromatography separation of modified and unmodified peptides. Based on the mentioned criteria, at least one peptide belonging to serum albumin was reliably identified as containing oxidized Lys, and quantified using label-free approach. Oxidation occupancy of this peptide in healthy control was found significantly lower compared with AD and pre-AD patients. Our data has shown for the first time that oxidized proteins can be detected in complex matrix (plasma digest) by standard proteomics method without enrichment and that the oxidation stress signature in at least one protein (serum albumin) correlates with AD progression.

Keywords: Alzheimer's disease, oxidative stress

POS-03-036 Glycomic Approach for Identification of Diagnostic Markers of the Acquisition of Drug Resistance Against Microtubule-Targeting Drugs

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Chemotherapy is mainly used in the treatment of leukemia because surgical treatment cannot be conducted. However, continuation of chemotherapy, often results in leukemia cells acquiring drug resistance. A distinct and simple diagnostic method for the acquisition of drug resistance is required. We focused on glycan structures on cell-membrane proteins to find a useful diagnostic marker of the acquisition of drug resistance. The leukemia cell line CEM was selected for acquired drug resistance against vincristine (VCR) and paclitaxel (TAX), which are major antitumor drugs targeting microtubules, and glycan structures on the cell-membrane proteins of these resistant cell lines were analyzed by LC-ESI MS. CEM cell lines acquired drug resistance against 3 nM VCR; they showed a significant decrease in the number of sialylated glycans and a significant increase in the number of desialylated glycans on both *N*-glycans and *O*-glycans as compared with CEM cell lines. CEM cell lines acquired drug resistance against 25 nM TAX slightly; the number of sialylated glycans increased and those of desialylated glycans decreased. To ascertain if identical alterations occurred in the blood of leukemia patients, the analytical conditions for LC-ESI MS of glycan structures on lymphocytes in blood were investigated. We established the conditions that could analyze glycan structures using 3 mL of blood. Currently, we are analyzing glycan structures on lymphocytes derived from the blood of patients with leukemia using the derived analytical conditions. We are also investigating a distinct and simple diagnostic method for drug-resistance acquisition using lectin.

Keywords: glycan, drug resistance, sialylation

POS-03-037 Resolving Double Disulfide Bond Patterns in SNAP25B Using Liquid Chromatography-Ion Trap Mass Spectrometry

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Complex disulfide bond patterns in synaptosomal-associated protein of 25 kD B (SNAP25B) are thought to regulate neurotransmitter release in response to oxidative stress. However, the steric feasibility of each possible disulfide pattern in SNAP25B has not been assessed. In order to assess the steric feasibility of hypothesized closely spaced complex disulfide patterning in SNAP25B and also the feasibility of identifying complex disulfide bond patterns with mass spectrometry, we have developed a novel probabilistic analysis to un-ambiguously resolve complex double disulfide bond patterns using an ion-trap mass spectrometer. We analyzed fragmentation patterns of singly linked peptides to determine likely fragmentation events in an ion trap mass spectrometer and observed double and single backbone cleavage along with heterolytic cleavage of the disulfide bond. We modeled these same events in the doubly disulfide linked SNAP25B peptide and used a cumulative hypergeometric distribution with top-down scoring to both identify, and differentiate these bonding patterns. Because of the presence of unique MS/MS peaks, two of the bonding patterns were directly identified. The third was assigned based on full chromatographic separation and confirmed by modeling triple breakage fragments. In total, this work demonstrates the feasibility-and also limitations-of identification of complex intra-disulfide patterns using ion trap based collision induced dissociation based fragmentation methods.

Keywords: oxidation, dsulfide, SNAP25

POS-03-038 UniCarbKB: Connecting Proteomics with Glycomics

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An essential requirement for glycomics and glycoproteomics to progress is the development of informatics tools to interpret and store diverse glycan data and enable public accessibility. Ultimately the analytical data must relate to the function of these glycans and their glycoconjugates.
The NIH Transforming Glycoscience: A Roadmap for the Future report (1) identified the hurdles and problems faced by life science research due to the disconnected and incomplete nature of existing glycodatabases. In 2011 we introduced UniCarbKB (2) as an international initiative that aims to collect, distribute and extend resources and practices from glycobiologists to the whole biological research community. The mission is to provide a comprehensive, high quality catalogue of published and experimental information on the carbohydrates attached to proteins, and to integrate this data with the other '-omics' knowledgebases.
I will present on behalf of many participants, a summary of the start we have made on establishing the infrastructure and content of the publically available UniCarbKB. This will include current work on the data integration from UniCarb-DB, GlycoSuiteDB, GlycoBase, SugarBindDB, EuroCarbDB, RINGS and PubChem. The establishment of a glycomics bioinformatics hub on the ExPASy server and the linking of UnicarbKB to the proteomics knowledgebase UniProt will be described. Other data initiatives, currently in their infancy, will also be reported on. In the future it is hoped that the UniCarbKB knowledgebase, centered on a reference database of curated glycan structures, will become the key resource of quality information for glycoproteomic research.

(1) Transforming Glycoscience: A Roadmap for the Future: The National Academies Press, 2012
(2) Campbell MP, Hayes CA, Struwe WB, Wilkins MR, Aoki-Kinoshita KF, Harvey DJ, Rudd PM, Kolarich D, Lisacek F, Karlsson NG, Packer NH (2011) Proteomics 11:4117-21.

Keywords: glycomics, glycoproteomics, glycoinformatics

POS-03-039 DJ-1 Isoforms are Altered in Mouse Hypothalamus Upon High-Fat Diet

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In order to identify proteins involved in central processes of energy intake we analyzed hypothalamic tissue of mice fed by either high-fat or control diet using 2D difference gel electrophoresis in combination with LC-ESI-MS/MS.
In three different mouse strains, we found DJ-1 to be altered in high fat compared to control animals. As a difference in the amount of DJ-1 could not be found on mRNA or whole protein level, we analyzed DJ-1 isoforms by isoelectric focusing in combination with Western blot analysis. Thus, we could detect a shift to acidic DJ-1 isoforms upon high fat diet similar to the shift already described for DJ-1 in neurodegenerative disease states. Mass spectrometric measurements suggest that an oxidation of a cysteine residue within DJ-1 to sulfinic/sulfonic acid accounts for this shift. This modification might be important for DJ-1's function in the context of oxidative stress as well as cellular survival and might link nutrition-induced effects to neurodegenerative disease states.
Keywords: obesity, thiol modification, protein isoforms

POS-03-041 Cell Surface Proteome Profiling to Discover Therapeutic Targets for Adult T-Cell Leukemia

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The number of those infected with Human T-cell leukemia virus type 1 (HTLV-1) is estimated to be more than 1 million in Japan. The infection of HTLV-1 induces onset of Adult T-cell leukemia (ATL) which is one of the lethal blood neoplasms. Although anti-CCR4 humanized antibody drug (Mogamulizumab) was approved for the treatment of ATL, overall response rate was 50% and, more importantly, adverse effects were observed in all Mogamulizumab-administrated cases. To discover novel molecular therapeutic targets for ATL, we comprehensively profiled membrane subproteome of $CD4^+$ T-cells in which HTLV-1 infected cells are enriched. Tryptic digests of $CD4^+$ T-cells were prepared from 14 normal donors (ND), 21 asymptomatic carriers (AC), 21 HTLV-1 associated myelopathy/tropic spastic paraparesis (HAM/TSP) patients and 13 ATL patients. To focus on cell surface proteome, N-glycosylated peptides were specifically enriched by Isotopic glycosidase elution and labeling on lectin column chromatography (IGEL) (Mol Cell Proteomics. 2010, 9(9), 1819) method using custom-made 96-well syringe robot. The LC/MS/MS analysis and subsequent data processing on Expressionist® server system (Genedata AG, Basel) detected and quantified non-redundant 146,323 peptides from 69 samples. The 2-step statistical selection composed of Kruskal-Wallis test and feature selection using machine learning finally identified 127 classifiers which represented molecular signatures for each of 4 clinical groups. In this session, we would like to illustrate some important molecular characteristics of identified therapeutic targets and shape the future direction of ATL therapy.
Keywords: glycosylation, cancer, cell membrane

POS-03-040 Recent Advances in Glycoprotein Analysis by Mass Spectrometry

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Large-scale analysis of glycoproteins from complex samples is developing rapidly. Byonic is the first proteomics search engine that can identify peptides carrying N- and O-linked glycans. This software provides multiple ways to search for glycopeptides, including preset glycan databases (tables) and/or entering specific glycans. The search strategy can of course impact results. Here we show how a progression of searches, from wider to narrower in both proteins and glycans, enhancing sensitivity and specificity for glycopeptide identification.
Data was obtained from the following samples: glycophorin-A, PSA, human blood serum enriched for glycoproteins, and secreted proteins from human endothelial cells. All data were acquired on Thermo Orbitrap instruments and included both HCD and ETD fragmentation.
Large numbers of glycopeptides are identified, carrying up to two N-glycans, one N-glycan and one O-glycan, and up to four O-glycans, with only occasional ambiguities in modification placement and mass distribution. Site localization is often uncertain however for O-glycans in HCD spectra. Focused protein databases containing 10 - 200 proteins, and focused glycan tables, significantly improve the sensitivity of glycopeptide searches relative to full-database searches. More complex searches are under study.
Keywords: glycoproteomics, glycosylation, search engine

POS-03-043 Glycan Structural Elucidation on a Novel Quadrupole Dual Cell Linear Ion Trap Orbitrap Hybrid Mass Spectrometer

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MS is a powerful tool for glycan structural elucidation. Permethylation in combination with MS^n is a critical aspect of this approach. Only MS^n truly characterizes a glycan structure as it allows identification of heterogeneity, branching pattern, linkages and resolution of isobaric structures. Traditionally, this workflow has been done using low energy CID, where several stages of MS^n are required to comprehensively elucidate a glycan. The primary advantage of HCD fragmentation is the production of glycosidic, cross-ring, and internal double cleavage ions at the MS^2 level, where branching, linkage and resolution of isobaric structures are derived from the latter two types of ions. The availability of HCD MS^n would enable comprehensive glycan structural elucidation at much lower MS^n stages. We have recently built a new quadrupole dual cell linear ion trap orbitrap hybrid mass spectrometer that has novel architecture and ion transfer path enabling HCD MS^nanalysis. Permethylated ovalbumin glycans were infused into the mass spectrometer and data-dependent top 20 MS^2 spectra were acquired. For each MS^2 spectra subsequent top 20 MS^3 were acquired. Due to the faster scan rate for HCD acquisition, the acquisition time is rapid. Additionally the informative HCD MS^2 spectra enabled selection of key fragment ions to be taken to MS^3 for structural elucidation. Using this approach we were able to differentiate various structural isomers of ovalbumin glycans. On low energy CID MS^n this type of analysis typically required MS^7 or MS^8 stage of fragmentation for clear structural differentiation. Similar acquisition strategy was employed and various structural isomers were identified on Bovine Fetuin.
Keywords: glycan, multistage fragmentation, quadrupole dual cell linear ion trap Orbitrap hybrid mass spectrometer

POS-03-044 Mass Isotopomer Analysis of Metabolically Labeled Nucleotide Sugars and N- and O-Glycans for Tracing Nucleotide Sugar Metabolisms

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Nucleotide sugars are the donor substrates of various glycosyltransferases, and an important building block in N- and O-glycan biosynthesis. Their intercellular concentrations are regulated by cellular metabolic states including diseases such as cancer and diabetes. In order to investigate the fate of UDP-GlcNAc, we developed a tracing method for UDP-GlcNAc synthesis and utilization, and GlcNAc utilization using $^{13}C_6$-glucose and $^{13}C_2$-glucosamine, respectively, followed by the analysis of mass isotopomers using liquid chromatography-mass spectrometry.
Metabolic labeling of cultured cells with $^{13}C_6$-glucose and the analysis of isotopomers of UDP-HexNAc (UDP-GlcNAc plus UDP-GalNAc) and CMP-NeuAc revealed the relative contributions of metabolic pathways leading to UDP-GlcNAc synthesis and utilization. In pancreatic insulinoma cells, the labeling efficiency of a $^{13}C_6$-glucose motif in CMP-NeuAc was lower compared with that in hepatoma cells.
Using $^{13}C_2$-glucosamine, the diversity of the labeling efficiency was observed in each sugar residue of N- and O-glycans on the basis of isotopomer analysis. In the insulinoma cells, the low labeling efficiencies were found for sialic acids as well as tri- and tetra-sialo N-glycans, whereas asialo N-glycans were found to be abundant. Essentially no significant difference in secreted hyaluronic acids was found among hepatoma and insulinoma cell lines. This indicates that metabolic flows are responsible for the low sialylation in the insulinoma cells. Our strategy would be useful for systematically tracing each stage of cellular GlcNAc metabolism.
Keywords: mass spectrometry, glycosylation, glucose metabolism

POS-03-047 Age- and Sex-Associated Differences in the Glycopatterns of Human Salivary Glycoproteins and Their Roles Against Influenza A Virus

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Recent studies have elucidated that expression of certain glycoproteins in human saliva are increased or decreased according to age, meanwhile, human saliva may inhibit viral infection and prevent viral transmission. However, little is known about the age- and sex-associated differences in the glycopatterns of human salivary glycoproteins and their significant roles against influenza A virus (IVA). Here we investigate the glycopatterns of human salivary glycoproteins with 180 healthy saliva samples divided into six age/sex groups using lectin microarrays and fabricate saliva microarrays to validate the terminal carbohydrate moieties of glycoproteins in individual saliva samples. Furthermore, we assess the inhibiting and neutralizing activity of saliva against two strains of influenza A (H9N2) virus. We find that seven lectins (e.g., MAL-II and SNA) show significant age differences in both females and males, and seven lectins (e.g., WFA and STL) show significant sex differences in children, adults and elderly people. Interestingly, we observe that elderly individuals have strongest resistance to IVA partly by presenting more terminal α2-3/6-linked sialic acid residues in their saliva, which bind with the influenza viral hemagglutinations. We conclude that age- and sex-associated differences in the glycopatterns of human salivary glycoproteins may provide pivotal information to help understand some age related diseases and physiological phenomenon.
Keywords: saliva, glycopattern, influenza A virus

POS-03-046 Modulation of the Intracellular Phosphorylation-Dependent Signaling Upon Sialoglycoconjugates Remodeling in Cancer

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Sialoglycoconjugates play a prominent role in cancer metastasis as result of aberrant sialyltransferases (STs) and sialyl hydrolases (SHs) expression and activity. Evidence shows that the level of STs and SHs expression is dramatically changed during tumorigenic transformation and invasiveness. Moreover, cell surface remodeling, catalyzed by extracellular or plasma membrane hydrolytic enzymes, has recently gained much attention due to the implication in the signal transduction mechanisms influencing the behavior of a biological system. However, no study has targeted in an unbiased way the modulation of phosphorylation-dependent signaling pathways upon STs and SHs silencing.
The analysis of isogenic non-metastatic (NM2C5) and metastatic (M4A4) human breast cancer cell lines identified a highly regulated sialyltransferase (STX) in different metastatic conditions using (glyco) proteomics, glycomics and glyco-gene microarray. This enzyme was shown to be important for the survival of several malignancies but did not affect non-cancerous cells. Using sensitive and robust mass spectrometry-based phosphoproteomics and sialioglycoproteomics we have investigated the quantitative changes in the Hela cells phosphoproteome upon modulation of the expression of STX sialyltransferase and Neu3 sialidase.
Quantitative phosphoproteomics using ITRAQ labeling and TiO_2 enrichment allowed us to identify the relation between sialic acid modulation and previously unreported phosphorylation-dependent signaling pathways. Moreover, this method clearly improves our understanding of the sialoglycoconjugates effect in "shaping" the phosphorylation pathways on a global and *in vivo* level.
Keywords: sialylation, signaling pathways, phosphoproteomics

POS-03-048 Global Substrate Profiling of the UDP-GalNAc: Polypeptide *N*-Acetylgalactosaminyltransferase Using a Human Proteome Microarray

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Proteome microarray, as a high throughput technology, has been widely applied in a variety of proteomics studies. One of the key application is post-translational modification (PTM) related study, which include phosphorylation, acetylation, ubiquitination and glycosylation. Mucin type *O*-glycosylation is the most abundant and complex form of protein *O*-glycosylation. Biosynthesis of mucin type *O*-glycan is initialized by a family of UDP-*N*-acetylgalactosamine: polypeptide N-acetylgalactosaminyl transferases (ppGalNAc-T, EC 2.4.1.41). Human ppGalNAc-T family contains 20 members, 15 of them have enzymatic activity. The 20 ppGalNAc-Ts display tissue-specific expression and substrate-specific activities. However, only a handful of *O*-glycosylated proteins have been reported as substrates of ppGalNAc-T(s). Thus, to decipher the substrate specificity of ppGalNAc-Ts, we have developed a strategy to identify potential substrates of ppGalNAc-Ts globally by taken advantage of the high-throughput capability of proteome microarray and the high specificity of click chemistry. We took ppGalNAc-T2 as an example and successfully identified 226 candidates. We have systematically screened potential substrates of ppGalNAc-T2 on proteome-wide and found some interesting potential substrates located in nucleus and cytoplasm. Gene ontology analysis showed that membrane-associated proteins were highly enriched. Intriguingly, intracellular proteins such as transcriptional factors and cytoskeleton related proteins were also enriched. Our strategy could be easily applied for other ppGalNAc-Ts, and this will greatly facilitate the construction of the complete ppGalNAc-T-substrate network for all ppGalNAc-Ts, and eventually help us to unveil the mysterious function of ppGalNAc-T systematically.
Keywords: *O*-glycosylation, proteome microarray, click chemistry

POS-03-049 Glycosylation of Human Plasma Clusterin Yields Novel Biomarkers of Alzheimer's Disease

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Clusterin is a highly-glycosylated secreted protein implicated in the pathogenesis of AD. Expression of the clusterin gene is significantly elevated in AD brain (May *et al*., 1990) and levels of plasma clusterin were also shown to correlate with AD progression (Thambisetty *et al.,* 2010). Since glycosylation plays an important role in physiological functions of clusterin (Stuart *et al.,* 2007) we hypothesised that the detailed profiling of plasma clusterin may reveal potential biomarker isoforms and also enable a better understanding of clusterin structure-function relationships.

Determination of glycosylation sites and structural characterisation of glycosylated peptides from human plasma clusterin was performed by LC/MS/MS following immuno-precipitation. Five out of six N-glycosylation sites were identified, two in the α subunit (α^{64}N and α^{81}N), and three in the β subunit (β^{64}N, β^{127}N, and β^{147}N). High heterogeneity of carbohydrate components was evident with 41 distinct N-linked glycopeptides detected. In general, clusterin glycopeptides are highly sialylated, and our results show the major glycoforms include bisialobiantennary, trisialotriantennary, and bisialotriantennary N-glycans with the bisialobiantennary structure being dominant at every glycosylation site.

We further hypothesised that the overall glycopeptide profile might be used as a diagnostic tool, since the difference in glycosylation patterns associated with distinct glycosylation sites may prove relevant to disease status. To explore this possibility, we compared the clusterin glycoform distribution in plasma obtained from a set of low atrophy (n=4) and high atrophy (n=4) subjects attending the memory clinic. The results of this analysis will be presented at the meeting.

Keywords: clusterin, glycosylation, biomarker

POS-03-050 N-Linked Global Glycan Profiling from Human Gastric Cancer Tissue Using High Performance Liquid Chromatography on a Microfluidic Chip and Time-of-Flight Mass Spectrometry

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Glyco-Proteome plays key roles in protein folding, cell-cell recognition, cancer metastasis, and the immune system. In cell, glycosylation comprise that many kinds of glycan structures associated with a specific glycoprotein have different functions. So, the biological meaning of glycosylation has made them a prime target for discovering biomarker in diseases. In this study, proteomic analysis was efficiently conducted to obtain glyco-proteome. This protocol was applied to profiling glycan from 25 patients of human tissue of gastric cancer. The greatest advantage of this method is only nano-gram or micro-gram of sample amount is required to process experiment. Identifying the numerous glycan, we have used new method to assess the diversity of the N-linked oligosaccharides without derivatization has been developed using on-line nano-liquid chromatography (nanoLC) and high resolution time-of-flight mass spectrometry. And profiling the glycoprotein, we have utilized lectin based glyco-capture method. As a result, we have identified hundreds of N-linked Glycan from Human gastric cancer tissue.

Keywords: N-linked glycan, gastric cancer, glycosylation

POS-03-051 Novel Glycan Column Technology for the LC-MS Analysis of Labeled and Native N-Linked Glycans

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The complex nature of glycans poses challenges to their characterization by LC-MS. Here we present a novel mixed mode stationary phase that provides superior selectivity and resolution compared to traditional modes. The column is based on innovative mixed-mode surface chemistry combining both weak anion-exchange (WAX) and hydrophilic interaction liquid chromatography (HILIC) retention mechanisms. We have characterized the structures of native and labeled N-linked glycans from various proteins using this new column, hybrid quadrupole-orbitrap mass spectrometer and bioinformatics tools. The column enabled separation of glycans based on charge: neutral, followed by acidic from mono- to penta-sialylated. Due to increased resolution and separation provided by the column we were able to identify more glycans, compared to commercial HILIC columns. To our knowledge this is also the first time the hybrid quadrupole-orbitrap mass spectrometer has been used for free glycan analysis. The primary advantage of this instrument is the ability to generate HCD fragmentation and detect them within the Orbitrap, providing HR/AM fragment ions. This allows for differentiation of near mass fragment ions which we observed to be useful for correctly assigning branching and linkage. The variation of collision energy can provide different fragmentation within the mass spectrometer. In order to maximize both glycosidic and cross ring fragments, we incorporated step-collision energy. The use of LC-MS/MS for glycan analysis increases the complexity of data analysis due to the large number of MS/MS spectra. We incorporated bioinformatics tool to simplify data analysis. We extended this workflow to characterize glycans from various biological sources.

Keywords: glycan, HILIC, Q Exactive

POS-03-052 Quantitative Mapping of Glycoprotein Micro- and Macro- Heterogeneity: An Evaluation of Mass Spectrometry Signal Strengths Using Synthetic Peptides and Glycopeptides

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Quantitative determination of site occupancy and glycan structure distribution remains a challenging task in glycoproteomics. Molecules frequently encountered within a glycoproteomics experiment such as unglycosylated, enzymatically deglycosylated and glycosylated peptides are best analysed using Mass Spectrometry, however these chemically diverse molecules are likely to exhibit very different ionisation strengths. Since this does severely impact quantitative data interpretation we investigated the potential of label free techniques to extract relative quantitative information on glyco-microheterogeneity using synthetically produced and thus exactly defined and quantified (glyco) peptides.

A library of 13 (N-glyco) peptides was produced by solid phase peptide synthesis. "Non-glycosylated", "deglycosylated" and N-glycosylated peptides were produced containing Asn, Asp, single N-Acetylglucosamine and biantennary, disialylated N-glycan structures, respectively, on the site of glycosylation. These standardised compounds were subjected to mass spectrometric analyses using a wide range of various ionisation and detection combinations to determine the different ionisation/detection yield and their principal implementation within a regular LC-ESI MS glycoproteomics experiment.

Glycopeptides containing a biantennary N-glycan showed significantly lower signal intensities compared to their "unglycosylated" and "deglycosylated" counterparts in an instrument dependant manner. Peptides mirroring PNGase F induced deamidation gave a lower ionization response compared to "Asn peptides" when mainly singly and doubly charged ions are formed (MALDI), which was not the case in setups where predominantly triply charged ions were detected (ESI). Effects on the signal intensity induced by the position of the glycosylated amino acid within the peptide sequence have been also observed.

Keywords: glycoproteomics, label-free quantitative proteomics, glycopeptide

POS-03-053 From Qualitative to Quantitative: The Evolution of Glycoproteomics

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Protein glycosylation is an important PTM that plays crucial roles in various biochemical processes. Comprehensive structural characterization of glycoproteins is necessary to fully understand the different functions that glycans confer to proteins. This includes localization of glycosylation site, determination of glycan structures and quantification of the various glycoforms. Previously, we reported on a fully automated workflow for glycopeptides characterization. Here we extend this workflow from qualitative to quantitative in order to identifiy and to quantify differential glycoforms of human prostate specific antigen (PSA) as a part of the 2012 ABRF Glycoproteomics Research Group (gPRG) study. All samples were analyzed either as intact or as LysC digested on an Orbitrap Elite with ETD. Byonyc was used to identify glycopeptides, Prosignt PC 3.0 was used for top down data analysis. Pinpoint 2.0 and Protein Deconvolution 2.0 were used for quantification. Our strategy consisted of using both top-down and bottom-up proteomics approaches to characterize PSA samples, and to gauge the advantages and limitations of each workflow for quantitative glycoproteomics. Both approaches correctly identified PSA and its isoform, as well as, the sites of glycosylation, including a second glycosylation site (Asn78). Glycoform profiling was performed employing both strategies. Significant differences in the composition of glycans were observed between PSA and the high pI isoform. Both approaches quantified more than fifty glycoforms. However, the quantitative results produced by each strategy were inconsistent. This difference could be explained by sample preparation and/or digestion efficiency. Several novel bioinformatics tools were developed and implemented for glycopeptides and glycoprotein identification and quantification. Proposed workflow can be used for simultaneous qualitative and quantitative glycoproteomics.

Keywords: glycoproteomics, quantification

POS-03-054 In-Depth Structural Characterization of Glycopeptides Using Complete Derivatization for Carboxyl Groups Followed by Tandem Mass Spectrometry

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Analysis of protein glycosylation, which is one of the most common forms of post-translational modification, is becoming an increasingly important aspect in proteomics. There are different levels in approaching protein glycosylation analysis (e.g. intact glycoprotein, glycopeptide, released glycan and hydrolyzed monosaccharide). Glycopeptide analysis using mass spectrometry (MS) provides valuable information on both glycan heterogeneity and glycosylation site(s) on a protein, allowing site-specific glycosylation analysis. Although structural information can be directly available from glycopeptide ions using tandem mass spectrometry (MS/MS) techniques, several limitations exist: (1) sialic acid residues are preferentially lost during both MS and MS/MS analyses; and (2) structure of the glycan moiety deduced from an MS/MS spectrum is somewhat ambiguous. Because of the inherent branch structure of glycans, positive-ion MS/MS spectra which exhibit successive losses of constituent glycan residues from simple glycosidic bond cleavages cannot provide sufficient structural features.

Here we show an improved method for in-depth analysis of glycopeptide structure. The method involves complete derivatization of carboxyl groups in glycopeptides. The derivatization reagents are easily removed by cellulose-based microcolumns prior to MS. This derivatization method for carboxyl groups mainly has two merits on glycopeptide analysis: (1) preferential losses of sialic acid residues during MS and MS/MS analyses are suppressed; and (2) in negative-ion CID, important glycan fragment ions such as D and E ions which define the specific composition of the antennae, are greatly enhanced. The combination of these two merits enables us to characterize detailed glycopeptide structures including sialylated glycopeptides.

Keywords: glycopeptide, structural characterization, MS/MS

POS-03-055 Glycomic Analysis Using Glycoprotein Immobilization for Glycan Extraction

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Glycosylation is one of the most common protein modifications and is involved in many functions of glycoproteins. Investigating aberrant protein glycosylation associated with diseases is useful in improving disease diagnostics. Due to the non-template nature of glycan biosynthesis, the glycans attached to glycoproteins are enormously complex; thus, a method for comprehensive analysis of glycans from biological or clinical samples is needed. Here, we describe a novel method for glycomic analysis using glycoprotein Immobilization for glycan extraction (GIG). Proteins or peptides from complex samples were first immobilized on solid support, and other non-conjugated molecules were removed. Glycans were enzymatically or chemically modified on solid-phase before releasing from glycoproteins/glycopeptides for mass spectrometry analysis. The method was applied to the glycomic analysis of both N- and O-glycans.

Keywords: glycoprotein

POS-03-056 Impaired Tryptic Proteolytic Activity at Citrullinated Amino Acids

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Protein citrullination, an enzyme catalyzed deamidation of arginine, is an important clinical biomarker in rheumatoid arthritis, and has been associated with several other diseases. The site specific characterization of citrullination using mass spectrometry remains problematic and often relies on a tryptic miss cleavage after citrulline. However, tryptic cleavage after citrulline has in some cases been reported, so we here investigate the cleavage properties of trypsin after a citrulline residue. In solution tryptic digest was performed on synthetic peptide sets containing either arginine or citrulline. The peptide sequences originated from disease associated *in vivo* citrullinated proteins, some reported as being C-terminal citrullinated peptides. Digested and undigested peptides were analysed using liquid chromatography/tandem mass spectrometry with electrospray ionisation in positive ion mode, and extracted ion chromatograms were constructed to identify the tryptic cleavage sites. The retention time difference between the citrulline and arginine peptides was, furthermore, investigated. Our results clearly demonstrate the inability of trypsin to cleave after citrulline residues. Furthermore, the shift in retention time between peptides containing citrulline and arginine was large enough on a 10 cm C18 column using a 30 min gradient to ensure that both citrullinated and uncitrullinated peptide can be identified when both peptides are present in a sample. Our study demonstrates that trypsin cannot cleave after a citrulline residue. As a result, a miss cleavage can be used to distinguish a citrullination from a deamidation of asparagine or glutamine, which is a common false positive as the modifications results in the same mass increase. Based on our findings, we present a verification strategy for reported citrullinated sites in modified peptides in proteomics shotgun experiments, which include the verified tryptic missed cleavage.

Keywords: citrulline, miss cleavage, retention time

POS-03-057 Crystal Structure of Tyrosylprotein Sulfotransferase: The Reaction Mechanism of Post-Translational Tyrosine Sulfation

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Post-translational protein modification by tyrosine sulfation regulates the physiological activity of proteins such as complement C4 and chemokine receptor CCR5. The protein tyrosine sulfation has an important role in the extracellular protein-protein interactions involving the inflammation, haemostasis, and viral infection. The sulfation reaction is catalyzed by the tyrosylprotein sulfotransferases (TPSTs) which transfer a sulfonate group from the active sulfate, 3'-phosphoadenosine 5'-phosphosulfate (PAPS), to tyrosine residues of target proteins in the Golgi lumen. Detailed mechanism of protein tyrosine sulfation reaction has still remained unclear. To reveal the reaction mechanism of protein tyrosine sulfation, we have demonstrated the crystal structure of the human TPST-2 complexed with a substrate peptide (C4P5Y3) derived from complement C4 and 3'-phosphoadenosine-5'-phosphate (PAP). Structural analyses and sulfotransferase activity assay using complementary mutants revealed the mechanism of dimeric structure and molecular basis for catalysis being an SN2-like in-line displacement mechanism similar to the other sulfotransferases including heparin sulfate sulfotransferase and cytosolic sulfotransferase. The peptide-binding site of TPST-2 appeared the substrate peptide in a deep cleft by using a short parallel b-sheet type interaction and the bound C4P5Y3 forms an L-shaped structure. These observations suggest a model for the sulfation of the substrates, e.g., the soluble complement C4 and the membrane-bound chemokine receptor, by TPST-2 in the Golgi lumen. **Nat. Commun.** 4, 1572, (2013). DOI: 10.1038/ncomms2593

Keywords: protein tyrosine sulfation, tyrosylprotein sulfotransferase, crystal structure

POS-03-058 Mass Spectrometric Identification of Glycosylphosphatidylinositol-Anchored Peptides

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Glycosylphosphatidylinositol (GPI) anchoring is a post-translational modification widely observed in eukaryotic membrane proteins. GPI anchors are attached to proteins via the carboxy-terminus in the outer leaflet of the cell membrane. GPI-anchored proteins (GPI-APs) are known to have important functions in receptors and enzymes. GPI-AP precursors contain a C-terminal hydrophobic sequence that is involved in cleavage of the signal sequence from the protein and addition of the GPI anchor by the transamidase complex. In order to identify proteins having a GPI anchor, it is essential to determine the GPI-anchored peptide sequence. Previously, efficient identification of GPI-anchored C-terminal peptides by mass spectrometry has been difficult, mainly due to the complex structure of GPI-anchor moiety. Here, we developed a method to experimentally identify GPI-APs and their GPI-anchor modification site (ω-sites). In this method, first GPI-APs were extracted from lipid-raft and then the GPI-anchor moieties are removed from them using phosphatidylinositol-specific phospholipase C and aqueous hydrogen fluoride. Finally, the peptide sequence was determined by mass spectrometry. Using this method, we successfully identified 12 GPI-APs in the cultured ovarian adenocarcinoma cells and 25 GPI-APs in the mouse tissues. The technique developed here is useful to identify efficiently the GPI-anchor modification sites in proteome analysis.

Keywords: GPI-anchor

POS-03-059 Functional Studies of Post-Translational Modification by Tyrosine Sulfation: Knockdown Analysis of Three Kinds of Zebrafish Tyrosylprotein Sulfotransferases

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Tyrosine *O*-sulfation is a wide spread post-translational modification of membrane and secretory proteins that occurs in multicellular eukaryotic organisms. It has been shown to be involved in the alteration of biological activity of proteins and modulation of extracellular protein-protein interactions. Tyrosylprotein sulfotransferases (TPSTs) catalyze the transfer of a sulfonate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the tyrosine residues of target proteins.

Zebrafish (*Danio rerio*) is a well-recognized model animal for the study of vertebrate developmental biology. Searches of the genome data base reveal genes encoding two *TPSTs* orthologs in many other vertebrate and invertebrate species. It is interesting to note that zebrafish has three kinds of *TPST* genes. Here, we analyzed phenotypes observed from experiments of the knockdown of the three *TPST* genes of zebrafish by using Morpholino antisense oligos (MOs).

In this study, we demonstrated three kinds of different patterns of deformities in brain or body trunk when zebrafish embryos were treated with MOs targeting each of the three zebrafish *TPST* genes. Furthermore, zebrafish embryo treated with the MOs against all three *TPST*s genes exhibited lethality in early development. In view of the lethal phenotype of triple knockdown embryos and nonfatal single knockdown embryos, tyrosine sulfation likely plays essential roles in maintaining life in early development.

Keywords: post-translational modification, tyrosine sulfation, tyrosylprotein sulfotransferases

POS-03-060 A Proteomics Strategy for Identification of FAT10 Modified Proteins and Specific Sites by Mass Spectrometry

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Ubiquitin-like protein FAT10 (HLA-F adjacent transcript 10) is uniquely expressed in mammals. The fat10 gene is encoded in MHC class I locus in human genome and related to some specific processes, such as apoptosis, immune response. But the biological knowledge of FAT10 is limited due to the poor identification of its conjugates. FAT10 covalently modifies proteins in eukaryotes. But only few substrates of FAT10 have been reported till now and no FATylated sites have been identified. Here we report a proteome-scale identification of FATylated proteins by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Totally we identified 182 proteins with high confidence as FATylated candidates. A total of 13 modified sites were identified for the first time by a modified search method of raw MS data. The modified sites are highly enriched with hydrophilic amino acids. Furthermore, the FATylation of hnRNP C2, PCNA, and PDIA3 were verified by co-immunoprecipitation assay. We confirmed that most of the substrates are covalently attached with a FAT10 monomer. The functional distribution of FAT10 targets suggests that FAT10 participates in various biological processes, such as translation, protein folding, RNA processing and macromolecular complex assembly. These results should be very useful for investigating the biological functions of FAT10.

Keywords: FAT10, mass spectrometry, post translational modification

POS-03-061 Nitroproteomics Analyses of Pituitary Adenomas

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Oxidative stress is extensively associated with tumorigenesis. A series of studies on stable tyrosine nitration as a marker of oxidative damage were performed in human pituitary and adenoma. The mass spectrometry characteristics of nitropeptides were analyzed and nitroproteomics of pituitary controls and adenomas was carried out. Enrichment of those low-abundance endogenous nitroproteins from human tissues or body fluid samples was the first important step for nitroproteomics studies. Mass spectrometry was the essential approach to determine the amino acid sequence and locate the nitrotyrosine sites. Bioinformatics analyses, including protein domain and motif analyses, were needed to locate the nitrotyrosine site within the corresponding protein domains/motifs. Systems biology techniques, including pathway analysis, were necessary to discover signaling pathway networks involving nitroproteins from the systematically global point of view. A total of eight nitroproteins and eight nitrotyrosine sites from pituitary control tissues, and nine nitroproteins, ten nitrotyrosine sites and three nitroprotein-interacted proteins from pituitary adenoma tissues were identified. Those nitrotyrosine sites were located within important protein domains/motifs. Two signal pathway networks were identified to involve those nitroproteins. Future quantitative nitroproteomics will discover pituitary adenoma-specific nitroprotein (s). Structural biology techniques such as X-ray crystallography analysis will solidly clarify the effects of tyrosine nitration on structure and functions of a protein. Those studies will eventually address the mechanisms and biological functions of tyrosine nitration in pituitary tumorigenesis and will discover nitroprotein biomarkers for pituitary adenomas and targets for drug design for pituitary adenoma therapy.
Keywords: nitroproteomics, bioinformatics, pituitary adenoma

POS-03-062 USP-t Elongates Telomerase Activity by Stabilizing Human Telomerase Reverse Transcriptase (hTERT)

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Proteolysis through ubiquitination plays to regulate function and stability of diverse cellular factors in extra- and intracellular processes. The major function of ubiquitination is not only regulation of protein half-life via 26S proteasome, but also control various signaling by ubiquitin-lysine chain specific conformation. Deubiquitinating enzymes (DUBs) act as a protease to modify ubiquitin chains for protects substrates from the proteasomal degradation, and regulates their activity. By immunoprecipitation and proteome analysis, we have observed that one of DUBs, USP-t binds heat shock protein 90 (HSP90). Previous studies revealed that HSP90 regulates human telomerase reverse transcriptase (hTERT) expression level. Among these evidences, we assumed that USP-t might be an asseccial DUB for hTERT. Co-immunoprecipitation showed that USP-t interacts with hTERT, and USP-test had deubiqutinating activity for ubiquitinated hTERT. Thus, USP-t protects hTERT protein expression levels from the treatment of geldanamycine (GA) known as a HSP90 inhibitor. Our data suggest that USP-t plays a specific DUB for the regulation of hTERT activity, and their interaction may lead to the regulation of both cancer cell and stem cell proliferation ability.
Keywords: deubiquitinating enzyme

POS-03-063 The Roles of HAUSP/USP7 Deubiquitinating Enzyme in Human Cancer and Apoptosis

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Herpes virus-associated ubiquitin-specific protease (HAUSP/USP7) is a well-known deubiquitinating enzyme that can control tumor cell proliferation and apoptosis by deubiquitinating its target proteins such as p53 and Mdm2. However, the molecular details of HAUSP-involved mechanisms are still unclear. Accordingly, to discover novel substrates for HAUSP and to investigate further possible intracellular processes which are involved in apoptosis or tumorigenesis, we performed two-dimensional electrophoresis (2-DE) and other proteomics-based approaches using HAUSP overexpressed human cancer cells. We confirmed 35 spots from MALDI-TOF/TOF analysis, which showed differences in their expression by HAUSP overexpression. Of 35 putative candidates, 4 proteins are associated with apoptosis pathway. Interestingly, the results from RT-PCR and ubiquitination assay showed that Ann protein, which is one of 4 apoptosis-related proteins, is mainly regulated by post-translational modification through ubiquitin-proteasome pathway (UPP) rather than transcriptionally regulated. Moreover, we found that HAUSP, as a deubiquitinating enzyme, not only binds to Ann protein but has ability to deubiquitinate and stabilize Ann protein *in vivo*. Although further functional studies are required to explore how HAUSP-Ann interaction is involved in apoptosis pathway, our study may provide insight into the role of HAUSP-mediated regulation of apoptosis.
Keywords: deubiquitinating Enzyme

POS-03-064 Identification of Novel Human N-Myristoylated Proteins Using cDNA Resource and Cell-Free Protein Synthesis System

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Protein N-myristoylation is a well-recognized form of lipid modification that occurs in eukaryotic and viral proteins. Many N-myristoylated proteins play key roles in regulating cellular structure and function. However, comprehensive identification of human N-myristoylated proteins has not been accomplished. In this study, in order to identify novel human N-myristoylated proteins, the susceptibility of human cDNA clones in human cDNA resources to protein N-myristoylation was evaluated by metabolic labeling of proteins expressed using an insect cell-free protein synthesis system. For this analysis, 178 cDNA clones with an N-terminal Met-Gly motif were selected as potential candidates from ~6300 Kazusa ORFeome project (KOP) human cDNA clones. The susceptibility of these cDNA clones to protein N-myristoylation was first evaluated using fusion proteins, in which the N-terminal ten amino acid residues were fused to an epitope-tagged model protein. Then, protein N-myristoylation on the gene product of the full-length cDNA was evaluated by metabolic labeling experiments both in an insect cell-free protein synthesis system and in transfected mammalian cells. As a result, the products of 35 out of ~6300 cDNA clones were found to be novel human N-myristoylated proteins. These novel N-myristoylated proteins contain not only physiologically important proteins such as protein kinases, phosphatases, E3-ubiquitin ligases, cytoskeletal regulating proteins, apoptosis related proteins, but also many integral transmembrane proteins that play critical roles in various cellular functions. These results indicate that the strategy proposed in this study is useful for the comprehensive identification of human N-myristoylated proteins from human cDNA resources.
Keywords: Protein N-myristoylation, cell-free protein synthesis system, comprehensive analysis

POS-03-065 Quantitative Analysis of Deamidation and Isomerization in β2-Microglobulin by ^{18}O Labeling

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Deamidation of Asn residues in proteins via the formation of a 5-membered succinimide ring intermediate is a nonenzymatic intramolecular reaction and, in general, occurs most rapidly at an Asn-Gly sequence. A protein containing this sequence would, therefore, be susceptible to modification and the result would produce a structural alteration in the molecule. A replacement of Asn with Asp could result in an increase in the overall negative charge but also an isomerization to isoAsp. Despite the fact that such a structural replacement could affect the functional property of a protein, estimation of the susceptibility of the Asn-Gly to deamidation/isomerization remains a difficult task. This is especially true for proteins that are subjected to enzymatic digestion during their characterization, since the above transformation could take place spontaneously during this treatment. To address this issue, we applied a stable-isotope ^{18}O-labeling method combined with nano-LC-MS/MS to examine the susceptibility of two Asn-Gly sites in β2-microglobulin (β2m) to the reaction. The method permits the reaction occurring in a protein to be distinguished from that during enzymatic treatment. When β2m was incubated for 60 days at 37°C, deamidation at Asn17-Gly and Asn42-Gly with half-lives of 33 and 347 days occurred, respectively. In addition, a comparison of the deamidated products to synthetic peptides revealed that 44% of the Asp17 and 96% of the Asp42 had been converted into isoAsp. Interestingly, such structurally altered β2m showed a specific affinity for divalent Cu^{2+} ions, which is thought to be a candidate for initiating fibril formation.

Keywords: deamidation, isomerization, ^{18}O-labeling

POS-03-066 USPJ is Critical for Regulating 14-3-3 in Cell Proliferation

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Deubiquitination is a process where ubiquitinated proteins can be reversed to counterbalance the ubiquitination process with the help of deubiquitinating enzymes (DUBs). Although, ~100 DUBs have been identified from the human genome, the functions and characterization of all the deubiquitinating enzymes are not completely understood yet. The removal of ubiquitin appears to be a highly regulated process that has been implicated in numerous cellular functions such as cell proliferation, gene expression, proteasome or lysosome-dependent protein degradation, DNA repair, kinase activation, and apoptosis. Here, we have characterized and reported a deubiquitinating enzyme USPJ that contains conserved Cys, Asp, and His residues, which are responsible for catalytic activity of DUBs. Deubiquitinating activity of USPJ was confirmed by *in vitro* and *in vivo* enzyme assays. RT-PCR analysis showed high expression levels of USPJ mRNA in intestine, testis, liver, brain and ovary. Additionally, USPJ itself was shown to be deregulated by the proteasomal degradation pathway. In a previous study, we reported that 14-3-3 protein inhibits apoptotic cell death and mediates cell proliferation in NIH3T3 cells. Here, we validated that USPJ interacts with 14-3-3 protein by immunoprecipitation assay and also demonstrate that 14-3-3 undergoes polyubiquitination through the ubiquitin-proteasomal pathway. Furthermore, we showed that functional association between USPJ and 14-3-3 results in the regulation of 14-3-3 proteins degradation. Taken together, USPJ was shown to be a specific DUB preventing 14-3-3 protein degradation which might contribute to malignant transformation, possibly providing a new target for therapeutic intervention in hematopoietic neoplasm.

Keywords: deubiquitinating enzyme

POS-03-067 N-Terminal Modification of Proteasome Subunit Rpt1 in Yeast

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The 26S proteasome is a multi-catalytic protease complex that degrades ubiquitinated proteins in eukaryotic cells. It consists of a proteolytic core (the 20S proteasome) as well as regulatory particles, which contain six ATPase (Rpt) subunits involved in unfolding and translocation of substrates to the catalytic chamber of the 20S proteasome. We previously characterized the N-terminal modifications of the six Rpt subunits of the yeast 26S proteasome. According to our studies, Rpt2 is partially N-myristoylated, and Rpt3, Rpt4, Rpt5, and Rpt6 are N-acetylated, while Rpt1 is unmodified. However, in light of the discovery of N-methyltransferase and the fact that yeast Rpt1 contains this enzyme's N-terminal recognition motif (Met-Pro-Pro-Lys), Rpt1 must be considered a candidate for N-terminal methylation. In this study, we used mass spectrometry to analyze the N-terminal modifications of the yeast Rpt1 subunit. Our results revealed that following the removal of the initiation Met residue of yeast Rpt1, the N-terminal Pro residue is either unmodified, mono-methylated, or di-methylated, and that this N-methylation has not been conserved throughout evolution. In order to gain a better understanding of the possible function (s) of the Pro-Lys (PK) sequence at positions 3 and 4 of yeast Rpt1, we generated mutant strains expressing an Rpt1 allele that lacks this sequence. The absence of the PK sequence abolished N-methylation, decreased cell growth, and increased sensitivity to stress. Our data suggest that N-methylation of Rpt1 and/or its PK sequence might be important in cell growth or stress tolerance in yeast.

Keywords: N-terminal methylation, proteasome, mass spectrometry

POS-03-068 Second Generation Electron Transfer Dissociation (ETD) on a Novel Hybrid Instrument with Improved Functionality, Increased Speed, and Robustness of Data

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A novel hybrid instrument, based on a mass resolving quadrupole, Orbitrap, collision cell, and dual linear ion trap (Q-OT-LT) architecture incorporates an improved ETD implementation. ETD apparatus improvements include a bright and stable glow discharge based ETD reagent ion source located ahead of the mass filter and a higher frequency RF axial trapping field (trap end-lens voltage) to improve ion confinement during ETD. The new instrument's design enables previously unavailable parallel/pipelined and multi-fill scan modes to minimize overall scan cycle times and minimize the need for spectral averaging. Further calibration of ETD reaction kinetics insures the shortest possible reaction times while maximizing product ion yields and spectral reproducibility. Collectively these developments constitute a true second generation ETD platform.

Breifly, reagent anions from a glow discharge source are introduced into the ion optics path ahead of the m/z filter where they are m/z selected, accumulated in the collision cell, and then transferred into the high pressure trap (HPT) of the dual region LT for the ETD reaction. Increasing the frequency of the RF axial confinement field during ETD from ½ to 2 times the quadrupole field frequency avoids parametric resonance excitation and ejection of low m/z (typically 120-130 Th) ions. ETD products may be directly transferred to the low pressure trap (LPT) or to Orbitrap for m/z analysis. Alternatively, ETD Products from multiple reactions can be accumulated in the LPT for collective m/z analysis with either analyzer.

Keywords: ETD

POS-03-069 Systems-Wide Analysis of Protein Ubiquitylation in Response to DNA Damage

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The integrity of the human genome is constantly challenged by external and internal insults that induce different types of DNA lesions. To counteract the potentially devastating effects of genomic instability, cells have evolved complex signaling networks that encompass dynamic changes in posttranslational modifications. The reversible modification of proteins by ubiquitin has been established as a key signaling component in DNA damage repair. We combine enrichment of ubiquitylated peptides with quantitative mass spectrometry to investigate proteome-wide and site-specific changes in ubiquitylation upon treatment of cells with ultraviolet radiation (UV). We quantify more than 6,700 unique ubiquitylation sites and demonstrate widespread regulation of proteins by ubiquitylation after DNA damage. Our results provide detailed insights into global, site-specific protein ubiquitylation changes in response to UV. We identified novel DNA damage-induced ubiquitylation sites on many proteins known to be involved in DNA repair. A majority of these sites are found in proteins functioning in nucleotide-excision repair consistent with the importance of this pathway for repair of UV-induced lesions. In addition, we show that a large fraction of upregulated ubiquitylation sites is located on proteins that have not previously been linked to the cellular response to DNA damage. Notably, a considerable proportion of ubiquitylation sites were downregulated after UV, suggesting that deubiquitylases or rapid protein degradation play widespread regulatory roles in DNA damage signaling.

Keywords: ubiquitin, DNA damage, quantitative proteomics

POS-03-070 Proteome-Wide Identification of Poly (ADP-ribosyl)ation Targets in Different Genotoxic Stress Responses

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Poly(ADP-ribos)ylation (PARylation) is a reversible post-translational protein modification found in higher eukaryotes synthesized by the catalytic activity of poly (ADP-ribosyl)transferases (ARTDs/PARPs). PARylation is involved in various cellular processes such as DNA damage response, transcription, energy metabolism and cell death. Supporting its role in DNA repair, specific inhibition of ADP-ribosyltransferase activity has recently been shown to constitute an effective target in treatment of several types of cancer. However, despite this recent scientific progress only little is known about the actual acceptor proteins of PARylation and how the modification regulates the functional role of these target proteins in mammalian cells. We performed a sensitive proteomics approach based upon quantitative mass spectrometry (SILAC) for macrodomain-based enrichment and identification of PARylated proteins that become covalently modified under different conditions of genotoxic stress. Our screen identified novel candidates not previously reported to be targets of PARylation, while confirming the majority of known PARylated proteins. Biochemical *in vitro* and *in vivo* validation of novel acceptor proteins confirmed that our methodology targets covalently PARylated proteins. Nuclear proteins encompassing nucleic binding properties were most prominently found to be PARylated upon genotoxic stress, in agreement with the nuclear functions ascribed to ARTD1/PARP1 and ARTD2/PARP2. Distinct differences in proteins becoming PARylated upon various genotoxic insults were observed. Most significantly, proteins involved in RNA metabolism are PARylated upon oxidative and alkylation induced stress, demonstrating that post-transcriptional processes are readily controlled through specific genotoxic stress-induced PARylation.

Keywords: poly(ADP-ribos)ylation, PARylation, genotoxic stress

POS-03-071 Analysis of Post-Translational Modifications in Human Transthyretin Associated with Familial Amyloidotic Polyneuropathy by Targeted LC/MS and Intact Protein MS

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Transthyretin (TTR) is an amyloidogenic tetrameric protein (55kDa) synthesized in liver and choroid plexus in brain. TTR is present in human plasma, transporting T4 hormone and retinol through the retinol binding protein (RBP). TTR is associated with several amyloidosis, namely familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC) and senile systemic amyloidosis (SSA) (1-2). Whereas FAP and FAC are caused by single point mutants, SSA is associated to the wild-type TTR. Variability of TTR is not only due to point mutations in the encoding gene but also to post-translational modifications (PTMs) at Cys10. Only around the 10-15% of the circulating TTR in plasma remains unmodified at this residue, being the most common PTMs the S-sulfocysteinylation, S-glycinylcysteinylation, S-cystinylation and S-gluthationcysteinylation (3). It is thought that PTMs at Cys10 may play an important biological role in the onset and pathological process of the amyloidoses related to TTR. The objective of our study is to analyze the most significant Cys10 PTMs present in TTR human serum samples of patients with TTR-related and to study the relationship between those PTMs and the clinical profile of the patients. We report here the development of the methodology for detection and quantification of PTMs in serum samples. It involves an enrichment step by TTR-immunoprecipitation followed by mass spectrometry analysis of (i) the intact TTR protein and (ii) targeted LC-MS analysis of peptides carrying the PTMs of interest, both performed on a UHR Q-TOF instrument (Impact, Bruker). Both methods developed have allowed the relative and absolute quantification of the selected PTM forms of TTR in plasma samples.

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Keywords: PTM quantification, UHR Q-Tof

POS-03-072 Site-Specific Quantitative Analysis of Protein Ubiquitylation in Cellular Signaling

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Posttranslational modification of proteins by ubiquitin is a fundamentally important regulatory mechanism involved in diverse cellular processes including protein turnover, endocytosis, inflammatory signaling, DNA damage repair and cell cycle progression. However, proteome-wide analysis of endogenous ubiquitylation sites remained a challenging task, and has typically relied on cells expressing affinity tagged ubiquitin. We combined single-step immunoenrichment of ubiquitin remnant (di-glycine-lysine) peptides with peptide fractionation and high-resolution mass spectrometry to investigate endogenous ubiquitylation sites in human cells. We have employed this method to identify more than 10,000 ubiquitylation sites and provide mass spectrometric evidence that a large part of the cellular proteome including numerous cell surface receptors and nuclear proteins is subjected to ubiquitylation. Importantly, when combined with a quantitative proteomic approach such as stable isotope labeling with amino acids in cell culture (SILAC) this method permits to quantify site-specific changes in ubiquitylation after cellular perturbations. We investigated the abundance of ubiquitylation sites in cells treated with the proteasome inhibitor MG132 and demonstrated that a subset of ubiquitylation sites present on histones and other nuclear proteins showed decreased ubiquitylation, while the majority of ubiquitylation sites showed a dramatic increase in abundance. Our results suggest that inhibition of the proteasome leads to a global imbalance in ubiquitylation that might explain the toxic effects of the proteasome inhibition on cells. We will further discuss novel results on the role of ubiquitylation in immune signaling and DNA damage response that were obtained by ubiquitin proteomics.

Keywords: ubiquitin, cellular signaling, quantitative proteomics

POS-03-073 Identification of Differentially S-Nitrosylated Proteins in Alzheimer's Disease Brain

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Introduction: S-nitrosylation, a reversible post-translational modification resulted by the covalent binding of nitric oxide (NO) with cysteine residues of target proteins with the formation of nitrosothiols (SNOs), extensively modifies the protein function and plays a key role in the pathology of multiple neurodegenerative diseases. Significant involvement of S-nitrosylation is also suggested in the progression of Alzheimer's disease (AD) pathology specifically in the formation and accumulation of misfolded protein aggregates.
Objective: In line with these observations, the identification of S-nitrosylated proteins can be a major step towards the understanding of relatively unknown mechanisms leading to neuronal degeneration.
Methods and Results: Present proteomic analysis identifies S-nitrosylated proteins in AD hippocampus, substantia nigra and cortex in comparison with unaffected controls using classical S-nitrosothiol detection methods combined with ESI QTOF MS/MS identification. Endogenous nitrosocysteines were identified in a total of 45 proteins, mainly involved in metabolism, signaling pathways, apoptosis and redox regulation, confirmed by REACTOME and KEGG pathway database analysis. Superoxide dismutase [Mn], fructose-bisphosphate aldolase C and voltage-dependent anion-selective channel protein 2 showed differential S-nitrosylation signal in AD brain regions. Extensive neuronal atrophy was also observed, which mainly due to increased protein S-nitrosylation. Insilico methods were used to assess the plausible cysteine modification sites via GPS-SNO 1.0 while functional annotations among the modified proteins were generated by STRING 8.3.
Conclusions: The findings will be helpful to characterize functional aberrations due to S-nitrosylation that may represent a convergent signal pathway contributing to NO induced protein misfolding and aggregation facilitating a better understanding of AD pathology.
Keywords: Alzheimer disease, S-nitrosylation, Brain

POS-03-074 Comparative Secretomics Reveals Wound Environment Acidification-Associated Secretome and Novel Microbial Virulence Factors for Group A *Streptococcus pyogenes* Infection

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Streptococcus pyogenes is a human pathogen responsible for various diseases. To cause diseases, *S. pyogenes* must adapt in adverse environments, such as acidic environment in the wound. The acid stimuli may trigger *S. pyogenes* invading from a mucosal to subepithelial tissue. However, how acid stimulates *S. pyogenes* to manipulate secretome for causing invasive infection is unclear. To investigate secretome change under acidic environment, a comparative secretomics by label-free LC-MS/MS was used to analyze the secretome from acidic and neutral conditions. The growth curves of *S. pyogenes* in acidic and neutral conditions are similar, which reveals that *S. pyogenes* can grow well during environmental acidification. The protein patterns on SDS-PAGE show prominent dissimilarity between the secretomes in acidic and neutral conditions. It demonstrates protein secretion is influenced by acid stress. The dynamic label-free LC-MS/MS profiling identified 172 proteins which are influenced by environmental acidification. Among these, 45 (26%) the identified proteins are predicted secreted proteins. Interestingly, the predicted secreted proteins occupy about 90% of protein abundance of secretome in acidic condition at stationary phase. In contrast, only 30% shows in neutral condition. It exhibits that acid is crucial for secreted protein expression. There are 21 pathogenesis-related secreted proteins effecting immune evasion, hemolysis, adhesion, tissue damage, and nutrient acquisition. The 24 non-pathogenesis-related secreted proteins could be potential virulence factors involved in invasive infection. Two known acid-induced proteins, SpeB and Pilin, are also observed. Several novel candidates, such as streptococcal histidine triad protein, CAMP, and biofilm regulatory protein A, are of special interests. This investigation provided key information for elucidating the broad influences and underlying mechanisms related to acidified environment for group A streptococcal infection.
Keywords: secretome, environment acidification, *Streptococcus pyogenes*

POS-03-075 Multiplexed Quantitative Analysis of 51 Proteins in Human Tears Using High Resolution Multiple Reaction Monitoring (HR-MRM) Mass Spectrometry

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Tear proteins are intimately related to the pathophysiology of the ocular surface of the eye. Many studies have demonstrated that the tear fluid is an accessible and useful source in studying ocular surface disorders and biomarker discovery. This study describes the use of a high resolution MRM approach to develop assays for biologically important tear proteins. Human tear samples were collected from 1000 consenting patients with no eye complaints (411 male, 589 female, average age 55.5 years, SD 14.5 years) using the Schirmer tear test strips and pooled into a single global control sample. Quantification of proteins is carried out by selecting "signature" peptides derived by trypsin digestion of the target protein. A 2-hour nanoLC-MS/MS run was used to separate the tryptic peptides and perform quantitation of human tear proteins in HR-MRM mode. Samples were analyzed in triplicate. Twenty-one high abundant proteins were further accessed for signal reproducibility. Fifty-three peptide assays that represent 51 high and intermediate abundant tear proteins were developed. All assays showed consistent retention time with a coefficient of variation (CV) of less than 2%. As for peak area, 17 out of the 21 assays showed a reproducible peak area with CV less than 20%. Some well characterized tear proteins, lacritin, mammaglobin-B, S100A4, S100A8, and prolactin inducible protein (PIP) showed the highest reproducibility of peak area, with CV less than 2%. These multiplexed MRM-based assays show great promise to be further developed for biomarker validation in human tear samples.
Keywords: high resolution MRM, tear proteomics

POS-03-076 Targeted Proteomics to Validate and Quantify One-Hit Wonders Proteins in Human Liver

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In shot-gun proteomics, protein identification and quantification based on peptide fragment sequences commonly exclude single hit protein identifications. Although rigid guidelines ensure high quality of the reported identifications and avoid the inflation of identification lists with erroneous entries, exclusion of single hit wonders may result in the loss of potentially valuable meta-data. Since the concept of proteotypic peptides are widely used in quantitative proteomics, retrial of these one-hit wonders will replenish our knowledge in gene-centric proteomics. We employed SRM to verify the one-hit wonders proteins in Chinese Human Liver Proteome Project dataset. Crude peptides were synthesized and used to develope SRM assays for target peptides. Proteins extracted from normal human liver were separated in SDS-PAGE and digested in split gel slice. Then the digests were subjected to LC- scheldued SRM analysis. Totally, 184 SRM assays were developed and expression of 57 target proteins were confirmed in normal human liver tissues. Among the proved 57 one-hit wonders, 48 proteins are of minimally redundant set in the PeptideAtlas data base, 8 proteins even have none MS-based information before.
Keywords: SRM, one-hit wonders,human liver

POS-03-077 The Human Embryonic Secretome

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Infertility is a growing problem worldwide and many children are conceived with the aid of fertility treatments. The selection of which embryo (or embryos) is to be transferred into the uterus is a critical step during *in vitro* fertilization (IVF) treatments in order to increase the probability of pregnancy. Today, embryo morphology is the most common method used to assess the developmental and viability potential of embryos. However, the success rates of fertility treatments are low and only about 30 % of IVF treatments result in pregnancies.
Little is known about the proteins that are produced by human embryos and secreted into the surrounding environment. Knowledge about these proteins and their functions may lead to a more comprehensive assessment of the embryos during their pre-implantation development and aid in the selection of the most viable embryos during IVF treatments.
The purpose of this project is to analyze conditioned human embryo media by advanced mass spectrometry to identify proteins secreted by human embryos. These proteins, together with candidate proteins from the literature, are analyzed by targeted mass spectrometry to assist in the development of biomarkers to distinguishing normal and abnormal embryonic development. The results may help increase the success rates of IVF treatments.
Keywords: In vitro fertilization, human embryo, secretome

POS-03-079 Targeted Analysis of *Salmonella* Effector Proteins Using Multiple Reaction Monitoring

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Salmonella enterica is a versatile pathogen capable of infecting diverse hosts and causing different diseases. In humans, *S. enterica* strains can cause typhoid or gastroenteritis, resulting in much morbidity and mortality throughout the world. Upon encountering a human cell, *Salmonella* delivers a choreographed series of virulence factors (also known as 'effector' proteins) into the cells. These effector proteins initially enable the bacteria to enter the host cell and then to target various natural processes within that host cell. To develop a clearer picture of the global effects of *Salmonella* on host cell processes, multiple reaction monitoring (MRM) assays for all known and potential new *Salmonella* effectors have been constructed by synthesizing at least two unique peptides per protein. Transitions were optimized by characterizing the synthesized peptides on a triple quadrupole mass spectrometer mainly focusing on optimizing collision energies. The three most intense fragment ions for each peptide were selected and used as transitions to detect the peptides in biological samples. A baseline level of effector proteins was established by analyzing *Salmonella* bacteria grown under effector secreting conditions. Finally, effector protein dynamics were investigated by analyzing *Salmonella* infected HeLa cells. This data shows how *Salmonella* takes control of its host cell by secreting specialized proteins at specific time points.
Keywords: multiple reaction monitoring, salmonella, quantitative proteomics

POS-03-078 Hepatocyte Pathway Alterations in Response to *In Vitro* Crimean Congo Hemorrhagic Fever Virus Infection

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Crimean-Congo hemorrhagic fever virus (CCHFV) is tick-borne virus responsible for hemorrhagic manifestations and multiple organ failure, with a high mortality rate. In nfected humans, damage to endothelial cells and vascular leakage may be a direct result of virus infection or an immune response-mediated indirect effect. The main target cells are mononuclear phagocytes, endothelial cells and hepatocytes; the liver being a key target for the virus which was described as susceptible to IFN host response and to induce apoptosis. However, to better understand the first liver cell alterations after virus infection, the protein profile of *in vitro* CCHFV-infected HepG2 cells was analysed using two oomplementary quantitative proteomic approaches, 2D-DIGE and iTRAQ. A set of 243 differentially expressed proteins was identified. Bioinformatics analysis (Ingenuity Pathways Analysis) revealed multiple host cell pathways and functions altered after CCHFV infection, with notably 106 proteins related to cell death, including 79 associated with apoptosis. Four protein networks emerged with associated pathways involved in virus entry and exit, protein synthesis, acute phase response, oxidative stress, ubiquitination, or lipid metabolism. These data suggest that CCHFV seems to hijack host proteins to promote its replication and spread. This work gives informations on virus-host interactions leading to CCHV pathogenesis, and offers an unparalleled opportunity of the description of possible targets for antiviral research
Keywords: Crimean-Congo hemorrhagic fever virus , deregulated pathways, phathogenesis

POS-03-080 Fibroblast Activation Protein (FAP) Degradomics

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Fibroblast Activation Protein (FAP) is a cell-surface anchored dimeric protease, closely related to Dipeptidyl peptidase 4 (DPP4). Both enzymes are also found in a soluble, secreted form. FAP expression is predominantly associated with disease states, such as cancer and liver fibrosis but few substrates of FAP are known. The aim of this study is to identify novel FAP substrates and downstream effects of knocking out FAP enzyme activity. Primary mouse embryonic fibroblasts (MEFs) were isolated from FAP gene knockout mice. These cells were immortalised by transduction with the SV40 Large T antigen. FAP and GFP were then cloned into an inducible expression system which produced both proteins in stoichiometric amounts. A second, FAP inactive, construct contained a serine to alanine substitution at the catalytic site. To identify substrates, Terminal Amine Isotopic Labelling of Substrates (TAILS) was employed to examine the differences between cell lines expressing functional enzyme (FAP e+) versus inactive FAP (FAP e-). Several protease-generated cleavage sites were identified in a number of small bioactive peptides as well as a range of ECM proteins, including type I collagen. In addition, the impact of FAP activity on the protein composition of the secretome was investigated using a quantitative gel-based proteomic strategy. These results implicated FAP in coagulation, fibrinolysis and wound healing. These data provide insights into FAP functions by elucidating its substrates and the global cellular effects of ablating its enzyme activity.
Keywords: serine protease, substrates, secretome

POS-03-081 Honeybee Worker (*Apis mellifera ligustica*) Larvae Have a More Diverse Hemolymph Proteome Than Pupae

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The honeybee hemolymph is vital for cellular and humoral immune responses in addition to transportation of nutrients. However, time-resolved changes have so far been limited to the investigation of a particular life stage. The proteome changes of the honeybee (*Apis mellifera ligustica*) hemolymph from the newly hatched larvae to five-day-old pupae were analyzed using 2-DE, mass spectrometry, bioinformatics and a western blot. Of the identified 80 protein spots that altered their abundances (49 non-redundant proteins), 59.2% (29 of 49) and 40.8% (20 of 49) were strongly expressed in the larvae and the pupae, respectively. The larval hemolymph had enhanced expressions of proteins related to metabolism of carbohydrates and energy, folding activities, development, cytoskeleton and antioxidant system, whereas proteins involved in food storage, metabolism of fatty acids and amino acids were abundantly expressed from the late larval to pupal development stage. The young larvae use multifaceted proteins to enhance their development process and as temporal humoral protection mechanisms until they establish high levels of immunity through aging. The pupae store energy proteins for their non-diet driven preparation for normal pupation. Our data reveals that the honeybee life transition matches with the indispensable functional adjustment of the hemolymph that serves as nutrition carrier and humoral immune system.

Keywords: honeybee, hemolymph, differential proteomics

POS-03-082 Absolute Quantitation of Yeast Kinases by Means of LC-MS/MS Using QconCAT and SRM Technologies

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Absolute protein quantification by MS is an important tool in assay development and systems modeling. Proteome dynamic range is the most challenging quantification barrier to date. This abstract presents the application of label-mediated targeted MS to quantify yeast kinases, which span a five-order dynamic range. QconCAT was used to create isotopically-labeled standards for 138 proteins and quantification was performed by scheduled SRM, investigating sensitivity, assay specificity and dynamic range.

A kinase QconCAT was expressed that comprises two isotopically labelled peptides for each of the targeted proteins and co-digested with a native yeast strain. SRM experiments were conducted with a nanoAcquity system interfaced to a Xevo TQ or Xevo TQ-S triple quadrupole MS. A RP gradient was employed using a vented trap-configuration. Quadrupole resolution settings of 0.7 Da and 0.4 Da were used, balancing the sensitivity of the mass spectrometers.

Experiments with unit mass resolution quantified 70% of the target proteins but had reduced success with low abundance proteins. For 147 peptides, the QconCAT and native counterpart were observed (A), affording direct quantitation. More than 77 showed inferred maxima, where the QconCAT peptide was observed, but the native peptide undetected (B). For 55 peptides, both the QconCAT and native peptide were undetected at biologically relevant levels (C). Reanalysis of the samples on a more sensitive MS and increasing Q1 and Q3 resolution to 0.4 Da, improved the success rate to 90%, quantifying proteins ranging from one million to fewer than 100 copies per cell. The type A, B and C peptides equaled 212, > 23, and 41, which increased the number of quantifiable proteins from 98 to 124. Both SRM assays were in good agreement (Pearson's r 0.93 r2 0.88) and correlate farily well with PAXdb (Pearson's r 0.96 r2 0.92). Moreover, 20 proteins were quantified that have not been reported previously.

Keywords: SRM, QconCAT

POS-03-083 The Profiling of the Effects of Adenomatous Polyposis Coli (APC) Mutation on the Cellular Proteome in Colorectal Cancer Cells

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Loss or mutation of adenomatous polyposis coli (APC), which is one of the tumor suppressor genes, is thought to initiate colon adenoma formation. Mutations of APC gene were associated with familial adenomatous coli (FAP). In addition, ~85% of sporadic colorectal cancers were reported to harbor APC mutations. However, the roles of APC in cancer development are not well understood. To obtain new understandings of cancer initiation underlying the aberrant Wnt pathway caused by APC mutation, we investigated the effects of APC mutant expression on cellular proteome using the FD-LC-MS/MS approach (Ref. 1-3). This approach allows the reproducible relative quantification of protein levels and requires an only small amount of samples. Using this approach, we found that 30 kinds of proteins were significantly altered in mutant APC-expressing cells. Remarkably, the level of annexin A2 was increased in APC mutant cells. Annexin A2 is aberrantly expressed in various human cancers and thought to be involved in invasion and metastasis in cancer cells. These findings provide clues to the mechanisms of tumor formation associated with APC gene mutations.

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Keywords: FD-LC-MS/MS, colorectal cancer, APC

POS-03-084 Proteomic Analysis of Oil Metabolic Pathway in *Arthrobacter nicotinovorans* MWB-30

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Arthrobacter nicotinovorans MWB-30, one of the oil degrading microbes, was isolated from crude oil contaminated seashore caused by oil spill incident in Taean, Korea. *A. nicotinovorans* MWB-30 exhibited 99.99% of sequence similarity with 16s rRNA of *A. nicotinovorans* DSM 420 (x80743). The strain was able to utilize diesel and kerosene, as well as crude oil as a sole carbon source for growth. Global proteomics was conducted to evaluate the catabolic difference and relationship on the degradation of specific substrates. Genome sequencing analysis of this strain, also conducted for identifies the bacterial enzymes responsible for specific substrate metabolism, which in turn ORFs were predicted in genome of MWB-30. Many of the enzymes related to the crude oil, diesel and kerosene metabolism exhibited broad substrate specificities, whereas some of the enzymes were specifically responded to the respective substrates. Therefore, catabolic enzymes of MWB-30 are composing of global and specific correlation for respective oil degradation and their metabolism.

Keywords: crude oil, diesel, kerosene, degradation, proteomics

POS-03-085 Comparative Proteomics for Yeast Interspecies Differences in Metabolic Pathway and Regulation of Protein Expression

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Comparative analysis of genome sequences and transcriptome among multiple species is powerful approach to reveal evolutionary processes and characteristics of individual organisms, based on differences in genomic architecture and regulatory mechanism of gene expression. On the other hand, interspecies differences in proteome to understand relationship of protein profile with species specific biological processes and evolution of regulation for protein abundance have been poorly studied.

In this study, we analyzed proteome expression pattern for multiple yeast species grown on different culture conditions to understand molecular differences involved in interspecies varieties. Eight yeast species grew with comparable growth rate on glucose medium. In contrast, four species of *Saccharomyces* genus, including *S. cerevisiae*, showed slow growth rate by two-to three-fold on glycerol and acetate medium, compared to four species belonging to different genus. Mass spectrometry-based quantitative analyses uncovered some groups of proteins involved in metabolic pathway that showed different abundance between *Saccharomyces* and other genus when grown on glycerol. It is suggested that such differences in abundance of proteins with specific functions could be responsible for disparity of growth rate among yeast species. Furthermore, protein expression from each duplicated gene with high conservation tends to have lower level in *S. cerevisiae*, compared to yeast species without whole genome duplication. Conserved duplicated genes, which have high copy number, seems to disperse expression responsibility on each gene. We will also report the effect of alteration of protein abundance on growth rate and quantitative analyses for the other carbon sources.

Keywords: comparative proteomics, metabolic pathway, yeast

POS-03-086 Improved Throughput and Reproducibility for Targeted Protein Quantification Using a New High Performance Triple Quadrupole Mass Spectrometer

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The SRM method on a triple quadrupole instrument has emerged as the method of choice for targeted quantitative proteomics. While designing a SRM assay, the peptide and transition selection heavily relies on the spectral library. It is critical that the MS/MS data collected on both the triple quadrupole instrument and the discovery platform have good correlation. We evaluated the correlation of MS/MS data collected on a new triple quadrupole mass spectrometer and a novel hybrid instrument, based on a mass resolving quadrupole, Orbitrap, linear iontrap (Q-OT-qIT) architecture to determine the success rate by applying discovery data directly to rapid SRM assay design for quantifying 12 standard proteins from a complex matrix. Two samples were prepared. In sample 1, 12 digested protein standards were spiked into *E. coli* matrix at same concentration of 5 fmol/ul. In sample 2, the 12 digested protein standards were spiked into *E. coli* matrix at various concentrations from 10 amol/ul to 100 fmol/ul. The sample 1 was fist analyzed using the new hybrid instrument to identify 12 proteins. The identified peptides and most strongest fragment ions were selected automatically for building an initial SRM assay for quantifying the 12 protein targets. After first run using this initial SRM assay, three peptides which provided most strongest signal intensities per protein were automatically selected to build a final optimized SRM assay. This optimized SRM assay was used to analyze both sample 1 and sample 2 with a nano flow rate separation. All spiked proteins were detected and the observed protein differential ratios between the two samples were in good agreement with the expected ratios.

Keywords: rapid SRM assay development, high throughput, reproducibility

POS-03-087 Integrated Proteomics Identified Novel Activation of Dynein IC2-GR-COX-1 Signaling by Suppression of NF1 Tumor Suppressor Gene Product, Neurofibromin, in Neuronal Cells

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NF1 tumor suppressor gene product, neurofibromin, functions partially as a Ras-GAP, and its loss is implicated in the neuronal abnormality of NF1 patients. However, the precise cellular function remains unclear. To examine the molecular mechanism of NF1 pathogenesis, we prepared NF1-knockdown PC12 cells, as a NF1 disease model, and analyzed their molecular expression profiles with a unique integrated proteomics approach, comprising iTRAQ, 2D-DIGE, and DNA microarrays, using an integrated protein/gene expression analysis chart (iPEACH). In NF1-knockdown PC12 cells showing abnormal neuronal differentiation after NGF treatment, of 3198 molecules quantitatively identified and listed in iPEACH, 97 molecules continuously up- or down-regulated over time were extracted. Pathway/network analysis revealed overrepresentation of calcium signaling and transcriptional regulation by glucocorticoid receptor (GR) in the upregulated protein set. The novel upregulated network we discovered, "dynein IC2-GR-COX-1 signaling", was then examined in NF1-knockdown cells. We confirmed that NF1-knockdown induces altered splicing/phosphorylation patterns of dynein IC2 isomers, upregulation/accumulation of nuclear GR, and COX-1 expression, in NGF-treated cells. Moreover, the neurite retraction phenotype observed in NF1-knockdown cells was significantly recovered by knockdown of the dynein IC2-C isoform and COX-1, and dynein IC2 knockdown significantly inhibited translocation/accumulation of nuclear GR and upregulation of COX-1 expression. These results suggest that dynein IC2 upregulates GR nuclear translocation/accumulation, followed by upregulation of COX-1, in this NF1 model. Our integrated proteomics strategy demonstrates that NF1-related neuronal abnormalities are partially caused by upregulation of dynein IC2-GR-COX-1 signaling, which may be a novel therapeutic target for NF1.(Kobayashi, Hirayama et al. Mol Cell Proteomics 2013 in press)

Keywords: Integrated proteomics, NF1, dynein IC2-GR-COX-1 signaling

POS-03-088 Altered Protein Expression of Rat Prefrontal Cortex Following Perinatal Asphyxia: Implications of Schizophrenia and Autism

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Background Human epidemiological studies have examined a specific subset of labor and delivery complications leading to hypoxia/ischemia at birth and consistently demonstrated a positive association with later development of schizophrenia (SZ) and autism. This has informed an animal model of perinatal asphyxia, in which rat pups were exposed to 15 min of intrauterine anoxia during Cesarean section birth. The exposed pups go on to develop behavioral and brain anatomical abnormalities which echo neurodevelopmental disorders like schizophrenia and autism. However, the underlying molecular pathways affected in this model have not been elucidated. **Methodology/Principal Findings** We harvested the prefrontal cortex (PFC), a region reliable associated with schizophrenia, from 6 and 12 week old rats exposed to to perinatal asphyxia, control Caesarian section or vaginal delivery. We investigated the proteomic profile using two dimensional differential in-gel electrophoresis and mass spectrometry, with targeted western blot analyses for confirmation. Proteomic profiling showed that mitochondrial associated enzymes were up-regulated, while signaling proteins like 14-3-3 and structural protein like neurofilament light polypeptide were down-regulated in the perinatally asphyxiated rats' PFC. Differences were most apparent at 12 weeks. **Conclusions/Significance** These results show that this rat model of perinatal asphyxia reiterates a number of pathological features of SZ and related neurodevelopmental disorders. In particular, our data fits with emerging evidence for mitochondrial-related dysregulation and disruption to signaling pathways in SZ and autism. This advances our understanding of etiological mechanisms and, in time, may inform novel treatment targets.

Keywords: 2D DIGE, schizophrenia, prefrontal cortex

POS-03-089 Novel Aspects in the Understanding of Honeybee (*Apis mellifera*) Venom from Electrical Stimulation and Manual Extraction from the Venom Gland: A Proteomic Study

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Honeybee venom is a complicated defensive insect toxin that has a wide range of pharmacological applications for human therapeutics. Manual (or reservoir disrupting) venom extraction from the venom gland (GV) and electrical stimulation (ESV) are the two major methods of obtaining honeybee venom. However, the composition of these two venom forms is still lacking a full characterization. The proteome of GV and ESV were compared using gel-based (one-dimensional gel electrophoresis, two-dimensional gel electrophoresis) and gel-free proteomics approaches. Of the 44 proteins identified in the two forms of bee venom, 9 of 17 toxins had higher abundance in ESV, five were abundant in GV, and three others showed no significant difference between the GV and ESV. Among these, four novel proteins (phospholipase A2-like, dnaJ homolog subfamily B, dehydrogenase/reductase SDR family member 11-like and histone H2B.3-like) and three phosphoproteins (icarapin-like precursor, phospholipase A-2 and apamin perproprotein) were identified. Due to the potential pharmacological substances that are found within in the toxins, the higher abundance of bee venom toxins in ESV suggests that the application of ESV is more efficient as a medical resource for human therapeutics. These findings provide previously un-described vital knowledge that significantly extends the understanding of the biochemical nature of bee venom.

Keywords: honeybee venom, electrical stimulation, venom gland

POS-03-090 Quantitative Proteomics Applied to Biobank Samples from Patients with Acute Myocardial Infarction Enrolled in SWEDEHEART, the Swedish Cardiac Registry

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The outcome after acute myocardial infarction (AMI) has improved considerably the last 10-20 years by new treatments with pharmaceutical agents and invasive procedures. However, for most therapies of AMI the beneficial effects are restricted to a small proportion of the patients while the side effects are common. Thus, there is a need to better identify patients who have a high likelihood of a beneficial effect and a high risk of side effects, respectively, in order to be able to use personalized treatment. There are certain subgroups of patients with AMI in which the outcome remains especially poor and in which considerable difficulties in selecting the most appropriate treatment exist. For the present project we have identified three such subgroups and we will focus our efforts to patients with AMI and coexisting renal dysfunction, atrial fibrillation or chronic obstructive pulmonary disease. The Centralized Region Skåne Biobank provides the samples from the SWEDEHEART Patient cohort. Using quantitative MS-based assays a large number of target proteins with putative links to cardiovascular and the coexisting diseases will be screened in a large patient cohort. The aim of the study is to develop a technology platform workflow that involves sample treatment, MRM analysis and biostatistical analysis, incorporating patient registry data.

Keywords: MRM, myocardial infarction, biobank

POS-03-091 A Qualitative and Quantitative Ion Mobility Enabled Data Independent SILAC Workflow

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SILAC employs the incorporation of isotopic labels into proteins for LC-MS based quantitative proteomics and mainly uses DDA based acquisition and informatics strategies. Due to the multiplexed nature of SILAC, sample complexity and co-isolation are increased, challenging specificity and thereby the qualitative and quantitative outcome. High resolution DIA methods, using IM separation, have the potential to overcome acquisition related issues. An informatics workflow is presented for the analysis of DIA SILAC data sets.
LC-MS data were acquired in LC-IM-DIA-MS mode using a Synapt G2-S. Nanoscale LC separation was conducted with a trap column configuration using a nanoAcquity system. WT and mutated Ba/F3 mouse cells were grown on SILAC media, digested and mixed at known ratios to study precision. Non-labelled UPS2 standard was spiked into labelled HEK-293 cells to determine workflow specificity and quantitation dynamic range. The accuracy was assessed by analyzing WT and labelled murine BW5147 lymphoma cells, replicating the experiment with reversed labelling order and contrasting the reciprocal response.
The precision study results illustrated a relative abundance distribution with median and average values of 0.47 and 0.48. The variance equalled 0.05 with the majority of the proteins illustrating the same fold-change. About 80% of had a relative expression between 0.4 and 0.6. The workflow specificity was high since searching UPS2 standard spiked into SILAC media mainly returned UPS2 identifications. Moreover, the results showed that the quantification dynamic range was at least three orders. The results were also contrasted with raw data read-out, illustrating that drift time separation was beneficial when co-eluting species could be resolved by ion mobility. Consequently, integration of the signal was improved. A reversed labelling experiment confirmed the observations with average protein and peptide quantitation CV values of 12.5% and 21.1%.

Keywords: SILAC, data independent acquistion (DIA), ion mobility (IM)

POS-03-092 Proteomics Profiling of Fuchs Corneal Endothelial Dystrophy (FECD)

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The cornea is the transparent tissue lining the anterior of the eye. It is divided into five layers: the epithelial layer, Bowman's layer, stromal layer, Descemet's membrane, and the endothelial layer, and comprises a total thickness of 500 μm. The uniqueness of the cornea is its transparency, its major function in focusing light to the retina, and its protection of the underlying ocular tissue from UV-damage.
FECD is a common disease of the cornea characterized by loss of endothelial cells as well as by deposits of extracellular material within the Descemet's membrane and the endothelial layer. This leads to leaking and thickening of the Descemet's membrane/endothelial cell layer (DM-ECL complex) resulting in corneal edema and, hence, reduced vision. To date, transplantation of a donor DM-ECL complex is the only treatment available.
The goal of this project is to initiate a discovery-based proteomics study using advanced mass spectrometry (MS) to determine the protein composition of the DM-ECL complex from FECD patients. Relative quantification using extracted ion chromatogram (XIC), iTRAQ, as well as emPAI is used to obtain knowledge on the pathophysiology of FECD. Comparing protein compositions of diseased and healthy tissue may give insight into the molecular mechanisms of FECD, which ultimately may allow non-surgical treatment of FECD.

Keywords: Fuchs endothelial corneal dystrophy, iTRAQ, XIC quantification

POS-03-093 Analysis of Proteins in the Brain Cortex Tissues of as Alzheimer's Disease Transgenic Mouse (TG)

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Many statistics claim that among the group of diseases which symptomologies are loss of both intellectual and social skills-dementia, Alzheimer's disease (AD) is the leading cause. Alzheimer's disease (AD) is a degenerative disease of the brain, herewith results into steadfast abatement of memory and mental utilities. In human brains, the proteasomal degradation of cytosolic, phosphorylation independent TAU is a potential link to the pathogenesis of neurofibrillary tangles (NFTs) in AD. In this study, transgenic (Tg) mouse model expressing neuron-specific anolase (NSE)-controlled human wild-type tau (NSE-htau23) was used, for it exhibit some of the typical AD pathological features. 2-dimensional electrophoresis (2-DE) has been performed to compare cortex proteins of transgenic mice (6 and 12-month-old) with those of the control group. Differentially expressed spots in different stages of AD were identified with ESI-Q-TOF (electrospray ionization quadrupole time-of-flight) mass spectrometry and Liquid chromatography/tandem mass spectrometry. GSTP1 and CAII which among the identified proteins, were down-regulated during the progression of AD. On the other hand; SCRN1 and ATP6VE1 were upregulated, but only in early-stages while they were down-regulated in the late stages. The proteins were further confirmed with RT-PCR at the mRNA level and with western blotting at the protein levels. In clinical applications, physical-neurological exam, mental status testing, neuropsychological testing, and brain imaging are being utilized to diagnose AD . Our discovery can fall into the entity of measurement of key proteins or protein patterns in blood, brain spinal fluid(biomarkers) which along with more advance brain imaging and mental abilities; considered as future frontiers in diagnosis of AD.

Keywords: Alzheimer's disease, 2-DE, neuron-specific anolase(NSE)

POS-03-094 Changes in Protein Expression Profiles of Brain Tissue during Cervical Cancer Development

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In cancer, it has been demonstrated that tumoral progression affects remote tissues and organs. The organ in charge of sending and interpreting stimuli from all systems is the brain. Hence, its participation during tumor development must be fundamental and so it might undergo modifications. In the present work, we found changes in protein expression profiles in mice brains due to the presence of remote 30, 45 and 50 day-old tumors from a cervical cancer cell line. We also observed that said expression profiles differ according to tumoral development. The identification of these differential proteins showed that there is an enrichment of cytoskeletal proteins overexpressed in brains of mice with tumors. Overexpression of these, among with chaperones and proteins of the 14-3-3 family, have been associated with cytoskeletal abnormalities and neurodegenerative diseases, suggesting possible brain damage due to the presence of tumors. Furthermore, this project is the first to describe brain tissue protein expression during cervical cancer development using a proteomic approach and let us propose a tumor-brain communication that could be of high relevance for cancer development. In addition, this work addresses to cervical cancer cell lines for its importance prevalence in women worldwide and especially in developing countries.

Keywords: tumor-brain communication

POS-03-095 Serine Protease HtrA1 Accumulates in Corneal Transforming Growth Factor Beta Induced Protein (TGFBIp) Amyloid Deposits

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Purpose: Specific mutations in the transforming growth factor beta induced (*TGFBI*) gene are associated with lattice corneal dystrophy type 1 and its variants. In this study, we performed an in-depth proteomic analysis of human corneal amyloid deposits associated with the heterozygous A546D mutation in *TGFBI*.
Methods: Corneal amyloid deposits and the surrounding corneal stroma were procured by laser capture microdissection from a patient with an A546D mutation in *TGFBI*. Proteins in the captured corneal samples and healthy corneal stroma were identified with liquid chromatography-tandem mass spectrometry and quantified by calculating exponentially modified Protein Abundance Index values.
Results: A C-terminal fragment of TGFBIp containing residues Y571-R588 derived from the fourth fasciclin 1 domain (FAS1-4), serum amyloid P-component, apolipoprotein A-IV, clusterin, and serine protease HtrA1 were significantly enriched in the amyloid deposits compared to the healthy cornea. The proteolytic cleavage sites in TGFBIp from the diseased cornea are in accordance with the activity of serine protease HtrA1.
Conclusions: Corneal amyloid caused by the A546D mutation in *TGFBI* involves several proteins associated with other varieties of amyloidosis. The proteomic data suggest that the sequence 571-YHIGDEILVSGGIGALVR-588 contains the amyloid core of the FAS1-4 domain of TGFBIp and point at serine protease HtrA1 as the most likely candidate responsible for the proteolytic processing of amyloidogenic and aggregated TGFBIp, which explains the accumulation of HtrA1 in the amyloid deposits.

Keywords: cornea, amyloid, emPAI

POS-03-096 Establishment and Application of a High-Quality Comparative Analysis Strategy of Low-Abundance Biomarker Peptide in Serum Based on Optimized Novel Peptide Extraction Method

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Low-abundance native peptides are an attractive target for the discovery of disease biomarkers. However, validating candidate peptides is difficult due to challenges associated with precise peptide identification and development of high-throughput assays using specific antibodies. Therefore, a highly reproducible and sensitive strategy based on effective peptide enrichment methods is needed to identify clinically useful biomarkers. We optimized our novel differential solubilization (DS) method [1] to selectively enrich peptides less than 6,000 Da, using tricine-SDS-PAGE to evaluate the optimization. The modified DS method was combined with LC-MS using conventional HPLC. The reproducibility and sensitivity of the proposed strategy were sufficient to enable discovery of low-abundance (ng/mL range) candidate biomarker peptides. A total of 40 serum samples collected pre- and post-surgery from renal cell carcinoma (RCC) patients were analyzed, resulting in discovery of 2 peptides that are upregulated and one peptide that is downregulated in pre-surgery RCC patients. These peptides were validated using 40 serum samples collected pre- and post-surgery from bladder tumor (BT) patients. Two candidate peptides that were upregulated in pre-surgery RCC patients were not upregulated in the sera of the pre-surgery BT patients. Finally, we propose 2 candidate marker peptides that could be used to detect RCC.

[1] Kawashima Y, Fukutomi F, Tomonaga T, Takahashi H, Nomura F, Maeda T, and Kodera Y, High-yield peptide-extraction method for the discovery of subnanomolar biomarkers from small serum samples. *J. ProteomeResearch*, **9**, 1694-705, 2010

Keywords: biomarker, peptide, serum

POS-03-097 Quantitative Proteome Analysis with Isotope Dimethyl Labeling to Identify TGF-beta-mediated Tumor Proteins Using the Metastatic Mouse Breast Cancer Model

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Transforming growth factor beta (TGF-beta) have complex roles in tumorgenesis with either as tumor suppressors or as pro-oncogenic factors in context-dependent manner. Although TGF-beta inhibitors have been proposed as anti-metastatic therapies for patients with many advanced stage cancer, how these inhibitors regulate the tumor-suppressive or tumor promoting effects of TGF-beta is poorly understood. Establishment of TGF-beta-related protein expression signatures may provide available biomarkers for clinical intervention to discriminate TGF-beta oncogenic effects from tumor suppressive effects. In the present study, we performed quantitative shot-gun proteomics approach coupled with stable-isotope dimethyl labeling to identify metastasis-promoting proteins with TGF-beta. We observed that systematic administration of TGF-beta receptor kinase inhibitor, SB-431542, significantly inhibited lung metastasis from transplanted 4T1 mammary tumor in BALB/c mice. We extracted proteins from SB-431542-treated tumor and "control" untreated tumor of metastatic sites and then dimethyl-labeled the proteins with triplex stable-isotopes of light (mixed replicate), medium ("control") and heavy (SB-431542-treated). The quadruplicate samples were further analyzed by LTQ-orbitrap velos mass spectrometry. We identified >2000 unique proteins and of those, 10% proteins were differentially expressed between the two groups (>1.5 fold). This study could determine tumor proteins in response to TGF-beta inhibitor in preclinical breast cancer model and provide clues for underlying molecular mechanisms associated with TGF-beta tumor-promoting activity.

Keywords: TGF-beta, metastasis, stable-isotope dimethyl labeling

POS-03-099 Elucidating the Functional Role of Frataxin (FXN) Over-Expression in HCT116 by a Quantitative Proteomics Approach

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Reprogramming of metabolic processes, or Warburg effect, has profound effects on cancer progression. Frataxin (FXN) is a nuclear-encoded mitochondrial protein initially implicated in the disease Friedreich's ataxia (FRDA). Recent evidence however suggest that FXN over-expression may have anti-cancer effects possibly mediated through alteration of cancer metabolism. To dissect FXN function in the development and progression of colorectal carcinoma, we investigated the expression changes that result from stable FXN over-expression (OE) in colon carcinoma cell line HCT116.

Based on iTRAQ analysis of FXN OE and control cells, a total of 522 proteins were significantly regulated, recapitulating significant effects on (i) mitochondrial oxidative phosphorylation, (ii) cellular redox homeostasis, and (iii) cell cycle/proliferation. Expression changes revealed by iTRAQ were validated by SWATH MS to increase the confidence of regulation. In addition, functional assays were used to validate the biological impacts of FXN over-expression. Increased oxidative phosphorylation and intra-cellular ROS in OF cells were demonstrated by JC10 measurement of mitochondrial membrane potential and DCF measurement of ROS respectively. Delayed entry into S phase upon FXN OE was confirmed through quantitative FACS analysis and semi-quantitative detection of G1/S cyclins.

These data suggest that FXN may be uniquely situated at the crossroads of cancer metabolism and oxidative defense, and that delineating the mechanistic importance of FXN may shed light on new therapeutic strategies that treat cancer from the metabolic perspective.

POS-03-098 iTRAQ-Based Quantitation and Validation by Multiple Reaction Monitoring to Identify Diabetes-Related Biomarkers in Sera from KK-A^y Diabetes Mice

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To identify candidate serum molecules associated with the progression of type 2 diabetes mellitus (T2DM), we carried out differential proteomic analysis using the KK-A^y mouse, a model of T2DM with obesity. We employed an iTRAQ-based quantitative proteomic approach to analyze the proteomic changes in the sera collected from a pair of 4 week-old KK-A^y versus C57BL/6 mice. Among the 227 proteins identified, a total of 45 proteins were differentially expressed in KK-A^y versus C57BL/6 mice. We comparatively analyzed a series of the sera collected at 4 and 12 weeks of age from KK-A^y and C57BL/6 mice for the target protein quantitation using multiple reaction monitoring, and identified 8 differentially expressed proteins between the sera of these mice at both time points. We also analyzed the serum levels of the target proteins in type 2 diabetic patients (n=5) and healthy control subjects (n=4), and found that 3 proteins still showed the differential expression between the two groups. Among them, we focused on the roles of serine (or cysteine) peptidase inhibitor, clade A, member 3K (SERPINA3K) during the progression of T2DM and/or diabetic vasculopathy. An *in vitro* assay revealed that the human homologue SERPINA3 increased the transendothelial permeability of retinal microvascular endothelial cells, which may be involved in the pathogenesis of T2DM and/or diabetic retinopathy. With the identified proteins, our proteomics study could provide valuable clues for a better understanding of the underlying mechanisms associated with T2DM.

Keywords: serum proteome, Type 2 diabetes mellitus, quantitative mass spectrometry

POS-03-100 Identification and Quantification of a Novel Prostate Specific Antigen Proteoform (SNP Leu132Ile) in Clinical Samples by Multiple Reaction Monitoring

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Prostate specific antigen (PSA) has been frequently employed as model biomarker in method developments, partly because standard immunoassays are widely available. We have designed a multiple reaction monitoring (MRM) assay to screen PSA in clinical samples (n=72), utilizing specificity and sensitivity of the approach for identification of PSA proteoforms. We report for the first time about the experimental proofs of a PSA proteoform, the product of SNP-Leu132Ile (rs2003783), observed in 9 samples in both heterozygous (n=7) and homozygous (n=2) expression profiles. Other isoforms of PSA derived from protein databases and targeted by MRM, were not identified by their unique proteotypic tryptic peptides. We also have utilized our MRM assay for precise quantification of PSA in clinical samples. The analytical performance provided high agreements between quantifications conducted with the 3 selected peptides (LSEPAELTDAVK, IVGGWECEK and SVILLGR) and a routinely used clinical immunoassay. We have also shown that the frequently used peptide IVGGWECEK is not unique for PSA and thus propose another tryptic sequence (SVILLGR) suitable for MRM-quantification of PSA in clinical samples. It is our conclusion that based on its frequency (ca. 10% worldwide), the mutant allele of LeuSNPIle should also be monitored in order to quantify PSA appropriately, using the signature peptide LSEPA (L/I) TDAVK, in samples with homogeneous and heterogeneous allele expressions.

Keywords: prostate specific antigen, MRM-MS quantification, mutant protein

POS-03-101 Stathmin-1 (STMN1) Silencing Restricts Metastatic Processes, Induces Chemosensitisation, and Potentially Inhibits EMT in CRC

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Colorectal carcinoma (CRC) is a rising concern in public health. Metastatic CRCs are particularly resistant to intervention and hence account for a large majority of global CRC mortality. Previously, we have shown that Stathmin-1 (STMN1) is implicated in CRC metastasis, since its up-regulation not only promotes metastatic behaviour *in vitro*, but also indicates poor CRC prognosis clinically. In this work, we propose and demonstrate that STMN1 silencing may be a promising form of therapy against metastatic CRC.
Targeted depletion of STMN1 (i) drastically reduced CRC metastatic phenotype *in vitro*, (ii) prominently reduced marker of EMT in primary CRC cells, and (iii) re-sensitized metastatic CRC cells to conventional CRC drug 5FU. Systematic iTRAQ profiling of STMN1 knockdown (KD) and control cells quantified the expression of 4562 proteins, and revealed that loss of metastatic potential in STMN1 KD cells was likely the summated effect of expression changes in numerous metastatic proteins and inhibitors. Increased cellular adhesion inferred from expression data was also confirmed biologically by confocal microscopy in stable STMN1 KD cells. Apparent chemo-sensitisation induced by STMN1 silencing was verified quantitatively by FACS, and shown by biochemical approaches to involve a novel Caspase 6-dependent mechanism.
Hence, STMN1 silencing appears to be a promising approach to prevent EMT, inhibit CRC dissemination, and improve chemo-sensitivity all at once. These findings, together with further validations, could potentially increase the efficiency of treating metastatic CRC and aid in reducing CRC-related mortality.
Keywords: stathmin 1, metastasis, colorectal cancer

POS-03-102 Dynamic Proteomic Survey on the Colon Tissue Interstitial Fluids (TIFs) during Tumorigenesis Process of APC^{Min} Mouse Model

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As the mediator in the microenvironment, tissue interstitial fluid (TIF) plays important roles in cell-cell crosstalking and tumorigenesis. To find the TIF proteins related with tumorigensis and understand their roles in tumor progression, we selected a mouse model of colorectal cancer, APC^{Min} mouse and collected colon tissues from APC^{Min} mice with 8, 13, 18 and 22-week old followed by TIFs preparation. To remove the age-related proteins, C57BL mouse whose genetic background is same with APC^{Min} mouse was used as a control. The TIFs from APC^{Min} and control mice were labelled by iTRAQ reagents. The Q Exactive Mass pectrometer was used to peptide identification and Scaffold was applied to peptide search and quantification.
Data quality analysis showed the iTRAQ label efficiency was 99.4% and the proteins overlap ratio of two injections was 79.4%. Combined the two injections, in total 1174 proteins were identified. With the fold change>1.3 and $p<0.05$, 322 proteins changed significantly compared with the control group. Fifty four percent differential proteins (174/322) were predicted as secretory proteins by SignalP and SecretomeP softwares. About 40% differential proteins (126/322) were reported to be mouse serum proteins, which could be potential biomarker candidates. By anlysis of the changed trend of all differential proteins with tumor progression, 42 proteins were increased and 25 proteins were downregulated sustainedly. These datas provide valuable clues for further investigation on biomarkers and mechanism of colorectal tumorigenesis.
Keywords: APC^{Min} mouse, tissue interstitial fluids, iTRAQ

POS-03-103 Confirming Different Roles of Embryo and Endosperm during Rice Seed Germination Though Proteomic Strategy

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Seed germination is a complex physiological process which allows the seed embryo to grow and develop into a photosynthetic organism. For rice, which is a typical monocotyledon plant, embryo and endosperm play different roles during seed germination. In this study, we split embryo and endosperm of rice seed and explored the changes in their proteome during germination. For embryo, both i-TRAQ and 2-D PAGE via MALDI-TOF MS methods were applied. Totally, 343 differentially displayed proteins including 191 down-regulated and 152 up-regulated were identified. We found that glycolysis was the main resource of energy during the early stage of germination, while TCA cycle was enhanced during postgerminative growth. Besides, starch synthesis was obvious during first 24 hours after imbibitions, sucrose was proved to be benefit for embryo starch synthesis, especially for amylose synthesis, and radicle growth during post-germination. Meanwhile,many secondary message signaling changing protein were identified, including G proteins, Ca^{2+} sensor proteins, lipid signal proteins, phosphoprotein interacting proteins and vesicle-mediated transport involved proteins. For endosperm, 110 changed proteins including 44 up-regulated and 66 down-regulated were identified. The up-regulation of starch hydrolytic and signaling proteins in endosperm seemed to be dependent on embryo, while the down-regulation of some transcriptional regulating and stress responsive proteins were independent on embryo.
Keywords: seed germination, embryo and endosperm, i-TRAQ

POS-03-104 Targeted Protein Quantitation for Plasma Samples by Scheduled MRM-HR Using a TripleTOF Mass Spectrometer

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Targeted quantitative analysis using MRM-HR (multiple reaction monitoring with a high resolution targeted quantitation strategy), a more recent approach developed from traditional MRM, has gained popularity in proteomics research as it provides greater sensitivity and specificity for analyte detection, and for its ease in up-front assay method development. The demand for targeted multiplexed mass spectrometry assays has risen as more extensive protein panels are required for quantitation across increasingly larger numbers of samples. By scheduling the collection of MRM-HR data (sMRM-HR) significantly more precursors can be monitored concurrently. In this study, 32 human plasma proteins were targeted for relative quantitation. Prior to sMRM-HR experiment, 1D nanoLC ESI MSMS IDA (information dependent analysis) was conducted to identify target proteins. Based on the IDA results, 70 precursors from 32 proteins were selected for sMRM-HR and MS/MS accumulation time was set at 100 milliseconds while maintaining an optimized duty cycle time of less than 2 seconds. We accessed the sMRM-HR workflow for multiplexing and its quantitative reproducibility. The assay development process as well as challenges of post-acquisition data analysis using Skyline software (University of Washington, USA) was also investigated. Furthermore, this study addresses and compares the limitations and advantages of MRM-HR and sMRM-HR.
Keywords: scheduled MRM-HR, MRM, quantitation

POS-03-105 High Resolution MRM Quantification of 300 Tear Proteins Using MS/MS^{ALL} with SWATH™ Acquisition and Its Application to Biomarker Discovery

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Many studies have demonstrated that the tear fluid is an accessible and useful source in studying ocular surface disorders and biomarker discovery. The aim of this study was to establish a robust, reproducible and rapid quantitative method for tear protein biomarker study from a large number of clinical samples. Human tear samples were collected from 1000 patients with no eye complaints (411 male, 589 female, average age 55.5 years, SD 14.5 years) using the Schirmer tear test strips and pooled into a single global control sample. A 2-hour nanoLC-MS/MS run was used to separate the tryptic peptides and MS data was recorded using MS/MS^{ALL} with SWATH acquisition on a TripleTOF® 5600 system. Data from three information dependent acquisition (IDA) experiments were combined and used as ion library for subsequent processing of SWATH acquisition data. This method was applied to compare the levels of tear proteins in both post-trabeculectomy glaucomatous eyes (n=11) and normal control eyes (n=8). For quantitation, 1487 peptides representing 474 proteins from IDA were used and the coefficient of variation (CV) of 808 peptides representing 298 proteins was below 20% for five replicates. Quantitative analysis revealed that 27 tear proteins were upregulated (ratio > 2.0) and 20 tear proteins were downregulated (ratio < 2.0) in post-trabeculectomy group as compared to control group. Tear proteomics results suggest that prolonged ocular surface inflammation after trabeculectomy may lead to increased wound healing and reduced surgical success. This study demonstrated that high resolution MS/MS based quantification of hundreds of tear proteins using MS/MS^{ALL} with SWATH acquisition is a powerful workflow for biomarker discovery.

Keywords: SWATH, tear Proteomics, high resolution MRM

POS-03-106 SILVER: An Efficient Tool for Stable Isotope Labeling LC-MS Data Quantitative Analysis with Quality Control Methods

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With the advance of experimental technologies, large-scale protein quantification is widely applied to quantitative proteomics. Different stable isotope labeling methods have been developed for the quantification of different samples based on mass spectrometry. Several software tools have been developed for single or multiple labeling techniques. Here, we present an efficient tool, named SILVER for quantification of the stable isotope labeling mass spectrometry data. SILVER implements novel quality-controlled quantification methods in spectrum, peptide and protein levels respectively. The advantages of SILVER were verified by applying it to the datasets with different isotope labeling methods and compared with MaxQuant and Proteome Discoverer. The results show that SILVER has higher accuracy and robustness. A suit of novel quantification confidence filters and indices contribute to the high confidence of the quantification results. What's more, it also includes a user-friendly visualization platform, named SILVER-view, which could provide some necessary statistics analyses and permit users to validate the results. SILVER is freely available under the GNU General Public License v3.0 at http://bioinfo.hupo.org.cn/silver.

Keywords: bioinformatics algorithm, quantitative proteomics, stable isotope labeling

POS-03-107 The Comparative Proteomic Study on Ana-1 and Ana-1-Derived Tumor Associated Macrophages

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Specialized group of macrophages called tumor associated macrophages (TAMs) has recently gained considerable attention due to their pivotal roles in the growth and invasion of tumor cells. However, the responses of macrophages to colorectal cancer inducement are controversial in numbers of published papers and the comprehensive functions of TAMs have yet to be explored in colorectal cancer. In this study, a macrophage cell line, Ana-1, was treated with the conditioned medium (CM) from a colorectal cancer cell line, CT26, for 48 hours. The protein abundances of CD206 and CD163 on cell membrane were examined by flow cytometry, and the mRNA levels of IL-10 and IL-6 were detected by Real-time PCR, respectively. Both data indicated that Ana-1 treated with CM from CT26 had been induced to TAM. To investigate the reason that Ana-1 was induced to TAM, the quantitative proteomic approach was applied to compare protein expression proflings of Ana-1 and Ana-1-derived TAM. Whole cell lysates from Ana-1 and Ana-1-derived TAM were digested by trypsin and then labelled with iTARQ reagents, followed by detection of the Q Extractive mass spectrometry. Total of 25226 peptides and 3359 proteins with two unique peptides were identified. Setting a cutoff as 1.2 fold change and p value<0.05, we found that 268 proteins were significantly alternated in response to the CM treatment, which were mainly involved in inflammatory response, cell growth and proliferation, cell-cell signaling and interaction. Among them, the elevated expression of IL-6/IL-10 and IL-1ra/CD14 were observed in Ana-1-derived TAM, which has been reported to relate with TAM phenotype. At the same time, the core enzymes of lipid metabolism and small molecule biochemistry pathways were obviously changed in Ana-1-derived TAM. These data provide valuable clues for further investigation on the interaction between macrophages and tumor cells.

Keywords: tumor associated macrophages(TAMs), conditioned medium(CM), iTRAQ

POS-03-108 Fast Stable Isotope Labeling of the Murine Intestinal Tract

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The surface of the gastrointestinal tract (GIT) is covered with mucus layers that protect it from bacteria. Disorders in forming proper mucus layers have been related to inflammatory bowel diseases as ulcerative colitis and Crohn's disease. The main protein components of the GIT mucus have been identified; however, the quantification of mucus components has been so far only performed in a label-free manner. Stable isotope labeling with amino acids in cell culture (SILAC) is the preferred method for precise relative quantification. Stable isotope labeling of live animals with a heavy amino acid-containing diet has been used to completely label mice over four generations (Krüger et al., 2008). This study also demonstrated that after 28 days of labeling the GIT epithelium proteins have an average labeling efficiency of more than 90%. The fast renewal of the GIT mucus layer has been confirmed by *in vivo* labeling of mucin glycoproteins (Johansson, 2012) and has led us to hypothesize that heavy labeled mucin proteins could be produced in less than 28 days and used in further studies as a standard. Metabolic labeling of mice has been used to study turnover in the liver, kidney, heart, brain and skeletal muscle (Claydon & Beynon, 2012); however, no study has used dietary administration of a labeled nutrient to measure the protein turnover rate for the fast renewing mucus proteins.

In the current study four mice were fed with Lys-6 labeled diet (Silantes). Mucus, epithelial cells and luminal content of different gastrointestinal segments at four time points (0, 7, 14 and 32 days) were collected and analyzed with LC-MS/MS. After 14 days more than 80% of the heavy label had been incorporated to the main mucus components. Based on the incorporation values over time we calculated turnover for mucus components and epithelial cells.

Keywords: stable isotope labeling of whole animal, turnover, Mucus

POS-03-109 Quantitative Proteomic Analysis of the Hippocampus in the 5XFAD Mouse Model at Early Stages of Alzheimer's Disease Pathology

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Alzheimer's disease (AD) is characterized by progressive memory loss accompanied by synaptic and neuronal degeneration. Although research has shown that substantial neurodegeneration occurs even during the early stages of AD, the detailed mechanisms of AD pathogenesis are largely unknown because of difficulties in diagnosis and limitations of the analytical methods. The 5XFAD mouse model harbors five early-onset familial AD (FAD) mutations and displays substantial amyloid plaques and neurodegeneration. Here, we use quantitative mass spectrometry to identify proteome-wide changes in the 5XFAD mouse hippocampus during the early stages of AD pathology. A subset of the results was validated with immunoblotting. We found that the mutant APP and PS1 proteins that are overexpressed in 5XFAD mice induce higher expression of ApoE, ApoJ (clusterin) and nicastrin, three important proteins in AD that are known to participate in amyloid-beta (Aβ) processing and clearance, as well as the neurological damage/glial marker protein GFAP and other proteins. A large subset of the proteins that were up- or downregulated in 5XFAD brains have been implicated in neurological disorders and cardiovascular disease (CVD), suggesting an association between CVD and AD. Common upstream regulator analysis of upregulated proteins suggested that the XBP1, NRF2 and p53 transcriptional pathways were activated, as was IGF-1R signaling. Protein interactome analysis revealed an interconnected network of regulated proteins, with two major sub-networks centered on APP processing membrane complexes and mitochondrial proteins. Together with a recent study on the transcriptome of 5XFAD mice, our study allows a comprehensive understanding of the molecular events occurring in 5XFAD mice during the early stages of AD pathology.

Keywords: Alzheimer disease, transgenic mice, amyloid beta

POS-03-110 Application of Nano-LC Tandem Mass Spectrometry for Screening the Differential Vitreous Protein Expressions in the Lens-Induced Myopic Chicks

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Myopia is known as the most common ocular disorder. Although the change of vitreous chamber depth in ametropic eye was well documented, vitreous proteins that may contribute to the eye growth are still largely unknown. Proteomics has shown dramatically advances to identify and quantify ocular proteins in recent decades. The present study aims to study the vitreous proteins in one of the popular myopic chicken (*Gallus gallus*) models using a non-gel proteomic approach. More than a hundred soluble vitreous proteins could be identified in normal days-old chicks. They were found to have wide variety of biological processes including cellular process, cell communication, transportation and developmental process. Most of them were found responsible for critical molecular functions such as binding, structural molecule activity, catalytic activity and receptor activity. The differentially expressed vitreous proteins in response to lens-induced myopic chicks were also investigated. Myopia and hyperopia were induced by -10.00 D and +10.00 D lenses in the right and left eyes, respectively, for 3 days and 7 days. The myopia growths were found by myopic refraction at both time points. Significant changes in ocular dimensions were characterized by A-scan ultrasound system. Using ICPL isotopes labeling, numerous differential protein expressions were identified by LCMS. Up-regulation of Apolipoprotein A-I, Ovotransferrin and Cystatin were found in response to myopic growth. This study is a crucial step in establishing a complete protein database in chick vitreous. It also demonstrated a feasible proteomic platform for further investigation of signaling pathway that might be related to myopia development.

Keywords: chicks, myopia, vitreous

POS-03-111 Proteomics Analysis of Endogenous Nuclear Receptor Proteins in Diet Induced Obesity Mouse Liver

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Obesity has increased dramatically during recent decades. Consequently, obesity and associated disorders such as nonalcoholic fatty liver disease (NAFLD) constitute a serious threat. In the liver, Nuclear receptor (NR) proteins direct a large array of key hepatic functions that include lipid and glucose metabolism, bile secretion and bile acid homeostasis. Derangements of nuclear receptor regulation and genetic variants may contribute to the pathogenesis and progression of liver diseases including cholestatic and fatty liver disease. However, little is known about the alteration of NR expression and their DNA binding activity in fatty liver due to low abundance and lack of high-throughput methods for detection at the protein level. Fortunately, we have developed an NRRE-pulldown method for profiling DNA binding activity of the NR transcription factor superfamily. In this study, the change of NR pattern in DIO mice liver was monitored and comprehensively compared by combining our NRRE-pulldown strategy with quantitative proteomics. Eighteen NR proteins or coregulators were dramatically upregulated in DIO mice liver, while 20 NR proteins or coregulators were dramatically downregulated. A set of potential NR or coregulator candidates were further validated by lost of function or gain of funtion *in vivo* with adenovirus-mediated gene silencing or overexpression. Our study uncovered the alteration of NRs and coregulators on protein level in a large-scale proteome aspect,which will expend people's knowledge of the biological functions of NR superfamily of transcription factors in liver disorder of obese subjects, and provide potential drug targets for treatment of obesity associated liver diseases such as hepatic steatosis and NAFLD.

Keywords: Nuclear receptor, proteomics, diet induced obesity

POS-03-112 Comparison of Cuprizone and Experimental Autoimmune Encephalomyelitis Multiple Sclerosis Models Using TMT and Label-Free Quantitative Proteomics and Translation to Patients

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Introduction and methodology

Multiple sclerosis is an immune-mediated disease characterized by inflammation, demyelination and axonal loss in the central nervous system (CNS), resulting in neurological disability in patients. We used both TMT-labeling and label-free quantitative proteomics to compare proteins expressed in the brain of mice representing two models of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE) and cuprizone (CPZ), in order to reveal potential biomarker candidates translatable to human CNS. First, pools of brain lysates (n≥5 individuals) were trypsinized, TMT-labeled, fractionated by Promix MP (60 fractions), and analyzed by LC-MS and SearchGUI/ PeptideShaker/ Reporter. Second, individual samples were trypsinized, analyzed by label-free quantification using an extended HPLC gradient LC-MS and results were analyzed with Progenesis.

Preliminary Data

By combining the protein identifications from TMT (4478) and label-free (2938) a total number of 5301 proteins were detected in mouse brain lysate for the two models, whereof 2115 were detected by both methods. Statistical analysis and filtering of expression data resulted in about 200 proteins which were altered in concentration level compared to CTR in CPZ and about 250 proteins in EAE. Candidates regulated in both the TMT (pools) and the label-free (individuals) experiment, and in both the cuprizone and the EAE model were focused on. As an example, CD9 antigen was significantly downregulated in both EAE and CPZ in TMT (same trend in the label-free), and this protein is associated with oligodendrocyte development. The CD9 human orthologue was also detected in human CSF and plasma. Translation of candidate proteins to human orthologues and validation in human cerebrospinal fluid and brain samples using selected reaction monitoring and immunohistochemistry is currently in progress.

Keywords: TMT-labeling and label-free quantitative proteomics, multiple sclerosis animal models, selected reaction monitoring

POS-03-113 Quantitative Proteomic Techniques: Exploring Protein Pathways and Potential Biomarkers in Dorsal Region of Rat Spinal Cord Associated with Neuropathic Pain

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Chronic neuropathic pain is a major clinical syndrome caused by disease or dysfunction of the nervous system and is often mediated by neuronal networks in the dorsal spinal cord. Unfortunately, the underlying of biological mechanisms of chronic neuropathic pain are still not well understood. In this study, we used a novel protein quantification and pathway analysis approach to identify the neuropathic pain regulated proteins and activated bio-pathways. The protein expressions in dorsal region of spinal cord were compared in three different rat groups, which are spinal nerve ligation (SNL) group, sham nerve injury (Sham) group, and naive rat group. From the quantification results, the protein expression levels of 3-hydroxyisobutyrate dehydrogenase, plasma membrane calcium-transporting ATPase 2, synapsin I, and cytoplasmic dynein1 were found significantly ($p<0.05$; fold change>2σ) decreased in SNL group compared with Sham group, and without significant changes between Sham and naive rat group. Some of these proteins have already been published as associated with pain, but not reported in neuropathic pain studies yet. It is of high expectation for biomarker discovery, although the results still need to be validated by further experiments, i.e. Western blot. The MS-obtained data along with additionally validated quantification results were also submitted in IPA® in order to identify the activated protein pathways affected by neuropathic pain.

Keywords: protein quantification, pathway analysis, neuropathic pain study

POS-03-114 Combination of a Discovery LC-MS/MS Analysis and a Label-Free Quantification for the Epithelial-Mesenchymal Transition Signature Characterization

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Disease phenotype reorganizations are the consequences of signaling pathway perturbations and protein abundance modulation. Characterizing protein signature during a biological event allows the identification of new candidate biomarkers, new targets for treatments, and selective patient therapy. For a long time, genomic studies have been used to understand pathological processes but proteomics studies become more and more useful as they provide a real and final status of a disease. The combination of discovery LC-MS/MS analyses and targeted mass spectrometry using Selected Reaction Monitoring (SRM) mode has emerged as an alternative technology to immuno-assay for biomarker identification and quantification owing to faster development time and multiplexing capability. In this study, some non transformed human breast epithelial cell line MCF10A, treated by TGF-b or stably transfected with the mutant K-ras^{v12}, two EMT inducers frequently involved in cancer progression, were used to characterize protein abundance changes during an EMT event in two different models. This study has been performed by comparing control cells versus stimulated cells after a LC-MS/MS analysis on a large-scale pool of proteins and a label-free quantification using MS1 filtering tool in skyline. The results revealed the regulation of several biological processes as translation/protein folding/proteasome and glycolysis but also RNA splicing and adhesion. This label-free method allowed a fast and repeatable quantification providing mass spectrum libraries that can be used for the development of a SRM method. Thus, a rapid measurement of an EMT signature together with specific signals could be used to improve tumor characterization and selective tumor therapy.

Keywords: label-free quantitation, single reaction monitoring, biomarkers

POS-03-115 Quantitative Proteomic Analysis of Serum Proteins in Patients with Gastric Carcinoma Using an Isobaric Tag for Relative and Absolute Quantification Labeling, 2DLC-MS/MS

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Gastric cancer is the second leading cause of cancer-related deaths worldwide. The mortality rate increased rapidly in Asia, especially in China. Until now there is no effective treatment method in gastric cancer patients before they show obvious symptoms for prevention and early diagnosis. In order to find out early disease specific biomarkers, two-dimensional liquid chromatography-tandem mass spectrometry coupled with isobaric tags for relative and absolute quantification (iTRAQ) labeling was employed to quantitatively identify the differentially expressed proteins among the different disease progress types like diffuse, intestinal and mixed type respectively. 235 non-redundant proteins were differentially expressed in total 876 identified proteins by this proteomic technique. In this study, the expression level of α-1-antitrypsin, ceruloplasmin increased, particularly in the early stage of gastric cancer. The expression level of three sub-types of 14-3-3 proteins, Rho GDP-dissociation inhibitor, platelet basic protein decreased. Those proteins are eventually related to cell signal pathway, lipid metabolism and tumor clotting mechanism. Among these 235 proteins, 27 proteins were selected to run the multiple reaction monitor (MRM) simulation by protein pilot software. Further experiments will be performed for clinical evaluation and validation. This might develop novel treatment method for gastric cancer.

Keywords: quantitative proteomic, gastric carcinoma, differential proteomics

POS-03-116 Biomarker Verification: Assessment of the Performances of LFABP as New CRC Marker on 4 Independent Cohorts of 798 Patients, Using ELISA, MRM and MRM3. Detection of CRC Early Stages and Adenomas

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Despite therapeutic progresses, survival rate at 5 years of colorectal cancer (CRC) is only 56%. CRC mass-screening has proven its efficacy to reduce mortality, but existing screening methods still lack performances. New methods, allowing compliance improvement and detection of colorectal dysplasia and early stages cancer, are critical.

Liver Fatty Acid binding protein (LFABP) has been identified using proteomics approaches on tissues. Further on, a validation process was settled to assess the clinical interest of the identified marker when tested in body fluids. Immunoassays (IA), commercial and home-made-automated, and multiple reaction monitoring and MRM3 assays were developed and used for LFABP testing.

Three independent cohorts of altogether 256 CRC patients and 465 healthy controls were assessed for LFABP doses in serum. All assays were performed in parallel on the whole second cohort (206 patients) for comparison between technologies. 77 colorectal adenomas were additionally tested. Automated IA 2C9G6 was analytically validated and a threshold could be set, allowing a true performance evaluation for adenomas and for CRC detection on the third cohort. LFABP allowed detecting 35% of CRC for a specificity of 98%. CEA and CA19-9 detected at the standard regulatory threshold respectively 25% and 13% of CRC. Moreover, LFABP allowed detecting 24.7% of the tested adenomas, and 7/11 of intra-epithelial CRC (Tis) among all patients.

This first study on 798 patients allowed developing analytically validated assays for LFABP in body fluids. MRM and MRM3 proved their efficiency as biomarker validation methods. LFABP showed promising performances to detect together precancerous and cancerous colorectal lesions. As a blood assay, LFABP dosage could help improving patient compliance, with subsequent mortality reduction. Alone, it could be a tool for CRC monitoring. Combined with other markers, the performances needed for CRC mass-screening are likely to be achieved.

Keywords: biomarker validation, MRM mass spectrometry quantification, colorectal cancer

POS-03-117 Identification of Novel Biomarkers for Prostate Cancer Radioresistance Using the Label-Free LC-MS/MS Approach

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Aim: Radioresistance is the major problem in current prostate cancer (CaP) radiation therapy (RT). Our objective in this study was to determine differential proteins in CaP radioresistant (RR) cell lines for biomarker candidates using a LC-MS/MS proteomic approach. **Methods:** Two CaP-RR cell lines (PC-3RR and LNCaPRR) were developed in our lab. After extracting and digesting the proteins from CaP-RR (PC-3RR and LNCaPRR) and CaP-control (PC-3 and LNCaP) cells. Samples were cleaned prior to MS using 3 passes through a C18 Stage-tip (proxeon) with elution in between passes. LC-MS/MS using the LTQ Orbitrap Velos ETD (Thermo Scientific, US) was used for a relative and quantitative analysis. The findings were statistically analyzed using Progenesis LC-MS software (Non-Linear Dynamics, UK) and all MS/MS ions filed were searched using the Mascot search engine for peptide and protein identification. The results were searched against the human non-redundant NCBInr database. **Results:** Total 513 proteins were found to be statistically significant differences between CaP-RR and CaP-control cells ($p \leq 0.05$, fold differences>3, power>80%). Of these 513 proteins, 186 proteins were up-regulated while 120 were down-regulated in PC-3RR cells. Whereas, 207 differential proteins, 110 were found higher while 97 proteins were lower in LNCaPRR cells compared to LNCaP cells. The several identified proteins are associated with CaP metastasis, progression, signaling pathways and radoresistance. **Conclusions**: Significant proteins were identified in the CaP-RR cells. These proteins associated with CaP radioresistance will be validated in human CaP-RR tissues. The characterization of these proteins is worthwhile in the future studies.

Keywords: prostate cancer, radioresistance; radiation therapy, LC-MS/MS; biomarkers

POS-03-118 Quantitative Analysis of Colorectal Cancer Secretomes Using MS/MS^ALL with SWATH™ Acquisition

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MS/MS^ALL with SWATH™ acquisition is a data-independent acquisition workflow that generates high resolution MS/MS spectra for all detectable analytes in a biological sample within a pre-defined mass range. By extracting high resolution XICs of any targeted analytes, MRM-like quantification can be performed. Here, we showed two studies utilizing SWATH Acquisition for the analysis of the secretomes derived from colon adenocarcinoma cell line HCT-116 and its metastatic derivative, E1. The first study is to utilize SWATH quantitative data to compare against an 8-plex iTRAQ® reagent experiment of HCT-116 and E1 secretomes. We observed that most of the differentially secreted proteins in the E1 cell line, such as SPARC, GDF15, PAI1 and MAN1A1 identified from iTRAQ analysis and subsequently confirmed by western blot, could also be readily quantified and validated using SWATH data. Secondly, we also utilized information-dependent acquisition (IDA) and SWATH acquisition to analyse the multi-lectin affinity chromatography (MLAC)-enriched glycoproteins from the secretomes of HCT-116 and E1 cells. A total of 679 proteins were identified, of which 404 were determined to be secreted and glycosylated. Of these, 182 secreted glycoproteins were differentially secreted in E1 cells. These results indicate that SWATH Acquisition is a highly promising approach that can be used for large-scale verification of iTRAQ data, as well as an unbiased label-free quantitative proteomics technique. Further evaluation of these secreted proteins as putative biomarkers for colorectal cancer metastasis in clinical samples is currently being investigated.

Keywords: MS/MS^ALL with SWATH acquisition, secretomes, colorectal cancer

POS-03-119 Proteomic Analysis for Discovering Therapeutic Targets and Prognostic Markers for Early-Stage Lung Adenocarcinoma

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To identify biomarkers and therapeutic targets for lung cancer with poor prognosis, we investigated proteins that expressed in surgically-excised cancer tissues from the patients at the early stage of lung adenocarcinoma. These cancer tissues included those resected from three patients with relapse after surgery within three years, defined here as "poor prognostic group". Frozen tissue sections were prepared and cancerous areas were removed by laser capture microdissection to extract soluble proteins from cancer tissues. The shotgun LC-MS/MS analysis detected and identified a total of 875 proteins in those cancer tissues. Relative quantitation analysis revealed that 23 proteins were preferentially expressed in the poor prognosis group. Among them, two proteins, 14-3-3 beta/alpha (14-3-3b) and calnexin (CANX) were considered to be involved in the relapse and malignant properties of lung cancer. Immunoblot and immunohistochemistry analyses confirmed the disease-associated expression of these proteins. In cell culture model with A549 Human alveolar adenocarcinoma cell line, the targeted depletion of either 14-3-3b or CANX resulted in the reduction of cell proliferation, invasion and migration. These results suggest the possible involvement of both 14-3-3b and CANX in malignant properties and poor prognosis of lung cancer. These proteins could thus be intriguing prognostic biomarkers and therapeutic targets in early-stage lung cancer.

Keywords: lung adenocarcinoma, therapeutic targets, prognostic prediction markers

POS-03-120 Gel-Based and Gel-Free Quantitative Proteomic Analysis in Oral Malignant Transformation

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Objective: This study was to improve the reproducibility and sensitivity of analysis related to oral carcinogenesis. **Methods**: Both gel-based fluorescent differential gel electrophoresis (2D-DIGE) and non-gel-based isobaric tags for relative and absolute quantitation (iTRAQ) coupled with liquid chromatography-tandem mass spectrometry have been used as screening tool for identification of differentially expressed proteins in oral malignant transformation. We comparatively analyzed the proteome profiles of multi-step tissues from normal to premalignant, finally to infiltrative cancer tissues identically from three patients. **Results**: 1,465 unique proteins were identified. Of those, 240 were differentially expressed between OSCC and normal tissues while 111 between normal and premalignant tissues, 202 between premalignant tissues and OSCC tissue. Subcellular location and functional annotation was done by *Gene Ontology* (GO). Pathway data was derived from KEGG. Moreover, we compared the different proteomic profiles between DIGE and iTRAQ. In general, nearly all the protein categories of DIGE was included in iTRAQ, but with different protein family members. The most significance is Annexins, S100 calcium binding proteins, Heat shock proteins and keratins. Up-regulation of Calcium regulation related proteins was validated in OSCC tissue microarrays by immunohistochemistry. The correlation with malignant transformation as well as recurrence and disease-free survival of patients with OSCC also was estimated. The activation of calmodulin-like 3 suppressed the migration and invasion properties of OSCC cells *in vitro*. **Conclusion**: Our study represents the successful complementary application with DIGE and iTRAQ to an investigation of oral malignant transformation, also provide valuable novel insights into the underlying mechanisms of oral carcinogenesis.

Keywords: quantitative proteomics, biomarker, oral malignant transformation

POS-03-121 Protein Dynamics of Human Cancer/Tumour Inducible Model Cell Lines Analysed by Next Generation Proteomics

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The study of human cancer/tumor model and discovery of biomarkers is a popular approch in reacent years, however, the most of candidates are not follwed up until detailed mechanism because a) too many candidate from out put, b) difficult to have a clear hyposethis from too much mixture of tissue sample, c) limitation of a huge data set analysis, etc. To avoid these problems, we used a conbination of oncogene/tumor suppresor inducible human model cell lines and a high-through put cell organera quantitative proteomic analysis termed SILAC. Here we present a successful model case of cell organera SILAC screening of human p14ARF tumour suppressor model cell line and discovery of the human Formin-2 (FMN2) protein as a novel component of the p14ARF tumour suppressor pathway. The ARF tumour suppressor is a central component of the cellular defence against oncogene activation in mammals. p14ARF activates p53 by binding and inhibiting HDM2, resulting, *inter alia*, in increased transcription and expression of the cyclin-dependent kinase inhibitor p21 and consequent cell cycle arrest. We show that FMN2 is upon p14ARF induction both at the mRNA and protein level that is independent of p53. FMN2 enhances expression of the cell cycle inhibitor p21 by preventing its degradation. FMN2 is also induced by activation of other oncogenes, hypoxia and DNA damage. These results identify FMN2 as a crucial component in the regulation of p21 and consequent oncogene/stress-induced cell cycle arrest in human cells. For novelty and importance of the discovery, this study has been filed and granted as a patent specification (PCT/GB2010/000448, 2010) to cover a potential usage of diagnosis methods of human disease. We foresee that a combination of human cancer/tumor model cell line and high through put cell organera SILAC proteomics is a very powerful approach to discover novel candidates of diagnosis and also to understand their biological mechanism in human disease.

Keywords: FMN2, ARF, SILAC

POS-03-122 Laser Capture Microdissection-based Quantitative Proteomics Analysis of Stromal Differentially Expressed Proteins in the Colon Carcinomas

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Tumor microenvironment plays very important roles in the carcinogenesis. A variety of stromal cells in the microenvironment have been modified to support the unique needs of the malignant state. This study was to discover stromal differentially expressed proteins (DEPs) that were involved in colon carcinoma carcinogenesis. Laser capture microdissection (LCM) was used to isolate the stromal cells from colon adenocarcinoma (CAC) and non-neoplastic colonmucosa (NNCM) tissues, respectively. Seventy DEPs were identified between the pooled LCM-enriched CAC and NNCM stroma samples by iTRAQ-based quantitative proteomics. Gene Ontology (GO) relationship analysis revealed that DEPs were hierarchically grouped into 10 clusters, and were involved in multiple biological functions that were altered during carcinogenesis, including extracellular matrix organization, cytoskeleton, transport, metabolism, inflammatory response, protein polymerization, and cell motility. Pathway network analysis revealed 6 networks and 56 network eligible proteins with Ingenuity pathway analysis. Four significant networks functioned in digestive system development and its function, inflammatory disease, and developmental disorder. It is the first report of stromal DEPs between CAC and NNCM tissues. It will be helpful to recognize the roles of stromas in the colon carcinoma microenvironment, and improve the understanding of carcinogenesis in colon carcinoma.

Keywords: colon carcinoma stroma, iTRAQ-based quantitative proteomics, laser capture microdissection

POS-03-123 Quantative Proteomic Analysis of RIP3 Mediated Cisplatin Sensitivity in ESCC Cells

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Recent studies showed that receptor interacting protein kinase 3 (RIP3) play a key role in caspase- dependent apoptosis and caspase-independent necrosis. Overexpression of RIP3 could switch apoptosis to necrosis. Our preliminary work indicated that RIP3 knockdown reduced cisplatin sensitivity in ESCC cells. However, how RIP3 takes part in cisplatin sensitivity is still unknown. We adopted quantative iTRAQ approaches to identify differentially expressed proteins between cisplatin-treated RIP3 knockdown cells and wide type cells. After 6h treatment of the 10μM cisplatin, proteins were extracted and digested with trypsin. Peptides were labelled with iTRAQ 4-plex reagents, mixed and separated into 60 fractions with off-line LC. Each individual fraction was identified by UPLC coupled with LTQ-Orbitrap MS/MS. Totally, 6964 protein groups were identified, and 6887 protein groups were successfully quantified. Among them, 685 proteins displayed RIP3-dependent changes in abundance, including 303 upregulated proteins and 383 downregulated proteins. Bioinformatic analysis showed that DNA damage repair, oxidative phosporylation and ribosome pathways were enriched in the upregulated proteins; while glycolysis, lysosome and fatty acid metabolism pathways were enriched in the downregulated proteins. These results suggested that RIP3 might mediate cisplatin sensitivity through multiple pathways. Proteins identified here might serve as biomarkers or drug targets. Knowledge of RIP3-regulated proteins could help us guide chemotherapy and develop new drugs.
This work was supported by grants of the National High-tech R & D Program (No. 2012AA020206, 2012AA02A503) and SKPBR of China (*Shouzhi Ma and Linhui Zhai are equal contributing authors).

Keywords: RIP3, quatative proteomics, iTRAQ

POS-03-124 Serum Proteome Analysis during Metastatic Processes of Hepatocellular Carcinoma and Function of Transaldolase and Secretory Clusterin

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Metastasis is the major cause for low therapeutic efficiency and poor prognosis of HCC patients. It was indicated that complex aggressive process of metastasis were closely related with tumor cell biological behavior (such as adhesion, motility and proliferation), tumor angiogenesis and so on. In this study the visible HCCLM3-R nude mouse xenograft model has been established and the iTRAQ based high throughput quantitative proteome analysis was provided for detecting differential key proteins in specific metastasis stages. The results are as follows:A total of 554 serum proteins were finally identified. 80 differential proteins were screened out and seven candidate proteins (FETUA, GRP78, ZA2G, LUM, AFAM, TALDO and ITIH3) were further validated by western blot. It was proven that Transaldolase (TALDO) in human HCC tissues and HCC cell lines was associated with its metastasis. Subsequent screening of TALDO expression in 72 serum samples revealed that the higher serum TALDO level in HCC patients with metastasis. A ROC analysis was a 77.8% of sensitivity and 86.1% of specificity for the predicting of HCC metastasis.By using the interracial research, a total of 20 putative human proteins were screened out and the secretory clusterin (sCLU) overexpression was closely associated with HCC metastasis. Tumor-derived sCLU facilitates hepatocellular carcinoma metastasis through inducing epithelial-mesenchymal transition via TGF-β-Smad3 signaling pathway.

Keywords: HCC metastasis, quantitative proteomics

POS-03-125 Comprehensive and Comparative Proteomics Reveals Alterations of Metabolomics between Monolayer and Three-Dimensional Cell Cultures

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Monolayer cell culture has been widely used in many fields of life science. However, it is known by now that the cells in monolayer culture do not represent the natural functions of their original tissues or organs. Recently, three-dimensional cell culture systems have been proposed as better models to mimic the *in vivo* function of cells. Among organs, liver is the one of organs that possesses complex metabolic systems. The functions are hardly reproducible in monolayer dishes of cell cultures. We have established a three-dimensional radial-flow bioreactor (3DRFB) which gives human liver cell lines the differentiated hepatic functions and morphological appearance. While comprehensive studies have been done for the cells from 3DRFB by using microarray, mRNA levels do not generally agree with protein expression levels. Here, we reveal the proteomes and the alteration of proteomes between monolayer and three-dimensional cultures of a human hepatocellular carcinoma-derived cell line FLC-4 (Functional Liver Cells-4). By using comprehensive and comparative mass spectrometry-based proteomics, we found about 10% of identified proteins appeared and about one fourth of the proteins disappeared only in the cells from 3DRFB. Many proteins which increased their expression or only appeared in the 3DRFB cells were involved in pathways of small molecule biochemistry, molecular transport, cell organization, cell movement, cell-cell interaction. Taken these results together, we are able to understand functional proteins and biological pathways of cells from 3DRFB comprehensively by identifications of the altered proteins and biological systems between these two distinct cell culture systems.

Keywords: liver, three dimensional cell culture, radial-flow bioreactor

POS-03-126 A Proteomic Investigation into the Molecular Mechanism of HIV Tat Induced Neuronal Apoptosis

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HIV related neurocognitive disorders affect up to 70% of HIV patients with varying degrees of severity. While there has been a great deal of work suggesting various viral and host molecules and pathways which may ultimately result in neuronal apoptosis, there are no all-encompassing data to consolidate these findings and provide insight as to how they may all function together. To this end, we performed SILAC based quantitative proteomic analysis on HIV-Tat treated neuroblastoma cells. Isolated protein was fractionated by PAGE and analysed by nLC-MS/MS on the orbitrap velos. Using MaxQuant, we identified 2791 unique protein groups with quantitation by minimum two unique peptides. Using the student's t-test, we identified 482 differentially regulated proteins which were analysed using Ingenuity Pathway Analysis (IPA). Herein, we present direct proteomic evidence for the entry of HIV-Tat into neurons as well as kinase cascades which ultimately results in apoptosis via key HIV-dementia associated signaling pathways. We also provide evidence for several pathways resulting in known HIV-dementia and Alzheimer's disease pathologies which are certainly capable of contributing to the apoptotic phenotype and warrant investigation as therapeutic targets. Our findings are highly congruent with literature regarding key molecular features in HIV-dementia. Together, these data provide a proteomic map of cellular pathways leading to neuronal dysfunction which can be used to test the effect of potential treatments and inhibitors used to query the involvement of specific HIV-Tat dysregulated pathways.

Keywords: HIV-dementia, quantitative differential proteomics, SILAC

POS-03-127 Screening for Protein Biomarkers in Alzheimer's Disease Using Multiplex Quantitative Mass Spectrometry

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by progressive memory loss and pathologically by the presence of neurofibrillary tangles and senile plaques. The etiopathology of AD is still unclear and new biomarkers might improve the chances for early diagnosis and prognosis. The major goal of this work was to quantitatively compare the brain proteomes of AD patients and controls in order to gain an improved understanding of the neuroproteome profiles in the AD pathology.

Proteins were extracted from temporal cortex biopsies of AD and control brains. Two different detergent based extraction techniques were used to comprehensively extract and dig deeper into the proteomes. The processing involved protein extraction and enrichment, fractionation, protein precipitation, tryptic digestion and stable isotopic dimethyl labeling according to protocols developed in our lab. Isotopically labeled and combined AD and control samples were analyzed by LC-MS/MS using a 7 T hybrid LTQ-FT mass spectrometer. Quantification and statistical evaluation was carried out with the freely available MSQuant software.

Our preliminary results revealed variation in the protein profiles between the AD and healthy control groups. Among the identified proteins, there are considerable up/down regulations of membrane, nucleus and cytoplasmic proteins that are associated with neurological disorders ($p<0.05$). Several of the significantly regulated identified proteins in temporal cortex are connected to decreased mitochondrial depolarization. Numerous proteins demonstrating major changes, of hydrophobic nature (membrane bound), were revealed as potential diagnostic biomarkers. Our findings may provide knowledge for unraveling new biomarkers for early diagnosis and prognosis in AD.

Keywords: Alzheimer's disease, dimethly labeling, quantitative proteomics

POS-03-128 Proteome Analysis of a Hepatocyte-Specific BIRC5-knockout Mouse Model during Liver Regeneration

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The Baculoviral IAP repeat-containing protein 5 (BIRC5 - also known as inhibitor of apoptosis protein survivin) is a component of the chromosomal passenger complex, which plays an important role in cell division. Since its discovery BIRC5 arrested attention because of its elevated expression in cancer cells as well as proliferating cells. Great affords have been made to understand the role of BIRC5 in cancer, but its role in regenerative processes like liver regeneration still remains to be further clarified.

We performed a label-free proteomics study to investigate the influence of a hepatocyte-specific BIRC5-knockout during liver regeneration after 70% hepatectomy. Liver samples of knockout and wild type mice were analyzed before and three days after hepatectomy. Subcellular fractionation of samples was applied to achieve higher proteome coverage. The samples were analyzed by LC-MS/MS using an Orbitrap Elite Instrument. Proteins were quantified with Progenesis-LC-MS and Ingenuity Pathway Analysis Software was used for data interpretation.

The analysis of the hepatocyte-specific BIRC5-deficient mice revealed alterations of protein networks linked to diverse cellular functions, which have not yet been described in relation to BIRC5. We observed higher expression levels of proteins connected to ubiquitinylation and vesicle trafficking. Proteins related to the transcription and translation machinery were found to be higher abundant in the BIRC5-kockout condition and showed elevated expression as a consequence of hepatectomy. We propose that these findings represent compensatory mechanisms as a result of BIRC5 ablation. The connection between BIRC5 and the affected cellular functions will be part of future studies.

Keywords: label-free proteomics, BIRC5, liver regeneration

POS-03-129 A Proteomic Analysis of Radio-Resistance in Breast Cancer Cell Lines.

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Ionizing radiations are one of the most important cytotoxic treatments adopted in cancer treatment. Toxicity largely depends on lesions caused to the DNA leading cells to respond by activating multiple response and repair mechanisms. Expression of DNA repair enzymes potentially provides cancer cells with a means to resist treatment and survive the irradiation. Here we present a quantitative shotgun proteomic characterization of the response of two breast cancer cell lines (carrying either the wild-type or mutated p53 gene) to both single or multiple doses of gamma-irradiation. Known as well as putative novel repair mechanisms were found at protein level, and linked to cell-cycle controllers. A preliminary phospho-proteome analysis is also described.

Keywords: SILAC radiotherapy DNA-repair

POS-03-130 Quantitative Proteome Analysis of Cervical Cancer Tissues Using a iTRAQ Approach

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The accurate understanding of tumour development relies on the comprehensive study of proteins. They are the main orchestrators of vital processes, such as signalling and metabolic pathways, which drive the carcinogenic process. Proteomic technologies can be applied to cancer studies to detect differential protein expression and to assess different responses to treatment and diagnosis. The cervical-uterine cancer (CuCa) is the third most common malignancy affecting women worldwide and is the major cause of morbidity and mortality in developing countries. There are some studies in the literature to describe the expression levels of proteins, but to our knowledge, there is no report published to date, about quantitative proteome analysis in cervical cancer tissues. We assessed highly overexpressed proteins in 5 different groups of cervical tissues types compared with normal tissue samples by liquid chromatography tandem mass spectrometry (LC-MS/MS) coupled with isobaric tags for relative and absolute quantitation (iTRAQ) technology. More than five hundred unique proteins were identified (FDR 1%). Several proteins were found to be significantly up-regulated or down regulated among the different groups analyzed. Results will be presented and further discussed. Part of this work was supported by PAPIIT-UNAM grant IN206113.

Keywords: quantitative-Proteomics, cancer, iTRAQ

POS-03-131 Label Free Quantitative Proteome Analysis on Cerebrospinal Fluid to Discover Severity Grade Markers for Human T-Cell Leukemia Virus Type 1 Associated Myelopathy/Tropic Spastic Paraparesis (HAM/TSP)

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Human T-cell leukemia virus type 1 (HTLV-1) associated myelopathy/tropic spastic paraparesis (HAM/TSP) is developed by the spinal cord inflammation fundamentally due to the HTLV-1 infection. The incidence of HAM/TSP is approximately 0.1% within the virus carriers after 30 years asymptomatic phase. Although appropriate evaluation of the disease status is essential for the decision of therapeutic intervention and for the control of patients' QOL, diagnosis is now totally dependent on the patients' motor manifestation with less objectivity.
Here we performed label-free quantitative proteome analysis for cerebrospinal fluids (CSFs) to identify severity grade markers for HAM/TSP. CSF specimens from 6 asymptomatic carriers and 51 HAM/TSP patients were collected and analyzed by LTQ-Orbitrap-Velos mass spectrometer. The raw data were transferred to Expressionist Proteome Server Platform (Genedata AG, Basel) and transformed into retention time - m/z 2D planes. The data processing module RefinerMS identified 68,077 multiply charged isotopic clusters as peptide derived ions. The peptides were ranked in increasing order by the *p*-value of Pearson's correlation coefficient between peptide intensities and severity grades. Finally peptides with top-100 *p*-values were extracted as the severity grade marker candidates and subjected to protein identification analysis based on 2D-LC/MS/MS protein identification data set (1,863 CSF proteins). Among 14 identified biomarker proteins, we further performed ELISA-based validation experiments for Myelin-associated cerebrospinal fluid protein (MCFP). The results of MCFP-ELISA measuring 51 CSFs or 105 plasma samples showed statisticallysignificant negative correlation between severity grades of HAM/TSP and MCFP levels, reflecting the progression of demyelination. We'd like to suggest a guideline model for HAM/TSP treatment.

Keywords: proteomics, mass spectrometer, leukemia

POS-03-132 Performance of the Label-Free Differential LC-MS/MS Incorporating Peptide Identification Items to Associate Peptide Detection Signals Among Measurements

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Label-free LC-MS/MS is a promising choice for proteomic differential analysis. With applicability to large sample sets, the methodology has spread especially over the fields of clinical biomarker discovery. To facilitate data processing, we developed the software named i-RUBY that enables fully automated signal alignment across LC-MS/MS measurements with the aid of peptide identification items. Here we further optimized our analytical platform based on label-free fashion. An Orbitrap mass spectrometer was used for high accuracy peptide mass measurement. A single LC-MS/MS measurement batch comprised a maximum of 20 trypsinized peptide samples: initial three repetitive runs for system equilibration, followed by 20 real samples and a peptide mixture from standard proteins at the preceding, middle, and succeeding positions of the real sample sequence. The analysis order of real samples was randomized across and within batches. In a signal alignment of the real samples, i-RUBY usually detected more than ten thousand peaks associated with reliable peptide identification(s). Measurement variability of the ion intensity within each measurement batch was assessed as the relative standard deviation (RSD) between the three standard sample measurements. RSDs fell below 10% for the relative ion signal intensity of the selected seven peptides. We applied the analytical platform to clinical studies including formalin-fixed and paraffin-embedded (FFPE) tissues. Data processing using i-RUBY and the following statistical analysis supported capability of the present analytical strategy.

Keywords: differential proteomics, label-free LC-MS/MS, statistical analysis

POS-03-133 Quantitative Mass Spectrometric Immunoassay of Clusterin

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Purpose: The objective of this work was to develop a proof-of-concept MALDI-based high-throughput mass spectrometric immunoassay (MSIA) for the quantification of human clusterin in plasma samples and to investigate plasma clusterin concentrations in multiple clinical groups related to diabetes and cardiovascular disease (CVD).
Experimental Design: Intact clusterin was extracted from plasma with MSIA affinity pipette tips followed by addition of a heavy-labeled peptide external standard, on-target tryptic digestion and analysis by MALDI-MS. A total of 100 samples from patients with normal glucose tolerance (NGT), clinically manifested pre-type 2 diabetes (preT2D), T2D, T2D with microalbuminuria, or T2D with established CVD were analyzed.
Result: Intra-assay precision was 9.78% (CV); three-day inter-assay precision was 17.3%. MSIA results were compared via Bland-Altman plot to those from a conventional ELISA, revealing a mean percent difference of 14.9%. Clusterin concentrations were 187.9±52.8 μg/mL (mean±SD) in NGT patients, 162.4±73.9 μg/mL in preT2D, 219.7±57.5 μg/mL in T2D, 150.3 ±35.9 μg/mL in T2D with microalbuminuria, and 146.5±45.1 μg/mL in T2D with established CVD.
Conclusions: Clusterin concentrations in plasma from T2D patients with microalbuminuria and T2D patients with established CVD were significantly lower than those in other T2D patients, suggesting an inverse association of clusterin concentration with CVD risk in the context of T2D.
Keywords: clusterin, cardiovascular disease, mass spectrometric immunoassay

POS-03-134 Absolute Quantitation of Plasma Biomarker Peptides Apl1β for Alzheimer Disease at fmol/ml Level Using SRM

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Recent advances in proteomic technology such as selected reaction monitoring (SRM) enabled the detection and quantification of specific proteins in complex samples. We have previously identified APLP1-derived Aβ-like peptides (APL1β 25, 27, 28) in human cerebrospinal fluid (CSF) and found that the ratio of APL1β28/total APL1β increases in CSF of Alzheimer disease (AD) patients compared with non-AD controls, which could be a novel surrogate marker for AD. However, examination of CSF is highly invasive for medical screening and less aggressive procedure such as blood test is needed. Thus, we investigated if APL1β could be detected and quantified in human plasma using SRM. Five optimal and validated precursor ion to fragment ion transitions of APL1β were developed on a triple quadruple mass spectrometer TSQ Vantage, and then three best responding transitions were used in final measurements. The resulting assays were then applied to detect and quantify the APL1β peptides in human plasma. To absolutely quantify the peptides, stable isotope-labeled reference peptides (SI peptides) were included in the plasma. Using the SI peptides for internal control, absolute concentrations of APL1β 25, 27, 28 peptides in control plasma were able to quantified. Strikingly, these plasma APL1β concentrations are around 1fmol/ml which are much lower than those in CSF, which are at pmol/ml level. Thus, SRM is a highly sensitive method to detect low-abundance proteins and peptides in complex samples such as plasma and has a potential to quantify over the whole range of plasma proteins.
Keywords: SRM, biomarker, Alzheimer disease

POS-03-135 Metabolomics Data Normalization Improves Correlation with NMR and Physiology Data

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LC-MS metabolomics data is generally very noisy as it is affected by many systematic biases, such as batch effect or day-to-day variation. Here we analyze an Intralipid infusion dataset where ten healthy males were infused with Intralipid in a double-blind, placebo-controlled experiment. Intralipid is a fat emulsion used as a component of parenteral nutrition for patients who are otherwise unable to get nutrition via an oral diet. In the very young Intralipid infusion can lead to a number of adverse effect as well as severe diseases. We thus explore changes in the metabolome of patients after infusion Intralipd (20% concentration) or Saline on different days.
We propose the use of singular value decomposition normalization for metabolomics data. The method works in several stages. First, we preserve the treatment group differences in the metabolomics data by estimating treatment effects with an ANOVA model (multiple factors can be used). We then use singular value decomposition of the residuals matrix to determine the bias trends in the data. Number of bias trends is estimated via a permutation and the effect of the bias trends is eliminated.
We show that a singular value decomposition-based normalization method removes bias of unknown complexity from the LC-MS metabolomics data allowing for improved differential expression analysis. Moreover, normalized data better correlates with the NMR and physiology data collected for the same biological samples. We advocate the use of singular value decomposition-based normalization for metabolomics data and explain ways to link LC-MS metabolomics data to NMR and physiology data.
Keywords: normalization, singular value decomposition, metabolomics

POS-03-136 Deconvolution of Overlapping Peptide Isotopic Clusters with EM Algorithm for Label-Free Quantification

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Even with today's high resolution instrument and LC separation techniques, for complex mixtures the overlapping of peptide isotopic clusters still cannot be avoided. This overlapping will hamper the quantification accuracy of the label-free method. Expectation maximization (EM) is a statistical algorithm which is used to deal with mixed distributions. In this poster, we present an EM based deconvolution algorithm for the overlapping peptide isotopic clusters. Here we use EM algorithm to auto-fit the component isotopic clusters for an overlapping peptide isotopic cluster. The algorithm is described as below: Step 1. Detect all the local maximum points on the LC-MS view; Step 2. Initialize a distribution model for each local maximum point and each possible charge. Each model represents a component isotopic cluster; Step 3. Use EM iteration to auto-fit the distribution for each component isotopic cluster; Step 4. Remove insignificant distribution models; Step 5. Do EM iteration again for all the remaining distribution models; Step 6. Report the position, duration and intensity for each component isotopic cluster. The algorithm is tested on two data sets. The first is from human samples. Every sample was done in triplicate at the biological level. In total 12 files are generated after being analyzed by LC coupled with Thermo LTQ-Orbitrap instrument. One file is picked to test the method proposed here. This file contains 4537 MS scans and 3252 MS/MS scans. After analyzed by the EM based algorithm, 8138 peptide isotopic clusters are reported. Among them 1825 (22%) clusters are overlapped with other clusters. The second set is from yeast samples. 6 files are generated after the sample is analyzed by LC coupled with Thermo Q Exactive instrument. One file with 35268 MS scans and 68128 MS/MS scans was analyzed by the algorithm. 96818 peptide isotopic clusters are reported. Among them 46193 (48%) clusters are overlapped with other clusters.
Keywords: label free quantification, EM algorithm, overlapping isotopic clusters

POS-03-137 Comparative Proteomic Analysis of Early Salt Stress-Responsive Proteins in Roots and Leaves of Rice

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Growth and productivity of rice (*Oryza sativa L.*) are severely affected by salinity. Understanding the mechanisms that protect rice and other important cereal crops from salt stress will help in the development of salt-stress tolerant strains. In this study, rice seedlings of the same genetic species with various salt tolerances were studied. We first used two-dimensional gel electrophoresis (2-DE) to resolve the expressed proteome in rice roots and leaves and then used nanospray liquid chromatography/tandem mass spectrometry (nano-LC/MS/MS) to identify the differentially expressed proteins in rice seedlings after salt treatment. The 2-DE assays revealed that there were 109 differentially expressed proteins in rice roots and 83 differentially expressed proteins in rice leaves. Functional classification analysis revealed that the differentially expressed proteins from roots could be classified into 18 functional categories while those from leaves could be classified into 12 functional categories. The proteins from rice seedlings that most significantly contributed to a protective effect against increased salinity were cysteine synthase, ATP synthase, quercetin 3-O-methyltransferase 1, and lipoxygenase 2. Further analysis demonstrated that the primary mechanisms underlying the ability of rice seedlings to tolerate salt-stress were glycolysis, purine metabolism, and photosynthesis. The findings reported herein provide a comprehensive profile of the metabolic mechanisms in roots and leaves that protect rice seedlings from salt-stress impacts.

Keywords: rice, salt stress, gel-based proteomics

POS-03-138 Novel Aspects of Understanding Molecular Working Mechanisms of Salivary Glands of Worker Honeybees (*Apis mellifera*) Investigated by Proteomics and Phosphoproteomics

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Honeybee salivary glands (SGs) are important exocrine glands. However, the molecular basis of how SGs fulfill their biological duty is still elusive. Proteomics and phosphoproteomics of cephalic SG (HSG) and thoracic SG (TSG) were compared between normal and single-cohort honeybee colonies. Of 113 and 64 differentially regulated proteins and phosphoproteins, 86 and 33 were identified, respectively. The SGs require a wide spectrum of proteins to support their multifaceted functions and ensure normal social management of the colony. Changes of protein expression and phosphoproteins are key role players. The HSG triggers labor transition from in-hive work to foraging activities via the regulation of juvenile hormone and ethyl oleate levels. The stronger expression of proteins involved in carbohydrate and energy metabolism, protein folding, protein metabolism, cellular homeostasis and cytoskeleton in TSG, support the gland to efficiently enhance honey processing by synthesis and secretion of saliva into nectar. The age structure of the colony is vital for increased production efficiency. This data reveals the molecular underpinning of SGs to accomplish their biological missions and provides new knowledge for the beekeeping industry for enhancing the maintenance and production efficiency of the colony and honey quality through manipulation of potential target proteins.

Keywords: honeybee, salivary gland, proteome

POS-03-139 High Resolution LC-MS/MS versus SOMAscan Proteomics Platform: An In-Depth Comparison of Two Quantitative Proteomic Technologies

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Measuring protein expression level differences is not only crucial to biological systems understanding but more importantly allows quantitative characterization of different biological states. Although the current preferred technique in quantitative proteomics is liquid chromatography coupled to mass spectrometry (LC-MS/MS), recently array based techniques have gained in analytical power and consequently popularity. Here we report a comparison of the analytical performances of high resolution LC-MS/MS and a DNA-aptamer-based protein profiling assay developed by SomaLogic. Human embryonic kidney cells (HEK293) were treated with the proteasome inhibitor MG132 or the stress hormone cortisol to quantify disturbances at the proteome level. After standard sample preparation, samples were divided and submitted to either the current SOMAscan™ assay measuring specifically 1129 proteins, or were quantified using stable isotope labeling in cell culture (SILAC) with subsequent pre-fractionation by in-solution isoelectric focusing and coupled to high resolution nano-LC-MS/MS. To assess analytical sensitivity within the frame of clinical measurements, samples were quantified both in the presence and absence of 50% serum. In total, 22 samples including biological and technical replicates have been analyzed with the SOMAscan™ assay and 264 separate MS runs were performed. Mass spectrometric data analysis and subsequent database search was performed using MaxQuant program suite. Statistical analysis and comparison of quantitative datasets were designed and performed using the R programing environment. Preliminary results demonstrate the complementarity of both techniques with limited overlap between both datasets, as expected when comparing targeted vs. exploratory approaches. Analytical performances and quantitative power will be discussed in details for both techniques.

Keywords: quantitative proteomics, LC-MS/MS, aptamer

POS-03-140 Urinary Proteome Variability Assessment of Normal Monozygotic Twins

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Determining the normal fluctuation of individual urinary proteins is important in providing threshold guidelines in biomarker discovery. For the quantitative variability assessment, monozygotic (MZ) twins could offer additional benefit of controlling for environmental factors by eliminating genetic variables. MZ twin pairs share essentially identical genomes at the DNA level and the differences within pairs could reflect environmentally influenced differences. Early morning urine samples from nine overnight fasted female MZ twin pairs were obtained for quantitative protein variability analysis. Urine samples were analyzed in duplicates by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Velos ion trap mass spectrometer. Six hour-long LC gradients were used to enhance protein identification and data were obtained by data dependent acquisition (DDA). Differences in protein quantity were determined by peptide spectral counting. The quantitative degree of urinary proteome variation was assessed by Spearman correlation coefficient and Ward hierarchical clustering analyses. Among the nine pairs, three twin pairs clustered together. When a subset of abundant proteins were subject for a further analysis, protein expressions varied by more than 1X10^^{2} among the nine twin pairs. These preliminary results indicate that urinary proteome variability exceeds the genetic boundaries of MZ twins, reflecting the impact of non-genetic environmental influences in biological samples.

Keywords: Urine, variability, twin

POS-03-141 Proteome Analysis of Ginseng Extract, Ginsenoside F2 and Ginsenoside Rg2 Treated Neuronal Cell

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Ginsenosides are a class of steroid glycosides, and triterpene saponins, found exclusively in the plant ginseng. Ginsenosides have been the target of research, as they are viewed as the active compounds behind the claims of ginseng's efficacy. Ginseng extract (GE) and ginsenoside F2 (F2), ginsenoside Rg2(Rg2) functional ingredient for Proteomic analysis techniques by Proteomic Studies analysis. Especially Recent studies have reported that Ginsenoside Rg2 has Metastasis inhibitory and antitumor activity effect. To investigate changes of the proteome analysis by functional ingredient of GE, F2 and Rg2, we used a quantitative proteomic approach using stable isotope labeling by amino acids in cell culture (SILAC), coupled to LC-MS/MS based proteomic approach. Using this approach, we identified more than 3331 proteins from SH-SY5Y cells cultured in two other physiological conditions. To analyze regulation of neuron proteome analysis with these data, we used proteome informatics tools including Gene Ontology, statistical tools, KEGG, and protein-protein interaction database. The results are visualized using Cytoscape, and its plugs. This analysis shows the functional networks information and quantitative proteomic of GE, F2 and Rg2 including cellular location, molecular function, and signaling pathway. Thus, these results reveal a previously unknown protective effect of nerve cells of GE, F2 and Rg2.

POS-03-142 Quantitative Proteomics to Study Neuromuscular Disorders

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Cellular accumulation and aggregation of misfolded proteins is connected to several neuromuscular disorders. Marinesco-Sjögren Syndrome (MSS; OMIM 248800) is a heterogeneous multisystemic disorder, although rare and genetic, the patients suffer from skeletal muscle, eye and brain abnormalities. Genetic studies show that mutations in the *SIL1* are responsible for this disease (~50% cases). SIL1 is a nucleotide exchange factor (NEF) for HSP70 chaperone BiP (GRP78). The latter is associated with the biosynthesis of secretory and membrane proteins in the lumen of endoplasmic reticulum (ER). However, it is not clear why the loss of SIL1 affects only certain tissues/organs. We therefore performed a comparative analysis using iTRAQ and label free approaches to investigate the underlying proteomic changes taking place due to disrupted protein folding mechanism in the lumen of ER. From our results we could confidently identify and quantify 4872 proteins from human lymphoblasts (unaffected tissue from patients) and nearly 1630 proteins from mice (*woozy*-mouse model of MSS) muscle tissue, respectively. In the both datasets, we found regulation of several proteins which are involved in the activation of unfolded protein response (UPR) pathway, like BiP and MHC class proteins. Coincidentally, our data correlates with the morphological findings observed in electron microscopic studies and the Western blot results. Moreover, a part of these proteins are also related to other neurodegenerative disorders and hence will be specifically monitored using targeted MS/MS approaches. Currently, analysis of *woozy* mice brain proteome is carried out with quantitative proteomics approaches. **Abbreviations:** SIL1: suppressor of Δire1 Δlhs1 double mutant number 1; HSP70: heat shock protein 70; BiP: immunoglobulin heavy chain binding protein; iTRAQ: isobaric tags for relative and absolute quantification.

Keywords: neuromuscular disorders, iTRAQ, UPR

POS-03-143 Hidden Proteome: Multiplex Quantitation of Low- and Ultralow-Copy Number Proteins in HepG2 Cells and Human Plasma

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Using ordinary SRM approach we have registered almost complete chromosome 18th proteome in human plasma and HepG2 cells. Still, there is some portion of proteins that remaine undetectable due to their low concentration. To discover and quantitate such "hidden" proteins we designed an experiment comprising reducing sample complexity, increasing the sensitivity and simultaneous qualification and quantitation using stable isotope dilution in QED-SRM mode. The problem of complexity of biological sample was resolved by fractionation. The resulting fractions were enriched by irreversible covalent binding, which allowed proteins immobilization and enhancement of digestion with trypsin. Quantitative analysis was performed in QED-SRM mode using multiplex calibration approach with stable isotope dilution. The defined attitude permitted simultaneous verification of the targeted peptides by full MS and following progressive MS/MS scanning of the isolated precursor ions overlaying SRM transitions. Quantitative analysis in reduction energy ramping manner was performed in the case where SRM transitions match the defined criteria after dd-MS/MS scanning. The correctness of the isolated and quantified peptides was verified by, firstly, MASCOT processing of the accumulated MS/MS data and matching them to the registered peptides; co-elution of native peptides with heavy internal standard peptides; and assay of the transitions stability and conformity using MLD (Mean Logariphmed Derivative) function. Thus, we registered up to 90% of proteins after fractionation and enrichment with sensitivity of 10^{-17} M. Only 70% of the verified proteins were quantified while the remaining proteins were registered with the signal outside of the LLOQ. Bioinformatic processing of the registered and quantified proteins interaction and molecular functions has been applied in order to eunsure the possibility of their adequate presence and qunttitation in HepG2 cells and human plasma.

Keywords: quantitation, QED-SRM, low-copy number

POS-03-145 Label Free Mass Spectrometry Proteome Quantification of Human Embryonic Kidney Cells Following 24 Hours of Sialic Acid Overproduction

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Background

Cell surface glycoprotein sialylation of one of the most ubiquitous glycan modifications found on higher eukaryotes. The sialylation pattern of cell is influenced by the environment but also by the Golgi sialyltransferase activity and flux of metabolites through sialic acid producing pathways. Altered cell surface sialic acid patterns have been observed in several cancers and other pathological conditions. In this experiment we examined the cellular proteomic changes that occur in HEK293 cells after 24 hours of sialic acid overproduction induced by excess N-acetylmannosamine.

Methods

Cellular lysates of N-acetylmannosamine and mock induced HEK293 cells were analyzed with high resolution mass spectrometer utilizing MS^E fragmentation and IMS separation. Proteins were quantified with label free protein quantification. We used multiple reaction monitoring to quantify the cellular sialic acid levels.

Results

Sialic acid production after N-acetylmannosamine induction was increased to almost 70-fold compared to non-induced control cells. MS analysis of cellular proteome identified 1802 proteins of which 105 displayed significant changes in abundance. Functional analysis of the changes in protein abundance revealed regulation of several cellular pathways including protein transport, metabolic and signaling pathways and remodeling of epithelial adherens junctions. We also identified several physically interacting co-regulated proteins in the set of changed proteins.

Conclusions

In this experiment we show that increased metabolic flux through Neu5Ac producing pathway affects the abundance of several protein transport, epithelial adherens junction, signaling and metabolic pathway proteins.

Keywords: proteomics, mass spectrometry, label free quantification

POS-03-146 Integrating Approach of Proteomics and Metabolomics in EAM Rat Model

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Myocarditis is a cardiac disease associated with inflammation and injury of the myocardium. Among the omics studies, proteomics and metabolomics provide protein and metabolite profiling and the integration of proteomics and metabolomics may provide synergistic information regarding the pathological mechanism of a variety of diseases including myocarditis. This study is to characterize alterations in the global proteome and metabolome that are related to the pathology of myosin-induced myocarditis using the integrated analysis of proteomics and metabolomics technologies. Mitochondrial dysfunction, activation of the unfolded protein response (UPR), and ERK-1/2 and RPS6 signaling were evaluated in EAM rat heart tissues.

Keywords: proteomics, metabolomics, myocarditis, label-free

POS-03-147 Proteomic Analysis of Human Obesity Reveals Differential Expression of the Epigenetic Factor HDAC4 and Role of Physical Exercise in Correcting Its Expression

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Background: Physical inactivity is a major factor in obesity development and complications and hence, regular physical exercise is prescribed as a part of the prophylactic management of obesity. Here, we used advanced proteomic profiling strategy to identify proteins that are dysregulated in obese subject and their potential modulation by physical exercise.

Methods: 48 non-diabetic male subjects (37 obese and 11 normal-weight controls) were enrolled in a defined exercise protocol for 3 months. PBMCs and subcutaneous adipose tissue biopsies were collected before and after exercise. Proteins were extracted from PBMCs, fractionated and digested on SCX beads and then analyzed by LC- Orbitrap Velos platform and label-free quantified. Circulating cytokine, metabolic and oxidative stress markers were investigated by multiplexing technology and commercial kits, respectively.

Results: Forty seven proteins were found to be differentially expressed between control and obese volunteers. In obese, upregulated proteins included thrombospondin 1 (TSP1), whereas the histone deacetylase 4 (HDAC4) protein was reduced. After exercise, expression of these proteins was restored to the levels observed in control group. The proteomic data was further validated by Q-RT-PCR in both PBMCs and adipose tissue as well as by immunohistochemistry. Our initial analysis on HDAC4 indicated that it negatively correlated with the metabolic markers leptin (P=0.008; r2=-0.59) and PAI1 (P=0.04; r2=-0.46) as well as with the inflammatory chemokines IP-10 (P=0.02; r2=-0.5) and MIP-1a (P=0.03; r2=-0.53).

Conclusions: HDAC4 mRNA and protein levels are reduced in obese subjects and are restored by physical exercise. The existence of a negative correlation between HDAC4 levels and Inflammatory and metabolic markers is suggestive of a protective role of HDAC4 against obesity and could therefore represent a potential target for the control and management of obesity.

Keywords: HDAC4, label-free quantitation, obesity

POS-03-148 Membrane Proteomic Analysis of the Effect of Lysophosphatidic Acid on Platelet-Monocyte Interaction in the Context of Atherosclerosis

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Introduction and Methods

Lysophosphatidic acid (LPA), a circulating phospholipid that accumulates in atherosclerotic lesions, plays a key role in atherosclerosis progression by stimulating platelets and therefore influencing platelet-monocyte interaction. In this study we used a monocytic cellular model, THP-1, to examine the effect of LPA stimulated platelet releasate (PR) on monocytes. THP-1 cells were isotopically labelled using SILAC to quantify protein amount changes. Heavy-labelled THP-1 cells were incubated with PR from LPA-stimulated platelets for 6 or 24 hours. We focused on the membrane proteome of THP-1 by employing a sucrose gradient ultracentrifugation membrane extraction method and two glycoprotein enrichment methods - lectin affinity chromatography and hydrazide chemistry.

Results

Over 700 membrane proteins were quantified. Among these, 49 proteins were upregulated in THP-1 activated by LPA-originating PR. By comparing these 49 proteins with those upregulated in THP-1 activated by thrombin-originating PR, we identified 7 proteins after 6 hours and 14 proteins after 24 hours specific to LPA but not thrombin stimulation, indicating a distinctive effect of LPA on platelet-monocyte interaction.

Conclusion and Future Work

We demonstrated in a model system that LPA affects platelet-monocyte interaction by activating monocytes with platelet releasate in a specific manner. In addition we identified several membrane protein markers specific to LPA activation. We will investigate these proteins to determine their roles in monocyte activation in the context of atherosclerosis. Furthermore we will apply this membrane proteomic approach to examine the effect of LPA on other types of platelet-monocyte interaction, e.g. platelet-monocyte aggregate formation.

Keywords: atherosclerosis, lysophosphatidic acid, quantitative membrane proteomics

POS-03-149 Towards the Identification of Yeast Endoplasmic Reticulum Phospholipid Flippase Using Quantitative Proteomics Approach

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Lipid movements across cellular membranes are essential for cell growth and survival. Phospholipids in cells are mainly synthesized in the cytoplasmic leaflet of the endoplasmic reticulum (ER) and the newly made phospholipids must be flipped to the lumenal leaflet to ensure bilayer stability and uniform membrane expansion. Since spontaneous flipping occurs very slowly it is widely thought that 'flippase' proteins are needed to facilitate the rapid, bi-directional, ATP-independent flip-flop of phospholipids between the cytosolic and lumenal leaflets of the ER membrane. These flippases have not yet been identified. The goal of the current study is to identify ER flippases in yeast *Saccharomyces cerevisiae* using a quantitative proteomics approach based on stable isotope labeling by amino acids in cell culture (SILAC). Yeast cells were grown in synthetic medium containing either 'light' or 'heavy' lysine. Proteins extracted from unlabeled cells were further fractionated by velocity sedimentation in a glycerol gradient and flippase activity of each fraction quantified by a reconstitution-based procedure. An aliquot of 'heavy' extract (containing equal amount of protein by weight) was added to each fraction. The fractions were then subjected to in-gel digestion followed by quantitative proteomic analysis using mass spectrometry. The data obtained were processed using MaxQuant and subjected to Spearman correlation analysis for identification of proteins with enrichment profiles matching that of the activity profile. These potential flippase candidates were tested for their activity. Methods and results will be discussed in detail.

Keywords: phospholipid flippase, *Saccharomyces cerevisiae*, quantitative proteomics

POS-03-150 Improving Throughput of Relative Protein Quantitation Using 10 Plex Isobaric Tags

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Amine reactive isobaric tags (TMT or iTRAQ) enable concurrent identification and multiplexed quantitation of proteins in different samples using tandem mass spectrometry. The number of samples that can be compared in a single experiment (multiplexing) is limited by the number of different reporter ions, currently 6 for TMT tags, and increasing this number is highly desirable. Here we demonstrate that a higher multiplexing rate up to 10 can be achieved utilizing the 6 mDa mass difference between 15N and 13C stable isotopes by adding four TMT127-130 variants in combination with high resolution mass spectrometry. HeLa cell lysate was labeled with TMT 10 reagents according to manufacturer's instructions. Six, eight or all 10 channel aliquots were mixed in 1:1 ratios. To assess the impact of interring peptides, the TMT 8 HeLa samples were spiked with TMT 10 labeled BSA. The samples were then analyzed using an Orbitrap Elite by LC/MS with HCD MS2 or multinotch MS3 fragmentations at a resolution of 30000 at *m/z* 400. The same series of analyses were repeated using a QExactive and a new ion trap/orbitrap hybrid mass spectrometer. It has been reported that increasing multiplexing by using larger tagging molecules significantly decreases the number of identified and quantified proteins and peptides. We have extended the multiplexing of TMT reagents from 6 to 10 without increasing tag size. Compared to unlabeled HeLa digest, we consistently identified at least 90% as many peptides in the TMT labeled sample. All three multiplexed samples constantly showed better than 95% of identified peptides were also quantified. The multinotch MS3 method demonstrated a comparable number of identified and quantifiable peptides with significantly improved quantification accuracy for spiked in BSA digest. The new hybrid instrument showed an overall improvement for this workflow compared to the current instruments.

Keywords: Isobaric tag

POS-03-151 A Six-Plex Proteome Quantification Strategy Reveals the Dynamics of Protein Turnover

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MS1 full scan based quantification is one of the most popular approaches for large-scale proteome quantification. Typically only three different samples can be differentially labeled and quantified in a single experiment. Here we present a two stages stable isotope labeling strategy which allows six different protein samples to be reliably labeled and simultaneously quantified at MS1 level. Briefly in the first stage, isotope lysine-d0 and lysine-d4 are *in vivo* incorporated into different protein samples during cell culture. Then in the second stage, three of K0 or K4 labeled protein samples are digested by lysine C and *in vitro* labeled with triple dimethyl groups, respectively. We demonstrated that this six-plex isotope labeling strategy could successfully investigate the dynamics of protein turnover in a high throughput manner.

Keywords: quantification, protein turnover

POS-03-152 Quantification of Peptides in Clinical Samples Based on High-Resolution Mass Measurements

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New hybrid mass spectrometers with high resolution and accurate mass (HR/AM) capabilities have opened new avenues in quantitative proteomics. Targeted analyses, routinely performed on triple quadrupole mass spectrometers using the selected reaction monitoring (SRM) mode, were replicated on a high-resolution quadrupole-orbitrap instrument (*Q-Exactive*) to improve the selectivity of the measurements. In this context, targeted measurements benefit from a narrow mass filtering window of the precursor ions together with the orbitrap HR/AM measurement of the fragment ions, while the multiplexing capability of the instrument was leveraged to measure precisely, using internal standards, a large number of peptides in a single LC-MS run. The quantification of peptides was performed in this parallel reaction monitoring mode (PRM), by extracting post-acquisition ion traces of specific fragment ions. The performance of the PRM technique was benchmarked against that of the reference SRM approach, and more specifically, the trapping capability proved beneficial for the enrichment of precursor ions of peptides in very tiny amounts (sub-amol level) and thus dramatically increase the signal-to-noise ratio.

The PRM technique was applied to the analysis of clinical samples with a complex background (*e.g.* plasma and urine) to demonstrate the benefits of the gain in selectivity and the identification of the fragments through accurate mass, to increase confidence in the measurements. More specifically, lung cancer candidate markers were analyzed by this technique to differentiate the disease stages andsubtypes. The results obtained by this technique allowed a clear discrimination of the different disease stages.

Keywords: quantification, high-resolution, PRM/SRM

POS-03-153 Liver Mitochondria Proteomics Employing High Resolution MS Technology

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To extensively mine the mitochondrial proteome, mouse liver mitochondria were isolated and purified in the presence of protease inhibitors. The protein fraction was digested with trypsin and analyzed by complementary LC-MS/MS workflows to maximize both qualitative and quantitative information on the mitochondrial proteome, phosphoproteome and acetylome. Nano-LC-MS/MS analysis on a hybrid Ion Trap-Orbitrap mass spectrometer was performed in a non-hypothesis driven manner, employing data analysis for both discovery and targeted validation approaches.

Here, we combine label-free and TMT (Tandem Mass Tag) based relative quantification for deep differential profiling of the liver mitochondrial proteome. Global label-free approach resulted in the identification over 1,300 protein groups at 1% FDR. Gene ontology annotation indicated that over 700 proteins identified are mitochondrial which represents a high number of specific identities for organ- and organelle-based enrichment.TMT labeling followed by chromatographic separation identical to the label free approach, further increased the number of identified proteins, with 98.3% of them successfully quantified. The multiplexing capabilities of TMT yielded a number of identified and quantified proteins similar to the label-free approach and with an equivalent statistical confidence, but with significantly less analysis time. Relative abundances of mitochondrial proteins determined by label free and TMT methods, respectively, were highly conserved. In phosphopeptide enriched samples >250 phosphopeptides were identified at 1% FDR representing a greater number of identified sites than previously reported (Ref1), which is of relevance towards the understanding of biological functions associated with the mitochondrion.

Ref1: Gnad et al. (2010), Mol Cell Proteomics, **9** (12):2642-53

Keywords: quantitation, liver, tandem mass tag

POS-03-154 Quantitative CID Cleavable Crosslink Strategy on a Benchtop Instrument Using All-Ion-Fragmentation and SILAC

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Mass spectrometry based crosslinking experiments have been one of the major successes in proteomics in recent years and have as a result become part of the standard proteomics toolkit. However, most current approaches rely on full database searches to determine the sequence identities of the crosslinked pair and are therefore limited in sample complexity, while lacking deterministic and quantitative properties. Alternative approaches use CID labile linkers, but these require expensive state-of-the-art mass spectrometry platforms and lack in sensitivity. Here we present a novel crosslinking approach designed to overcome these limitations with SILAC labeling in conjunction with CID cleavable crosslinkers on the Q Exactive benchtop mass spectrometry platform. As this platform lacks true ms3 capabilities, we utilize pseudo-ms3 as All-Ion-Fragmentation (AIF) to separate the crosslinked peptides prior to further analysis. The combination of full scans and AIF scans allows for deterministic assignment of the two crosslinked peptides both during acquisition as well as during the analysis of the recorded data. Analysis of the recorded data is performed with MaxQuant, which has been extended to support this type of data. The method is validated on the recently published TRIC/CCT chaperone complex for which we approach the sensitivity of non-cleavable crosslinking approaches, while opening up the possibility to analyze higher complexity samples for which MaxQuant Real-time is used to improve the acquisition.

Keywords: crosslinking, LC/MS, real-time control

POS-03-155 Compared Performances of Different Targeted Proteomics Approaches on a Benchtop UHR-Q-TOF

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Mass spectrometry is a popular tool for candidate protein biomarker discovery. Validation of biomarker candidates is typically performed through a targeted quantitative proteomic approach involving multiple-reaction monitoring (MRM) and stable isotopically labeled standards (SIS). This approach requires a priori knowledge of the target, precursor/product ion transitions and time-consuming method preparation, and prevents post-analysis data mining. High resolution systems, such as QTOFs, are now able to address these limitations, while providing comparable selectivity. We report here the evaluation of a benchtop UHR-Q-TOF system for the targeted quantitation of peptides in a plasma tryptic digest. A freeze-dried plasma tryptic digest that was spiked with 43 SIS peptides and was re-suspended to obtain a final concentration of 0.2 μg/μl of plasma digest and 2fmol/μl of the tryptic peptides. The resuspensed solutions (5 μl) were injected in triplicate on a 100 μmX2cm Pepmap pre-column (Dionex, USA) and separated on a 75 μmX25cm PepMap UHPLC column (Dionex, USA) with 2 μm particles. A CaptiveSprayTM source was used as an interface with an ImpactTM benchtop UHR-Q-TOF system, operated in the HrXIC or Middle Band CID modes. All results were processed in Skyline software. Preliminary results reveal high quantification efficiency for the target peptides using both a pure MS-based data acquisition method (HrXIC) and a middle-band CID quantification method, where quantification was based on specific fragment ions. As an additional merit, the latter method also provides qualitative data in the same run. This poster will present a comprehensive comparison of the targeted quantitative proteomic methods and lay the foundation for future applications.

Keywords: targeted proteomics, high resolution, skyline

POS-03-156 Global *In Vivo* Terminal Amino Acid Labeling for Exploring Dialyzed Serum Cultivation Induced Proteins Differential Expression in SILAC Studies

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In vivo termini amino acid labeling (IVTAL), a recently developed quantification method taking the advantage of reliable metabolic labeling and accurate isobaric MS2 quantification, has been successfully applied in quantifying cell systems. However, only a part of specific peptides sequence digested by Lys-N and Arg-C can be used for MS2 quantification. Here, we optimized IVTAL by combining metabolic labeling and isotopic dimethyl labeling for tryptic peptides quantification, forming a global *in vivo* terminal amino acid labeling (G-IVTAL) strategy. It maintained the advantage of high accuracy and reliability quantification, as well as two times improved the qualitative and quantitative data scale in 1:1 mixed HepG2 analysis. Furthermore, up to 81.78% of identified proteins were confidently quantified in G-IVTAL strategy. When being used to address the influence of dialyzed serum in HepG2 growth, 111 proteins with over two fold differential expression were discovered. Functional annotation revealed that fatty acid metabolism and cell viability were significantly inhibited after dialyzed serum cultivation. Western bolting analysis of MTDH, BCAP31 and GPC3 verified the expression difference in G-IVTAL data. Therefore, when using dialyzed serum containing SILAC medium to study the differential proteomic profiling of HepG2 cell, the reference data set of 111 proteins provided here could assist the acquiring of more accurate and reliable quantitative information.

Keywords: isobaric MS2 quantification, SILAC/AACT, isotopic dimethyl labeling

POS-03-157 Quantitative Proteomics Analysis Reveals the Significant Changes on the Cell Shape and Energy Shift After IPTG Inducing Via SILAC Approach on *Escherichia coli*

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Stable isotope labeling by amino acids in cell culture (SILAC) increases the sensitivity of quantitative proteomics, and has been widely used in yeast, cultured mammalian cells, and even some multicellular organisms. However, the lack of optimized SILAC media limits its application in *E. coli*, the most commonly used model organism. We optimized SILAC medium for non-auxotrophic *E. coli* (SILAE) based on the commonly used M9 medium to increase growth and to support complete labeling of the whole proteome in 12 generations.To investigate the sensitivity and accuracy of our quantitative proteomics platform with *E. coli*, we applied swapped SILAC labeling workflow using *E. coli* BL21 (DE3) cells hosting a pET expression vector before and after IPTG induction of vector-encoded recombinant proteins. In present study, we identified 1261 proteins with a significant change in abundance. Pathway analysis with these changed proteins suggested that the size and shape of the induced cells might be changed. Moreover, cell growth and fissiparism were inhibited accompanied with the down-regulation of proteins related to energy and metabolism, cell division and the cell cycle. In addition, the pathways for cell wall biogenesis and cell motility also changed.Taken together, the results of our study confirm that we have developed a medium suitable for efficient and complete labeling of *E. coli* cells, and established a data filtering and evaluation strategy for SILAC-based quantitative proteomics studies of *E. coli*, which could also be used as a system for stable-isotope-labeled recombinant protein production.

Keywords: quantitative proteomics, *Escherichia coli*, SILAC

POS-03-158 A Hybrid Multiple Reaction Monitoring Method Using mTRAQ/iTRAQ Labeling for Multiplex Absolute Quantification and Validation of Human Colorectal Cancer Biomarker

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Well-established biomarker verification assays are urgently required to improve the efficiency of biomarker development and facilitate the deployment of novel clinical tests. Benefitting from lower development costs, multiple reaction monitoring (MRM) has been used for biomarker verification as an alternate to immunoassay. For the verification of candidate biomarkers, large numbers of samples are needed to be examined. However, in general MRM analysis, only one sample can be quantified in a single experiment, which restricts its application. Here, a MRM-based quantification approach combining mTRAQ for absolute quantification and iTRAQ for relative quantification was developed to increase the throughput of biomarker verification. In this strategy, internal standard peptides labeled with mTRAQ reagents Δ0 and Δ8 respectively, were mixed equally and used as double references, while 4-plex iTRAQ reagents were used to label samples as an alternative to mTRAQ Δ4. Thus, quantification of target proteins in four different samples can be achieved in a single run. In addition, double references were used which increased the reliability of the quantification results. Using this method, three biomarker candidates, ademosylhomocysteinase (AHCY), cathepsin D (CTSD) and lysozyme C (LYZ) were successfully quantified in colorectal cancer (CRC) tissue specimens of different stages with high accuracy, sensitivity and reproducibility. To summarize, we demonstrated a promising quantification method for high-throughput verification of biomarker candidates.

Keywords: MRM, iTRAQ, mTRAQ

POS-03-159 Increasing the Breadth and Depth of Multi-Notch MS3-based TMT quantitation using a hybrid Q-OT-qIT Mass Spectrometer

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Multiplexed quantitation *via* isobaric tags provides an avenue for mass spectrometry based proteome wide quantitation experiments to move towards greater parallelization. Recent advancements - i.e., the inclusion of TMT reporter ion isotopologues and MS3 isolation with multiple precursor notches - have increased the multiplexing capacity, accuracy, and sensitivity. However, with these advances the instrument duty cycle increased significantly. Herein, we describe the application of these methods to a new mass spectrometer. By the clever arrangement of its hybrid components, and the careful sequencing of the ion injection, manipulation, and analysis events, the additional time requirements are largely mitigated. Therefore we are able to accurately quantify unprecedented numbers of proteins in a given time frame. Recent advances in TMT labeling and MS3-based methods have allowed for increased multiplexing capacity (10-plex) and improved ratio accuracy and sensitivity. However, with these advances have come significant time penalties. Longer transients are required to resolve the isotopologues, and the MS3 scans require large precursor populations and injection times. When these time intensive MS3-methods are performed on the Q-OT-qIT, these bottlenecks are largely mitigated - e.g., while the instrument is acquiring the longer transient it is concurrently accumulating/fragmenting ions for the next scan. As such, we collect ~50% more spectra during a typical 90-minute analysis (i.e., 14,000 precursors interrogated vs. 9,000, respectively - same AGC target and max IT). Hence, we can produce data of similar or better quality than an Orbitrap-Elite with substantially lower accumulation times, which translates into more quantitative measurements of low abundance peptides and proteins.

Keywords: TMT, Multi-Notch, Q-OT-qIT

POS-03-160 Development of SRM Methods for the Detection and Quantification of Chromosome 16 Proteins

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Detection and quantification of chromosome 16 proteins in biological matrices by SRM procedures is one of the major goals of the SpHPP chr16 Consortium. Two strategies have been undertaken to tackle chr16 proteome: one focused on proteins with experimental evidences and the second to the search for the missing proteins, which is based on the expression of recombinant proteins in a cell free translation system to gather MS information to define the SRM methods to be used in real biological samples. A group of chr16 known proteins were selected according to their score in GPMDB and their tryptic peptides were assayed in biological matrices to select the best transitions. Upon optimisation, methods were developed for 50 proteins allowing detection in total cell lysates of MCF7, Ramos and CCD18 cell lines. Cross validation was performed in three independent labs combining data from ABSciex 4000 and 5500 QTrap instruments. Among all peptides assayed, those observed in parallel shotgun proteomic analysis of the same cell lines were preferentially observed and confirmed with synthetic peptides, supporting the strategy of collecting shotgun MSMS data to facilitate the design of SRM methods. Moreover, an SRM method for detection and quantification cardiotrophin 1, an important hepatoprotective factor, in complete plasma samples has been developed. A database collecting all SRM data is under construction.

Keywords: Human Proteome Project (HPP), selected reaction monitoring (SRM)

POS-03-161 Proteomic Analysis of Whole Glomeruli in Patients with IgA Nephropathy Using Micro-Sieving

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Background and Purpose: Immunoglobulin A (IgA) nephropathy (IgAN) is one of the most common primary glomerular disorders in the world. Pathogenesis of IgAN is still unclear. We recently established a method to isolate glomeruli from renal samples obtained by single needle biopsy, named as "micro-sieving". To identify glomerular proteins related to the pathogenesis of IgAN, we analyzed protein profiles of glomeruli obtained by micro-sieving in patients with IgAN. Material and Method: Glomeruli were obtained by micro-sieving from renal biopsy samples of five patients with IgAN and five patients with minimal change nephrotic syndrome (MCNS). Then proteins, extracted from the isolated glomeruli, were separated by two dimensional fluorescence differential gel electrophoresis (2D-DIGE). By this method protein spots intensity of which was different between the IgAN and MCNS groups were detected and further identified by MALDI-TOF/MS. Some of the identified proteins were evaluated by immunohistochemistry. Result: By 2D-DIGE, we detected in total 1170 protein spots matched among the samples. We found that intensity of 22 out of the 1170 spots was significantly higher in the patients with IgAN than in those with MCNS. Similarly, we found that intensity of 12 out of the 1170 spots was significantly lower in the patients with IgAN than in those with MCNS. Fifteen out of the 34 spots were successfully identified by MALDI-TOF/MS. Among them, we focused on two proteins, α-actinin-4 and glycine amidinotransferase, which intensity was significantly higher in IgAN. Also in immunohistochemistry, the expression of α-actinin-4 increased in the patients with IgAN than in those with MCNS. Conclusion: Use of micro-sieving enabled us to obtain only glomerular proteins from renal biopsy samples and thus to analyze protein profiles of glomeruli. Future analysis of the identified proteins would help understanding of the pathogenesis of IgAN as well as MCNS.

Keywords: IgA nephropathy, micro-sieving, alpha-actinin-4

POS-03-162 Whole Cell Proteome Quantification and Cell Shaving Analyses of the *Staphylococcus aureus* Response to Oxacillin

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Antibiotic resistance is a phenomenon that is becoming widespread across the globe and poses a significant threat to human health and clinical practices. Medical treatments that were once effective are now proving to be unsuccessful in treating common infections. One important pathogen known to cause severe morbidity and mortality that has undergone extreme antibiotic resistance is *Staphylococcus aureus*. However many organisms maintained in laboratory conditions lose their original characteristics due to passaging events. Here we adapted S. aureus COL to high levels of salt and oxacillin and investigated its response to different growth conditions by quantitative proteomics and cell surface shaving proteomics. Initially cell shaving experiments were performed between COL and the highly adapted strain (APT) by incubating entire cells in isotonic buffers for 15 min with trypsin. An additional false positive control was incubated in parallel where cells had no trypsin then followed by supernatant digestion. These fractions were acquired on an LTQ-Velos Orbitrap in top20 CID mode. Employing novel hypergeometric distributions and Bayesian inference allowed the localization of 150 proteins with 62 predicted surface exposed proteins (2027 peptides), 76 predicted cytoplasmic proteins (539 peptides) and 12 unknown (57 peptides). Additionally, whole cell lysates were purified, digested and labelled with iTRAQ tags for quantitative analysis of the S. aureus proteome under antibiotic adaptation. Samples were fractionated using HILIC and analysed either with a nanoUPLC coupled to an LTQ Orbitrap XL (using CID/HCD top 5) or nanoHPLC coupled to an LTQ Orbitrap Velos (HCD only top 8). This resulted in 1802 protein identifications (50% of the predicted proteome). This is one of the most extensive S. aureus profiling studies to date using novel cell shaving analyses and clustering analysis of iTRAQ data has revealed important pathways associated with antibiotic adaptation.

Keywords: cell shaving, surface proteomics, statistical methods

POS-03-163 Proteome-Wide Dysregulation by G6PD Reveals a Novel Protective Role for G6PD in Aflatoxin B1-Mediated Cytotoxicity

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Glucose-6-phosphate dehydrogenase (G6PD) is pivotal to NADPH production and cellular redox balance. Cells with G6PD deficiency are susceptible to oxidant-induced death at high oxidative stress. However, it remains unclear what precise biological processes are affected by G6PD deficiency due to altered cellular redox homeostasis, particularly at low oxidative stress. To further explore the biological role of G6PD, we generated G6PD-knockdown cell clones using a lung cancer line A549. We identified proteins differentially expressed in the knockdown clones without the addition of exogenous oxidant by means of isobaric mass tags (iTRAQ) labeling coupled with multidimensional liquid chromatography-mass spectrometry (LC-MS/MS). We validated a panel of proteins, which showed altered expression in G6PD-knockdown clones and were involved in metabolism of xenobiotic and glutathione (GSH) as well as energy metabolism. To determine the physiological relevancy of our findings, we investigated the functional consequence of G6PD depletion in cells treated with a prevalent xenobiotic aflatoxin B_1 (AFB_1). We found a protective role of G6PD in AFB_1-induced cytotoxicity possibly via providing NADPH for NADPH oxidase to induce epoxide hydrolase 1 (EPHX1), a xenobiotic metabolizing enzyme. Collectively, our findings reveal for the first time a proteome-wide dysregulation by G6PD depletion under the condition without exogenous oxidant challenge, and suggest a novel association of G6PD activity with AFB_1-related xenobiotic metabolism.

Keywords: glucose-6-phosphate dehydrogenase, aflatoxin B1, iTRAQ

POS-03-164 Quantitative Proteomic Approach to Identify Proteins

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Oestrogen has been shown to facilitate multi actions in regulating downstream cellular signals such as transcription, proliferation, and differentiation processes. However, the complete molecular mechanism involved in oestrogen modulation in human cerebral microvascular endothelial cells (HCMEC) is still unclear. Identification and quantification of proteomic changes will allow targeted research into protein networks associated with cerebrovascular disease related to oestrogen deficiency. In this study, interaction between 17β-estradiol and oestrogen receptors was investigated by incubating HCMEC for 24 hours with 17β-estradiol followed by Proximity Ligation Assay (PLA). Interactions were recognized as red dot formations on the cells. In order to systematically profile the changes in protein expression, the cells were fractionated into three different components; 1) membrane proteins 2) cytoplasm proteins, and 3) nuclear proteins respectively. The protein extracts were tagged using iTRAQ labeling and analyzed by LC ESI MS/MS. A total of 2350 unique proteins were identified and the expressions of 317 proteins were significantly altered ($p<0.05$) following treatment with 17β-estradiol. Ingenuity pathway analysis was performed for the regulated proteins and the signalling events directed to *Eukaryotic Initiation Factor 2 (eIF2)* signalling was identified as the major pathway. In addition, 2 other pathways were identified in membrane and cytoplasm components, i) *hypoxia signalling* and ii) *glycolysis signalling*. In nuclear fraction, i) *granzyme signalling* and ii) *Eukaryotic Initiation Factor 4 (eIF4) & p70S6K signalling* are highly stimulated. Thus, a combination of high throughput technique together with bioinformatics analysis is valuable in providing insights of molecular signalling events of 17β-oestradiol modulations in HCMEC.

Keywords: oestrogen, protein signalling pathways, protein interactions

POS-03-165 Advanced SILAC Proteomic Analysis of Human Protein Replacement Stable Cell Lines Established Using snoMEN-PR Vector

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The study of the function of many human proteins is often hampered by technical limitations, such as cytotoxicity and phenotypes that result from overexpression of the protein of interest together with the endogenous version. We had demonstrated the snoMEN (snoRNA Modulator of gene ExpressioN; Ono and Lamond, "Targeted Modulation of Gene expression", PCT/GB2008/003211, 2008) vector technology for generating stable cell lines where expression of the endogenous protein can be reduced and replaced by an exogenous protein, such as a fluorescent protein (FP)-tagged version at the 10th HUPO annual congress in 2011. Here we present the detailed analysis of snoMEN-PR stable cell lines using quantitative proteomics methods, termed SILAC and SILAC-IP (stable isotope labeling by amino acids in cell culture - immuno purification). SnoMEN are snoRNAs engineered to contain complementary sequences that can promote knock-down of targeted RNAs. We have established and characterised two such partial protein replacement human cell lines (snoMEN-PR). Quantitative mass spectrometry was used to analyse the specificity of knock-down and replacement at the protein level and also showed an increased pull-down efficiency of protein complexes containing exogenous, tagged proteins in the protein replacement cell lines, as compared with conventional co-expression strategies. The snoMEN approach facilitates the study of mammalian proteins, particularly those that have so far been difficult to investigate by exogenous expression and has wide applications in basic and applied gene-expression research especially for SILAC-IP proteomics.

Keywords: SILAC, snoMEN, human protein replacement

POS-03-166 Quantification and Mathematical Modeling of the CD95 Death Inducing Signaling Complex

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Apoptosis plays a key role in the development and homeostatis of multicellular organisms. Deregulation of apoptosis can lead to several diseases, such as autoimmune diseases, neurodegenerative diseases or cancer. Apoptosis can be induced by extra- as well as intracellular stimuli. The CD95-induced apoptotic pathway is one of the best-studied signaling pathways. The CD95 death inducing signaling complex (DISC) is essential for the initiation of CD95-mediated apoptotic and non-apoptotic responses. The CD95-DISC comprises CD95, FADD, procaspase-8 (casp8), procaspase-10 (casp10) and c-FLIP proteins. Casp8 is activated at the DISC following LZ-CD95L stimulation leading to the formation of the active caspases and apoptosis initiation. The aim of our study was to provide insight into the stoichiometry of the DED proteins in the DISC. After stimulation of SKW6.4 cells with LZ-CD95L immunoprecipitations (IPs) were performed using an anti-CD95 antibody. Protein quantification was achieved applying the AQUA technique. To reduce complexity proteins from the IPs were separated by one-dimensional gel electrophoresis. Gel slices corresponding to FADD, casp8, casp10 and c-FLIP were spiked with 50 fmol of corresponding heavy AQUA peptides prior to tryptic digestion and Orbitrap analysis. Mass spectrometry of six replicate IPs revealed a stoichiometry of 1 : 2 : 0.3 : 0.1 for FADD, casp8, casp10 and c-FLIP in the CD95-DISC after strong apoptotic stimulation of SKW6.4 cells. Together with mathematical modeling our results led to an improved chain model of the CD95-DISC.

Keywords: Absolute quantification, CD95-DISC, stoichiometry

POS-03-167 Further Exploration of Plasma Biomarkers for Alzheimers Disease Using Isotopic Tandem Mass Tags and A Combined Targeted /Non-Targeted LC/MS/MS Method

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We have previously developed a nine-protein Selected Reaction Monitoring assay for Alzheimer's disease based on in silico-predicted proteotypic peptides which attained moderate performance in a trial with 1,000 samples. To further improve performance we set up a hybrid LC/MS/MS method with an inclusion list of 95 predicted tryptic peptides from our nine proteins combined with data-dependent selection when no target peptides were present. Samples were selected to create a balanced subset in terms of the center, gender and disease status. Male (n=5) and female (n=5) subjects classified as either AD, mild cognitive impairment (MCI) or elderly controls were chosen from each of three clinical centres . Each plasma sample was digested with trypsin and individually labelled with the TMT0 reagent. An aliquot of a reference pooled plasma digest labelled with a single TMT6 reagent was then added to each specimen. Mass spectrometry data was acquired using the LTQ Orbitrap Velos. A total of 1630 peptides representing 152 proteins were detected in >70% of all samples and 1104 of these were used for panel selection using the Group Method of Data Handling (GMDH Shell 2.8). A panel of four peptides achieved 58% sensitivity with 98% specificity for Alzheimer's disease. A six-peptide panel could differentiate Mild Cognitive Impairment with sensitivity of 71% for specificity of 95%. These data confirm the value of using isotopic TMT reagents in a hybrid targeted/non-trageted LC/MS/MS discovery paired with GMDH for optimal panel selection

Keywords: tandem mass tags, combined targeted /non-targeted LC/MS/MS, plasma biomarkers for Alzheimers disease

POS-03-168 Phosphoproteomic Analysis of ABA Signaling Pathways in *Physcomitrella patens*

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Abscisic acid (ABA) is a major phytohormone that is critical for plant's responses to environmental conditions. Recent studies clarified the major ABA signaling pathway in plants as follows; in the absence of ABA, protein phosphatase type 2C (PP2C) shuts off ABA signaling by direct dephosphorylation of SNF1-related protein kinase (SnRK2). In the presence of ABA, PYR/PYL/RCAR proteins inhibit PP2C in an ABA-dependent manner, resulting in SnRK2 activation and phosphorylation of SnRK2 substrates. The core ABA signaling system seems to be conserved from bryophytes to higher plants, suggesting that some ancient ABA signaling systems could be involved in bryophytes (1). To investigate the ABA signaling systems in bryophytes, we enriched phosphopeptides from wild-type and ABA hypersensitive/insensitive mutants of *Physcomitrella patens*, and then the peptides were analyzed with a LC-MS/MS instrument (LTQ-Orbitrap). The LC-MS/MS-derived data covered a total of 4,698 phosphopeptides belonging to 2,007 proteins. Furthermore, we identified 6,545 phosphorylation sites in which the ratio of each phosphorylated residue, serine, threonine and tyrosine, was calculated to be 79.1%, 17.3% and 3.6%, respectively. Now we are analyzing quantitative data for each peptide to find differentially regulated phosphoproteins in response to ABA. Previously we analyzed thousands of phosphoproteins in Arabidopsis by a same approach (2), enabling us to compare phosphoproteome data between bryophytes and angiosperms in near future.

1. Umezawa, T. et al. (2010) *Plant Cell Physiol.* 51: 1821-1839.
2. Umezawa, T. et al. (2013) *Sci. Signal.* 6(270): rs8.

Keywords: phosphoproteomics, signal transduction, abscisic acid

POS-03-169 Detection of Cellular Response to an *In Vitro* Challenge with Bacterial Gram-Negative Lipopolysaccharides (LPS) in Peripheral Blood Mononuclear Cells (PBMCs)

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Sepsis results from severe local or systemic infections such as appendicitis, pneumonia, bacteremia, diverticulitis, pancreatitis, and necrotizing fasciitis. Fatality from sepsis is typically 25-50% (1). Early diagnosis of sepsis is critical in patient survival. Gram- negative bacteria account for 50% of all cases of sepsis, and a major component, LPS, contributes greatly to septic shock. In this study, we look at global protein profiling of mononuclear cells from LPS challenged whole blood. Mononuclear cells are easy to collect, have little of the protein dynamic range difficulties associated with plasma, and are directly associated with many diseases such as cancer, autoimmune disease, and infection. In addition, they are responsive to many immune state conditions, making them ideal targets for biomarker discovery experiments.

The large number of human proteins and their associated post translational modifications (PTM) represent a challenge for MS-based biomarker discovery. In order to allow the detection of differentially expressed proteins and peptides, instrumentation should provide enough quantitative full-scan measurements while simultaneously providing MS/MS fragmentation data to allow sequencing of as many peptides as possible. In this experiment, *ca* 4000 proteins per sample were identified, quantified and analyzed for trends. Peptides that were more than 2 fold increased at the final time point and that demonstrated a steady increase over all time points *versus* control samples were selected for an additional acquisition experiment with the instrument in a targeted quantitation mode. The results from the described experiments using an *in vitro* model suggested several putative marker candidates that could be selected for further verification experiments.

Keywords: sepsis, quantification, cell signalling

POS-03-170 Integrating Genetics and Phosphoproteomics Reveals a Protein Phosphorylation Network in the Abscisic Acid Signaling Pathway in *Arabidopsis*

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Abscisic acid (ABA) is a phytohormone that regulates diverse plant processes, including seed germination and the response to dehydration. In the major ABA signaling pathway, three members of SNF1-related protein kinase 2 (SnRK2) family transmit ABA-induced signals through phosphorylation of downstream substrates. To identify such substrates, we screened thousands of phosphoproteins in Arabidopsis by mass spectrometry-based phosphoproteomics. We identified proteins that were phosphorylated in Arabidopsis wild-type plants, but not in mutants lacking SnRK2s (*srk2dei*), treated with ABA or subjected to dehydration stress. Comparative analysis revealed that 35 peptides were differentially phosphorylated in wild-type but not in *srk2dei* plants. Biochemical and genetic studies of candidate SnRK2-regulated phosphoproteins showed that SnRK2 promoted the ABA-induced activation of MAPK(s), AtMPK1/2; that SnRK2 mediated phosphorylation of Ser^{45} in a bZIP transcription factor, AREB1, and stimulated ABA-responsive gene expression; and that a previously unknown protein, SnRK2-substrate 1 (SNS1), was phosphorylated *in vivo* by ABA-activated SnRK2s. Reverse genetic analysis revealed that SNS1 acts as a negative regulator of ABA responses. Thus, by integrating genetics with phosphoproteomics, we identified multiple components of the ABA-responsive protein phosphorylation network (1).

1. Umezawa, T. et al. (2013) Sci. Signal. 6: rs8.

Keywords: phosphorylation, hormone, plant

POS-03-172 A Proteomic Investigation of Proteasome Malfunctioning

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Inhibition of the proteasome is pro-apoptotic in most cell types. In cancer therapeutics, the induction of apoptosis using chemical agents is widely used, for instance for the treatment of myeloma patients. Here, we aim to dissect the functional modules of the proteasome by profiling the dynamic proteome and ubiquitinome as a result of proteasome dysfunctioning. We study the effect of proteasome malfunctioning on the global cellular *Drosophila* S2 cellular proteome using a SILAC-based nanoLC-MS/MS (Q Exactive) approach. Inhibition of the proteasome is accomplished by either using chemical agents (MG132 and lactacystin) or by using selective RNAi knockdown constructs against different proteasomal subunits. For the identification of the dynamic pool of ubiquitinated proteins we use a recently developed protocol based on immunoprecipitation of peptides derived from ubiquitinated proteins. Roughly 5,000 proteins are identified and quantified routinely in these SILAC screens. After relatively short incubation times with drugs, approx. 100 proteins are specifically found to be upregulated and/or accumulated. After longer incubation times and upon RNAi of knockdown of proteasomal subunits, the abundances of several hundreds of proteins are altered. Proteins that show severe and relatively fast upregulation and/or accumulation are associated with functional categories such as stress response, cell cycle regulation, apoptosis and the ubiquitin-proteasome system. In addition, the pool of ubiquitinated proteins is upregulated after proteasome inactivation. Strikingly, there is little overlap between the sets of proteins with increased abundances and proteins showing increased ubiquitination ratios. Currently, we are investigating the target specificity of various proteasome-bound deubiquitinating enzymes by analysis of the dynamic ubiquitinome. Results are expected to give more insight into the mechanism and specificity of the proteasome functioning.

Keywords: proteasome, ubiquitinome, SILAC

POS-03-171 Comprehensive Study of Redox Proteomics in THP-1 Cells Using a Modified Biotin-Switch Assay

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Platelet-monocyte aggregates circulating in blood are established early markers of atherosclerosis. It is known that oxidative stress caused by increased levels of reactive oxygen species (ROS) effects signal transduction in blood vessels, such as monocyte adhesion to endothelium, and platelet aggregation. ROS can participate directly in these processes or via oxidized low-density lipoproteins. We hypothesise that lysophosphatidic acid (LPA) released from atherosclerotic plaques can induce oxidative stress in the platelet-monocyte system, facilitating aggregate formation. To measure the redox status of cells in this process, a flow cytometry based method was set up to measure ROS and glutathione content in stimulated THP-1 cells or a mixture of platelets monocytes. We observed a significant increase of intracellular hydrogen peroxide (H_2O_2) in THP-1 cells upon treatment with H_2O_2 or platelet releasate. Based on this result, we investigated the effect of 100 μM H_2O_2 treatment on redox protein changes in a modified biotin-switch assay. Combined with stable isotope labeling of amino acids in cell culture (SILAC), we were able to quantitatively determine thiol/disulfide changes in THP-1 cells. About 400 cysteine-containing peptides were quantified in each experiment. In 32 of these peptides, corresponding to 29 proteins, it was consistently observed that over 50% of the free cysteines formed disulfide bonds in response to H_2O_2. These included proteins from the redox system, channel proteins on the cell membrane, kinases and mitochondrial proteins. In next step, we will apply the modified biotin-switch assay to platelet releasate treated THP-1 cells to quantitatively study the redox protein changes.

Keywords: oxidative stress, Platelet-monocyte aggregation, quantatitive proteomics

POS-03-173 Variability of Signature Peptide Production in Bottom-Up Proteomics

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Targeted proteomics is important in detection and quantification of biomarkers. The analysis is carried out by liquid chromatography with tandem mass spectrometry (LC-MS/MS). This is often done using a bottom-up approach where trypsin cleaves the protein of interest to yield peptides. The signal intensities of the signature peptides are then used to determine the parent protein concentration. Somewhat problematic, though, is the fact that the digestion of proteins does not go to completion, or that produced peptides tend to decrease in signal intensity during overnight digestion. This can lead to varying ratios between signature peptide and respective parent protein, thus causing trouble when it comes to the quantification.

The digestion process needs to be further investigated in order to make it more robust and reproducible. It is essential to understand the underlying mechanisms that can tribute to variability in peptide production during the digestion process and to find which parameters are influencing these. To investigate these aspects of tryptic digestion we have been studying digestion profiles of several produced peptides originated from different model proteins. By looking at peptide digestion profiles we can see how the recovery of the digestion is changed with time. Comparison of several of these profiles can reveal general patterns, which again can help us discover some of the underlying mechanisms.

Keywords: tryptic digestion, signature peptides, quantification

POS-03-174 Discovery of Subnanomolar Serum/Plasma Biomarkers Inclusive of Albumin-Bound Molecules by Novel Sample Pretreatment and High Quality Comparative Analysis

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Proteins and peptides in serum/plasma reflect physiological and pathological states in humans and are attractive targets for the discovery of disease biomarkers. However, the existence of high abundant proteins and the large dynamic range of proteins/peptides make the quantitative analysis of low abundant molecules extremely challenging. Especially, detailed analyses of low abundant proteins and peptides inclusive of degradation products of proteins are difficult due to the interference each other and the presence of the bound forms. Therefore, effective pretreatment of samples with high reproducibility is essential for effective biomarker discoveries. We developed a novel pretreatment method (DS method; Kawashima, et al. *J. Proteome Research*, **9**, 1694-705, 2010), which makes it possible to extract and enrich low abundant peptides and proteins including the ones bound to carriers such as albumin with high yield and high reproducibility. In this work, the discovery and small-scale validation studies were conducted for the analyses of low abundant peptides (molecular weight of less than 5,000) by high quality label-free comparative analysis using LC-MS. The following high throughput (150 samples/day) large-scale study by SRM successfully validated the discovered results. Furthermore, for the analyses of proteins selectively enriched by the DS method, dimethyl labeling of tryptic peptides followed by high-resolution LC-MS analysis was applied to comparative analysis of low abundance proteins. The potentiality of these comprehensive analysis methods for peptides and proteins in serum/plasma based on the DS method is shown to be prominent for the analysis of deepest proteome and peptidome zone by the discovery of biomarkers of renal cell carcinoma and the other diseases.

Keywords: biomarker, serum, plasma

POS-03-175 Revealing the Membrane Proteome, Phosphoproteome and Sialiome of Human Embryonic and Neural Stem Cells

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Human embryonic stem cells (hESCs) can differentiate into human neural stem cells (hNSCs), which can further be differentiated into motor neurons, making these cells potential source for treatment of neurological diseases. Membrane-associated proteins are very important in cellular signaling and their function and activity are frequently regulated by post-translational modifications such as phosphorylation and glycosylation. In order to obtain more information about important membrane proteins and modification sites involved in the differentiation of hESCs to hNSCs and also investigate potential new markers for two stages, we have performed a comprehensive mass-spectrometry-based quantitative proteomics and PTMomics study. This approach employed membrane purification followed by peptide dimethyl labeling and peptide enrichment using SIMAC and TiO_2. Using this strategy we identified a total of 5105 proteins whereof 57% contained transmembrane domains or signal peptides. The enrichment strategy yielded a total of 7894 phosphorylated peptides with ≥ 90% confidence in site assignment and 1810 unique formerly SA-glycopeptides. Several proteins were identified as significantly regulated in hESCs and NSC, including proteins involved in the early embryonic and neural development. In the latter group, we could identify potential NSC markers as Crumbs 2 and several novel proteins. Motif analysis of the altered phosphosites showed a sequence consensus motif (RXXpS/T) significantly up-regulated in NSC emphasizing the importance of calmodulin-dependent protein kinase-2 for this stage. A total of five protein/modifications were validated by selected reaction monitoring (SRM). Collectively, this data represent the most diverse set of post-translational modifications reported for hESCs and NSCs contributing to improve our understanding on the differentiation process.

Keywords: phosphorpoteome, sialiome, stem cell

POS-03-176 Quantitative Phosphoproteomics Studies Reveal Different Signaling Events for Cultured Distal Convoluted Tubular Cells Upon Vasopressin or Angiotensin II Stimulation

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The thiazide-sensitive NaCl cotransporter (NCC) plays an important role in sodium reabsorption at the distal convoluted tubules (DCT) of the kidney. Both angiotensin II (ANG II) and vasopressin are hormones known to transiently induce the phosphorylation of NCC in DCT, which regulate NCC's apical trafficking and its transport activity. However, detailed signaling cascades and their interplay due to the acute stimulation of DCT by these two hormones remain unclear.

Here we carried out large scale quantitative phosphoproteomics study in cultured mpkDCT cells to map the global changes in protein phosphorylation events upon individual treatment using ANG II or V2-selective analog dDAVP. Preliminary experiments were carried out using the dimethyl labeling quantitative approach. IMAC followed by TiO_2 was used for phosphopeptide enrichment. Phospho-enriched fractions were then analyzed by LC-ESI-MS/MS. In total, we have identified 723 quantifiable phosphopeptides, 1053 different phosphorylation sites. From phosphorylation sites increased upon dDAVP treatment but decreased upon ANGII treatment or vice versa, we performed kinase prediction using NetworKIN. Results indicated that ANG II stimulation may increase activity of kinases CDK, MAPKs or GSK, while dDAVP stimulation may increase activity of kinases PKA, DMPK, PIM2, PAK, or CLK. cAMP assay showed that dDAVP induced an increase in cAMP level comparable to forskolin, while ANG II did not lead to any significant increase. Elevated cAMP level upon acute dDAVP treatment supported PKA signaling cascades identified from proteomic analysis. Our results suggested that highly distinct signaling cascades were involved in cultured mpkDCT cells upon acute treatment by either dDAVP or ANG II.

Keywords: phosphoproteomics, quantitative, DCT

POS-03-177 A Proteomics-Based Study to Reveal Profilin1-Induced Molecular Changes in Breast Cancer Cells

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Despite early screening programs and new therapeutic strategies to improve well-being, breast cancer is still the leading cause of death in women. Death usually occurs following cancer cell invasion into the surrounding tissue and metastasis. Profilin-1 (Pfn1), a ubiquitously expressed actin-binding protein, is downregulated in several different types of adenocarcinoma and suppresses tumorigenicity and metastatic dissemination of breast cancer cells. However, molecular changes in breast cancer cells associated with perturbation of Pfn1 have not been characterized. The objective of this study was to determine profilin1 overexpression induced changes in cellular proteins which have been implicated in breast cancer progression. We used three quantitative proteomic approaches: (i) two-dimensional electrophoresis coupled with mass spectrometry (LC-MS/MS), (ii) 8-plex iTRAQ and (iii) label-free LC-MS quantification, to provide preliminary results of the effect of Pfn1 overexpression on the proteome of MDA-MB-231 breast cancer cell line. In particular, we found proteins associated with *adhesion*: filamin A, filamin B, galectin-1 and galectin-3 *protrusion at the leading edge*: coronin 1B (lamellopodia), plastin-3 (filopodia), *polarity and migration*: Vacuolar protein sorting-associated protein 28 homolog, *migration and metastasis:* NME2 and *cell proliferation*: 26S protease regulatory subunit 6A, transketolase, protein S100-A6, 14-3-3 proteins, stratifin, proliferating cell nuclear antigen, stathmin. These findings will likely provide novel insights into the multiple underlying mechanisms of how Pfn1 might regulate breast cancer progression.

Keywords: profilin, breast cancer, progression

POS-03-178 Using 2D-PAGE and iTRAQ Proteomics Approaches to Investigate the Effect of Cigarette Smoke Fraction Exposure on Primary Normal Human Bronchial Epithelial (NHBE) Cells

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Philip Morris Products S.A.

In order to update and advance our knowledge regarding the impact of cigarette smoke on the human bronchial epithelium, a systems biology approach that combines state-of-the-art proteomics, together with gene expression analyses and toxicological endpoints, was developed. Primary normal human bronchial epithelial (NHBE) cells derived from a healthy non-smoker adult donor were used and were exposed for 24 hours to a solution of phosphate buffered saline through which smoke from the reference cigarette 3R4F had been bubbled (smoke bubbled PBS, sbPBS). The dose applied was selected based on survival assay results showing less than 20% cytotoxicity after 24 hours of exposure. Protein lysates obtained from NHBE cells exposed to sbPBS, and control exposed only to sPBS, were analyzed using 2D-PAGE and iTRAQ (isobaric Tags for Relative and Absolute Quantitation) proteomics approaches to detect differentially expressed proteins. Three technical replicates were analyzed to assess repeatability and reproducibility across measurements. Analyses for the verification and quantification of selected targets identified from the 2D-PAGE and iTRAQ proteomics approaches, either western blots or selected reaction monitoring-like approach (SRM-like) approaches were performed. The generated proteomic datasets of differential proteins and the selected verified ones will be presented. Those results will complement the analyses performed on the other endpoints (gene expression and transcriptomics) captured within this study and will further establish the foundation of PMI's systems biology approach to assess the impact of modified risk tobacco products (MRTPs).

Keywords: differential proteins, iTRAQ, 2D-PAGE

POS-03-179 Protein Expression Analysis of Soft-Tissue Sarcomas

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Soft-tissue sarcomas (STS) are a rare type of malignancies, accounting for approximately 1% of all cases worldwide. Rarity is not a hinder for diversity, however, as there are more than 50 subtypes of STS recognized by the World Health Organization (WHO). These tumors typically exhibit extensive genomic aberrations and complex karyotypes. This complexity, together with the rarity of these tumors, have resulted in a relatively low concordance amongst pathologists in classification of particular subtypes.

Leiomyosarcomas (LMS), in particular, are a rather malignant subtype of soft-tissue sarcomas that display smooth muscle differentiation. Gene expression studies often cluster LMS samples together with, or as closest neighbor to, undifferentiated pleomorphic sarcomas (UPS). While gene expression patterns of many histotypes of soft-tissue sarcomas have been studies previously and some prognostic biomarkers have been identified, few studies have focused on protein expression patterns of these tumors. Similarly, an overwhelming majority of studies published to this date focus on differential expression of individual genes or proteins.

We have investigated protein expression profiles of STS tumors from various histotypes, using various proteomics techniques. Unsupervised hierarchical clustering based on protein expression data from 2D-gel studies have led us to choose 20 tumors, histologically classified as LMS or UPS, for further analysis using tandem mass-spectrometry. Here we demonstrate possible approaches for studying the differences between proteome of pleomorphic STS tumors, as well as strategies for inference of pathways likely to be effected by these differences.

Keywords: soft-tissue sarcoma, quantitative proteomics, functional analysis

POS-03-180 Mechanism Survey on Cell Senescence Induced by the Knockdown of Cathepsin D Through Quantitative Proteomic Approach

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Cathepsin D (Cath D) is one of the major proteases in the lysosome in eukaryotic cells. Various cell models have demonstrated the enzymatic activity of cathepsin D is required to combat exogenous stresses. Based on our early observations, the knockdown of Cath D reduced proliferative and tumorigenic abilities of HeLa cells, induced significant G2/M arrest and cell senescence. To understand the underlying mechanisms, we conducted a quantitative proteomics using stable isotope labeling with amino acids in cell culture (SILAC) to determine protein abundance changes regulated by Cath D knockdown in the nuclear, cytoplasmic and lysosomal fractions, respectively. Using LTQ-Orbitrap Velos Mass spectrometer and Maxquant analysis, we identified 839, 2699, 916 and proteins in nucleus, cytoplasmic and lysosome frations, respectively. Interestingly, 21 proteins involved in combating oxidative stress and detoxification were down-regulated, especially in the cytoplasmic and lysosomal fractions. In accordance with this, we observed a surge of Reactive Oxygen Species in the Cath D knockdown cells, accompanied by increased nuclear localization of NF-kB p65 subunit and evidenced DNA damage. Importantly, the ROS scavenger NAC partially reversed the senescent phenotype induced by the knockdown of Cath D. Bioinformatic analysis indicated that the transcription of most anti-oxidant and detoxifying enzymes we identified are regulated by the transcription factor NRF2. As expected, we found the nuclear abundance as well as the transcriptional activity of NRF2 was decreased by the knockdown of Cath D. Our data indicated that the involvement of Cath D in senescence was likely due to the attenuation of NRF2 signaling.

Keywords: SILAC, subcellular fractionation, cathepsin D

POS-03-181 Why Less is More When Generating Tryptic Peptides for Bottom-Up Proteomics

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Trypsin is the most frequently used proteolytic enzyme in mass spectrometry based bottom-up analyses. The enzyme generates peptides of mass and charge that is well suited for current MS-technology. Preparing your protein sample for analysis by mass spectrometry suffers from being labour-intensive and time-consuming. It is a general opinion that the enzyme digestion has to be applied overnight to be efficient. In contrast to traditional digestion methods our alternative method demonstrates that by optimizing a few steps in a trypsin digestion protocol we obtained a rapid and efficient in-solution digest protocol that produced higher protein amino acid coverage, number of peptides generated and more reproducible ion abundances compared to longer digestions. A short incubation time of 5 minutes yields higher number of peptides which together overlap more of the amino acid sequence of the protein compared to peptides generated by longer digestion times. Long trypsin treatment time leads to an increase of small peptides that is not detectable by LC-MS/MS analyses. Slow rate non-tryptic digestion and possibly slow-rate modifications by buffer components of tryptic peptides is a contributing factor for loss of ion intensities during extended digestion time. We suggest that in bottom-up MS-based proteomics it might be beneficial to produce incomplete trypsin digests. This might be achieved by reduction of trypsin treatment time from overnight to 5-minute digestion.

Keywords: bottom-up proteomics, sample preparation, trypsin digestion

POS-03-182 Rapid and Simple Method of Tamm-Horsfall Protein Removal for Urinary Exosomal Protein Identification

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Background: Urinary exosomes are membrane vesicles secreted by renal epithelial cells, and are expected as a new biomarker source. In the electrophoretic analysis of urinary exosomal proteins, urinary Tamm-Horsfall protein (THP), which is the most abundant protein in the urine of healthy subjects, co-precipitates in the exosomal fraction, thus preventing identification of the characteristic exosomal proteins. Here, we validated the utility of polyvinylidene difluoride (PVDF) membrane filtration for urinary THP removal with maximum recovery of urinary exosomal proteins. Methods: Urinary exosomes were purified from a pooled urine sample of healthy subjects by 2-step centrifugation method following DTT treatment to dissolve the THP aggregation. Isolated exosomal proteins were analyzed by two-dimensional electrophoresis (2DE). PVDF membrane filtration was performed (1) before $17{,}000\times g$ centrifugation, (2) before $200{,}000\times g$ ultracentrifugation, or (3) after $200{,}000\times g$ ultracentrifugation. The resulting 2DE protein profiles were compared with that of the unfiltered sample. Protein spots were subsequently trypsin-digested and analyzed by LC-MS/MS. Results: The numbers of urinary exosomal protein spots for treatments (1) and (2) were 39 and 41, respectively, which were significantly lower than that the number for the unfiltered sample 104. The 2DE pattern for treatment (3) showed the best protein recovery 143 spots, among which 76 proteins were identified by LC-MS/MS analysis, and some of which have not previously been published or listed in the major database for urinary exosomes. Conclusion: We determined that the appropriate timing for the PVDF membrane filtration for THP removal, and this easy-to-use approach facilitated improved identification of urinary exosomal proteins.

Keywords: urinary exosome

POS-03-183 Depletion of Abundant Plasma Proteins by Irreversible Trapping in poly-*N*-isopropylacrylamide-Acrylic Acid Hydrogel Particles

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Protein and proteome analysis of human blood plasma presents a challenge to current analytical platforms, such as mass spectrometry. High abundance plasma proteins interfere with detection of potential protein biomarkers that are often three to ten orders of magnitude lower in abundance. We report the application of pH-sensitive poly *N*-isopropylacrylamide - acrylic acid hydrogel particles for removal of abundant plasma proteins, prior to proteome analysis by mass spectrometry. A particular group of proteins that includes some of the most abundant in plasma, such as albumin or transferrin, are irreversibly trapped and depleted from the plasma sample at plasma amounts above a determined threshold. When this threshold is reached, these proteins remain permanently trapped as a result of the increasing interactions between particles and proteins, and the dynamic behaviour of the particles. Our optimized hydrogel-based protein depletion protocol enabled subsequent detection of low abundance proteins (ng/ml range), facilitating the development of a targeted quantitative analysis of the low-abundance biomarker proteins insulin-like growth factors (IGF) 1-2, IGF binding proteins (IBP) 2-7, acid labile subunit (ALS), kallikreins (KLK) 6/7, serine protease inhibitor kazal-type 5 (ISK5) and platelet factor 4 (PLF4) using LC-SRM-MS/MS. This novel use of hydrogel-based particles for protein depletion prior to proteome analysis opens new perspectives for protein biomarker discovery and mass spectrometry based diagnostic and prognostic methods.

Keywords: sample treatment, plasma depletion, hydrogel particles

POS-03-184 Selective Preparation of Faecal Material for Parasite Proteome and Biomarker Identification

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Proteomic studies of parasites are important for deeper understanding of the pathogenesis of infections and preliminary work has concentrated on parasitic cell cultures. *Giardia duodenalis,* a protozoan parasite, is transmitted by the faecal-oral route and is commonly associated with gastro-intestinal conditions in a wide range of mammalian and other species. Genetic characterisation studies report a range of host specific and cross species genotypes in mammals. Little is known about *Giardia* infecting dogs (*Canis familiaris)* and associated transmission mechanisms, both intra-species and cross-species, although evidence suggests *Giardia duodenalis* has zoonotic potential. A trial was conducted with a variety of methods to describe the *Giardia* proteome, as it exists within a canine faecal sample. The methods selected were based upon existing literature[1] for examination of faecal proteomes, although none were specific for canine faeces. Another was selected upon the fact that *Giardia* cysts are the functional structure found in faeces.[2] A third method selected considered the hepato-bilary conditions of the digestive tract and likely physio-chemical principles in activation of digestive processes and parasite infection mechanisms[3]. Water soluble fractions of faecal material were the central concept for protein and peptide profiling combined with variation of extraction and resolubilization of protein fractions, paralleling conditions experienced by the parasite in the digestive tract. Using a 2D LC MALDI shotgun approach this research has identified candidate proteins for further biomarker studies, and the potential for differentiation between host and zoonotic forms of *Giardia* is yet to be resolved.

POS-03-185 Quantitative Analysis of Whole Proteome Using Denator in Two Human Cancer Cell Lines Treated with Doxorubicin

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Proteomics early in 1970s described as protein chemistry is an emerging field in understanding the protein functions and interactions inside a cell. The major problem in proteomics analysis is sample degradation as it generates protein/peptide fragments that can interfere with analytical results. An important step in preventing such artifacts is to preserve the native, intact proteome as early as possible during sample preparation prior to proteomic analysis. Hence we are interested in designing a pipeline for quantitative and qualitative shot gun proteomics. Here in this study we aim at capturing the whole proteome intact from cell line sample by thermal stabilization. Control and Doxorubicin treated A549 and MCF7 cells cultured in TC flask were harvested and divided into three aliquots, processed and evaluated for the effect of thermal stabilization/protease and phosphatase inhibitor cocktail and for heating efficiency on protein stability before protein extraction. The isolated proteins were subjected to in-solution tryptic digestion and the isolated peptides were processed for MS/MS analysis using Agilent 6550 Q-TOF. Protein quantification was carried out using database search sorcerer and CORRA and the peptide picture of the samples were compared. The tissue stabilizing technology of Denator effectively preserves the proteome of the sample by means of rapid and reproducible heat denaturation (90-95^{0}C). The results of the study shows increased number of peptides and proteins in Denator treated samples compared to samples processed with inhibitors. The efficient heating system in Denator denatures the protein effectively and presents increased number of peptide fragments from low abundance proteins.

Keywords: denator, cancer, heat stabilization

POS-03-186 Electrostatic Repulsion-Hydrophilic Interaction Chromatography for Profiling of Human Urine Shotgun Proteome

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Measurement of urinary albumin levels is commonly used to diagnose a patient with diabetic nephropathy (DN); however, a major limitation of this method is the difficulty in the diagnosis of early DN. Here, we compared the performance of Strong Cation Exchange chromatography (SCX), high pH Reversed Phase chromatography (hpRP), and Electrostatic Repulsion-hydrophilic Interaction Chromatography (ERLIC) as a first dimension in human urine shotgun proteomics. Significantly more proteins and peptides were identified by ERLIC method, and 1321 proteins and 32400 unique peptides were identified with high confidence from two technical replicates. In addition, ERLIC is significantly better at the identification of highly hydrophobic peptides. The results indicate that ERLIC is a more convenient and more effective alternative to SCX and hpRP for the fractionation of peptides. Optimization of ERLIC-RP conditions was achieved by using urine samples, which were from a type 2 diabetes mellitus patient with or without DN (DN+ and DN–, respectively). Consequently, 323 and 211 proteins were identified with a Mascot score >20 in DN– and DN+, respectively, of which 79 proteins were shared commonly. Further quantitative analyses using more urine samples derived from DN patients could contribute to identify novel markers for the early diagnosis of DN.

Keywords: electrostatic repulsion-hydrophilic interaction chromatography, human urine shotgun proteomics, two-dimensional liquid chromatography

POS-03-188 Development of Universal Protocols for Clinical Tissue Phosphoproteomics Optimized for Formalin-Fixed Paraffin-Embedded Specimens

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Archives of clinical tissues have been stocked typically in formalin-fixed and paraffin-embedded (FFPE) state in almost all clinical institutes for several decades, and therefore the accumulated specimens can be useful for retrospective research. This fact motivates us to achieve proteome and phosphoproteome analysis of FFPE clinical samples. However, FFPE specimens have inadequate properties to be analyzed, e.g. low recovery of proteins and artificial modifications by formaldehyde-induced cross-linking, leading to the low efficiency in protein identification as well as the significant decrease in quantitative accuracy. Recently, we introduced a novel protocol for protein extraction and digestion to maximize the proteome coverage, which we call phase transfer surfactant (PTS)-aided method. PTS method made it possible to extract 100 μg of protein from a few FFPE slides and identify more than 1500 phosphopeptides in combination with hydroxy acid-modified metal oxide chromatography (HAMMOC) using titania. In this study, we further improved our PTS method by utilizing novel deparaffinization protocols, to achieve more effective protein extraction and reduce the processing time. The optimized protocol was applied to FFPE slides of various human cancers and healthy control samples, and the obtained phosphoproteomes were compared. Furthermore, phosphoproteomes of freshly frozen tissues from cancer patients and healthy controls were also analyzed, and the difference between FFPE specimens was evaluated. In our system, almost all clinical samples including FFPE and freshly frozen specimens can be retrospectively analyzed without any analytical and biological discrimination.

Keywords: phosphoproteomics, FFPE, clinical tissue

POS-03-187 Middle-Down Proteomic Analysis of Embryonic Proteins Secreted Prior to Implantation

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Bottom-up proteomics is the method of choice for high-throughput proteomics; however, the technique fails to identify viable biomarkers in many application areas. By generating mid-range (3-7 kDa) peptides, the sample complexity is decreased and analysis of a single peptide can offer more extensive sequence information and reliable protein identification. The aspartic protease Candida albicans Sap9 generated peptides in the desired mass range, and was found to be active at pH 3.5-6, maintaining its activity at temperatures 15-45 ˚C. After optimal digestion conditions (pH 5.5, 25 ˚C, enzyme: protein ratio 1:10, 30 minutes), peptides were desalted using C18 and C4 ZipTip (Millipore). The combined eluents were separated on a nano-LC C8 column (150 mm, 100 A, 5μm, Thermo Scientific) and analyzed with an Orbitrap Elite ETD FTMS. MS scan was performed at the resolution of 60,000 at m/z 400; HCD and ETD fragment ions were scanned at 15,000 resolution (3 microscans). As a result, 43 out of the 48 proteins in the Universal Proteomics Standard (UPS1, Sigma) were identified from a single 60 minute LC-MS/MS experiment, the average sequence coverage of proteins was 42%. The same experimental conditions were applied to the analysis of secreted embryonic proteins from the culture medium. A differential proteomic profile of the viable and non-viable embryo secretome has been previously reported, and several biomarker proteins indicative of embryonic survival were expected to be present. In our hands, and as reported in the literature, typical bottom-up proteomics identified only a handful of proteins, and with low sequence coverage. Sap9 digestion of the proteins resulted in enhanced sequence coverage of these embryonic proteins and identified more proteins compared to bottom-up. In addition, several phosphorylation sites were reliably assigned. A middle-down proteomics approach is under development for quantification of targeted biomarker candidates.

Keywords: middle-down proteomics, embryonic secretome, biomarker identification

POS-03-189 Differential Solublization Method to Extract Low-Molecular-Weight Proteins/Peptides for Successful Serum SRM Analyses

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Background: Selected reaction monitoring mass spectrometry (SRM-MS) has been more frequently used to measure low abundance proteins/peptides in serum/plasma, owing to its high sensitivity and selectivity. However, the existence of high abundance proteins and the large dynamic range of serum proteins/peptides make SRM analysis challenging. Without sample preparation, limit of the sensitivity of the SRM is about microgram per milliliter. More recently, low abundance proteins/peptides are measured by combination of immunoprecipitation and SRM analysis. However, antibody with sufficient specificity for the target proteins/peptides is generally not available, and development of specific antibody requires a significant amount of time and effort.

Materials and methods: Venous blood samples were obtained from four healthy volunteers (HVs; two males and two females) who tested negative to human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and syphilis. The LMW proteins/peptides enrichment from homogeneously pooled serum was performed as according to Kawashima et al. Enrichment LMW proteins/peptides separated by SDS-PAGE was identified by in-gel tryptic digestion of the proteins followed by LC-MS (GeLC-MS). Database search and analysis was used Mascot and Scaffold.

Results: Analysis of 20μL of pooled serum by GeLC-MS was identified 456 proteins. Chemokine and interleukin were included in the identified 456 proteins. Identified peptide fragments in pooled serum were detected in three-fourth healthy volunteers. One of the identified chemokine was measured 52.5±10.9 ng/mL by ELISA.

Conclusions: Our developed the DS method has the potential to be useful as pretreatment for serum SRM analyses for biomarker validation.

Keywords: serum, low-molecular-weight proteins/peptides, SRM

POS-03-190 Selective Analysis of Cell Surface-Associated Proteins: Characterization of Intracellular Proteins That Are Released from Damaged Cells and Interact with the Surface of Human Monocytic Cell Line U937

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Various intracellular proteins released from damaged tissues have been utilized as established clinical markers. However, functional consequences of protein leakage in the extracellular space remain largely unknown. To characterize intracellular proteins potentially functioning in the extracellular space and affecting cellular responses, cell surface-associated proteins were selectively identified using a combination of cell surface labeling and mass spectrometry-based protein identification technology. A human monocytic cell line U937 was incubated in the presence or absence of a fibroblast cell lysate, followed by labeling with a membrane-impermeable reagent, sulfo-NHS-LC-biotin. U937-bound proteins were then eluted from the cells under mild denaturing conditions, and analyzed by Western blotting using avidin-peroxidase. Biotinylated proteins were detected exclusively in the lysate (+) sample, demonstrating cell surface association of proteins derived from the fibroblast cell lysate. After tryptic digestion and avidin affinity chromatography, the lysate (+) sample was subjected to nanoflow liquid chromatography/quadrupole-time-of-flight mass spectrometry analysis. From 2,185 unique peptides carrying one or more biotin labels, we assigned 454 proteins including 405 proteins that are predicted to have neither signal sequences nor transmembrane segments. The U937-associated proteins included a variety of high-abundance proteins, such as heat shock proteins, cytoskeletal proteins, and metabolic enzymes. Interestingly, a number of clinically important molecules, such as lactate dehydrogenases, high-mobility group box 1, and autoantigen La, were also included in the U937-associated proteins, suggesting potential involvements of these molecules in monocyte-mediated inflammatory responses. These results may shed light on functional aspects of common biomarkers in disease progression and/or tissue repair.
Keywords: cell surface labeling, intracellular protein, tissue damage

POS-03-191 Evaluation of OFF Gel- Based Prefractionation Approach in Combination with In-Solution or Ultra Filtration Protein Digestion

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There is a general agreement that prefractionation of proteins or peptides prior to LC-MS/MS analysis has dramatic impact on the number of proteins ultimately identified. However, considering compatibility and feasibility, it is not clear which is the best digestion method should be combined to this technique in order to finally achieve more complete in-depth analysis. To this end, we prefractionated rat kidney proteins into 12 fractions and digested them with either In-solution or on an ultrafiltration membrane (MWCO= 10K) after buffer exchange. Digests were then analyzed using 3 different mass spectrometries (Agilent, Themo Q-exactive, and Thermo Velos) for conclusive results. Our finding showed that uniquely identified protein from in-solution digests was higher than its counterpart (ultrafiltration method) with 964 proteins (3292 peptides). Among them 592 proteins were significantly abundant using spectral count label free quantification. To figure out this disparity, comparison of physico-chemical properties of protein candidates in both proteomes showed that small sized proteins were less abundant in untrafiltration method compared to in-solution digested group. Most notably, smallest protein detected in ultrafiltration method was 178 aa compared to in-solution method (79aa). To further confirm this relevance, a positive correlation ($R=0.73$, $P<0.05$) was confirmed between protein length (aa) and fold changes abundance between both groups suggesting loss of small sized protein at the buffer exchange step prior to protein digestion in ultrafiltration procedure. Our conclusion revealed superiority of In-solution digestion over ultrafiltration method when processing prefractionated OFFgel samples.

POS-03-192 Method Development for Metaproteomic Analyses of Biofilms from Methanogenic Consortia Degrading Terephthalate

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Biofilms are microbial consortia composed of various trophic groups of microorganisms grown in a close approximation on the surface of supporting media and represent an important biological process for water/wastewater purification, bioenergy recovery, and environmental remediation. In biofilms, the cellular activities can be regulated through signaling based gene expression and nutrient exchange associated with cell-cell and cell-environment interactions. Therefore, understanding of these cellular mechanisms that are usually involved with functions of membrane proteins provides fundamental information for improving the efficiency of biofilm-related processes. Because of hydrophobic nature, and low abundance, as well as many biogenic matrixes known to interfere with common proteomic operations and nano LC/MS/MS technology, extraction methods to facilitate the protein recovery from biofilm samples must be developed. In this study, we compared the efficacy of two sample preparation methods [Triton + Guanidinium thiocyanate extraction; RIPA buffer extraction] for the metaproteomics analysis of a terephthalate-degrading methanogenic biofilm at 55°C in an attempt to gain better insight into the expression of membrane proteins. Subsequent nano LC/MS/MS analysis showed that the RIPA buffer method resulted in the greater protein extract efficacy than the Triton buffer method, while >16% of total proteins identified were associated with the membrane. In addition to histidine kinases, F420-reducing hydrogenase, we successfully observed the electron transport complex and many transporter proteins for ions and amino acids, suggesting the active cellular interactions among cells and with environments. Overall, the developed extraction protocol combined with nano LC/MS/MS analysis can improve the metaproteomic studies on microbial functions of biofilms from the wastewater treatment systems.
Keywords: biofilm, metaproteomic, membrane protein

POS-03-193 Application of Group Specific Anti-Peptide Antibodies for Immunoaffinity Enrichment of Signature Peptides of Allergens from Food Samples

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For people suffering from food allergies, prior knowledge of their presence in food stuffs is of paramount importance. Indeed listing allergens on the packaging of food products is now common place in many countries, even though reliable methods for determining many such allergens are challenging. Thus, the food industry has taken the precaution of labelling products with "may contain traces of allergen X" in order to protect itself from litigation. Currently immuno- and PCR assay are available on the market and more recently mass spectrometry based MRM assays were introduced. However, application of these techniques is limited due to allergen alterations which occur during food processing, and is compromised by the complex and diverse sample matrices. Recently Heick *et al.* demonstrated the superiority of their LC/MS/MS assay over ELISA Kits for the determination of milk allergens in processed food (Heick, J.*et al.* (2011) *J AOAC Int* 94, 1060-8) and established a generic MRM screening method for the simultaneous detection of seven major allergens (Heick, J. *et al.* (2011) *J Chromatogr A* 1218, 938-43).

We hypothesised that the incorporation of immunoaffinity enrichment based methods for multiple allergen peptide enrichment using group specific anti-peptide TXP antibodies could broaden the applicability of such MS based assays by enabling application of a generic sample preparation step for reliable protein quantification from different food matrices. Data will be presented comparing TXP-based immunoassays to established sample preparation protocols. The advantage of such enrichment techniques for the detection of partially modified allergen peptides will also be discussed.
Keywords: food allergen, group specific immunoaffinity enrichment, MS quantification

POS-03-195 Boronate Affinity Materials for the Selective Enrichment of Glycoproteins for Proteomic Analysis

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Because many glycoproteins (including glycopropeptides) of biological importance are present in very low abundance in samples while interfering substances are usually present in high abundance, the selective enrichment of glycoproteins is a key and challenging issue in proteomic analysis. Boronic acids can react with glycoproteins to form covalent complexes at basic pH while the complexes dissociate when the environment is switched to acidic pH. Due to their unique capability of selective extraction of glycoproteins, boronate affinity materials have been getting increasing attention in proteomics in recent years. In our lab, a series of boronate affinity materials have been developed. A number of boronate affinity magnetic nanoparticles and macroporous monoliths that exhibit excellent selectivity towards glycoproteins have been developed. Based on these materials, the selective enrichment and mass spectrometric identification of intact glycoproteins and tryptic glycopeptides has been demonstrated. Also, a strategy for fine-tuning the selectivity towards two sub-classes of glycoproteins, sialylated and nonsialylated glycoproteins, has been proposed. Besides, boronate affinity magnetic nanoparticles with greatly enhanced affinity have been developed, which allowed for the extraction of glycoproteins of concentration as low as 2 x 10^{-14} M. More challengingly, some important progresses in biomimetic materials with enhanced specificity have been achieved. A macroporous monolith that shows protein A-like specificity towards antibody has been developed, which provided a facile platform for antibody purification and antibody removal from complicated samples. Moreover, molecularly imprinted polymers (MIPs) with high specificity towards given glycoproteins have been developed, which permitted the effective extraction and removal of specific glycoproteins in samples.

Keywords: glycoprotein, boronate affinity, selective enrichment

POS-03-196 A Graphene Based Soft Material Platform for Facile One-Step Glycan Enrichment and Derivatization for MALDI-TOF-MS Analysis

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As one of the most important types of biomolecules, glycans of glycoproteins involve in the control of many crucial biological processes, such as protein folding and stability as well as cell-cell recognition and interaction. In this work, we demonstrated the application of reduced graphene oxide (rGO) as a soft material platform for facile one step glycan enrichment and derivatization with high specificity and efficiency for MALDI-TOF-MS analysis.
Using well dispersed rGO as a soft material platform for glycans enrichment and derivatization is beneficial in two aspects. First, the high specific surface area, flexibility and low steric hindrance of free rGO leads to improved enrichment and derivatization efficiency. Second, the combination of enrichment and derivatization of glycans in one step leads to larger improvement in glycan detection sensitivity in MS and evidently reduced processing steps and sample loss. Successful applications of this method in enrichment and derivatization of standard oligosaccharides, glycans from standard glycoproteins and human plasma glycoproteins have been demonstrated and resulted in 30-50 times enhancement of MS signal intensity. Obviously increased number of identified glycoforms was achieved compared with that obtained using either isoniazide derivatization or HILIC enrichment, indicating promising potential of this method in glycoproteomics/glycomics study.

Keywords: glycan, enrichment, derivatization

POS-03-197 Accurate Quantification of Peptides for Mass Specrtometric Analysis: A Modified Micro-Scale Bicinchoninic Acid Assay

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With the advent of precision mass spectrometers with high sensitivity and resolution, proteomics has become one of the important approaches for discovery of biomarkers and disease-related proteins. It has been well admitted that the outcome of MS analysis is dependent on peptide loads onto LC-MS/MS. We have developed a high-sensitivity, high accuracy procedure for quantification of peptides to optimize peptide load to achieve high-performance, qualitative and quantitative proteomic analysis. This procedure is based on a modified bicinchoninic acid (BCA) assay developed by Kappor et al [Anal Biochem, 2009. 393, 138-140]. We found that accurate estimation of peptide amount was possible in the range of 0.01 - 1.0 mg of peptides using the original Kappor's method, and that color development at 562 nm was instable when the "standard" incubation conditions (37 °C for 30 min) was used. Alteration of incubation conditions (60 °C for 30 min, "enhanced" procedure) and reducing the scale of reaction mixture from 1 mL to 100 μL resulted in improved stability of color development and high sensitivity in peptide quantitation in the range of 0.05 - 1.5 μg. We reported examples of peptide quantitation using samples prepared from tissue laser-microdissected from clinical samples including breast cancer and needle biopsy specimens of kidney cortex. Peptide amounts recovered from laser-microdissected breast cancer tissue were found to be around 2 μg/mm^2 of section. We also evaluated effects of different amounts of peptide load on the number of identified proteins using a LTQ-Orbitrap mass spectrometer. We concluded that peptide quantification prior to LC-MS/MS analysis is prerequisite to generate comparable datasets to be qualitatively and quantitatively compared, and that the micro-scale modified BCA peptide assay has enough sensitivity and accuracy, enabling us to perform high-quality MS analysis.

Keywords: peptide assay, LC-MS/MS, clinical application of proteomics

POS-03-198 iTRAQ Proteome Analysis of Inner Cell Mass Derived Versus Epiblast Derived Murine Embryonic Stem Cells

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Beside murine embryonic stem cells (mESC) generated from blastocysts, pluripotent mouse stem cells can be derived from epiblast cells of post-implantation embryos and are referred to as epiblast stem cells (mEpiSC). Compared to mESC, mEpiSC share similar pluripotency factors like Nanog, Oct3/4 and Sox2 but differ significantly in their state of pluripotency. Both types of stem cells can be derived from embryos generated by fertilization (FT) or by somatic nuclear transfer (NT). In this study we performed a 4-plex iTRAQ LC-MS/MS based proteome analysis facilitating a multiplexed comparison of the four indicated types of stem cells. In total, 4 biological replicates were analysed from which 1650 proteins could be quantified. 234 of these proteins showed significant abundance alterations (log2 fold change $\geq |0.6|$, $p \leq 0.05$) between FT/NT-mESC and FT/NT-mEpiSC, amongst them pluripotency and DNA repair associated proteins. Moreover, several cytoskeletal proteins are enriched in mEpiSC, which may reflect a progressed differentiation state of epiblast derived stem cells. Beside the substantial proteomic differences originating from the developmental stage of the embryo used for stem cell generation, 44 proteins with different abundance between FT and NT derived stem cells were observed. Among them several cytoskeleton proteins but no known pluripotency associated proteins were found.

This work was supported by EU FP 7 ("Plurisys" HEALTH-F4-2009-223485; "Epihealth" HEALTH-2012-F2-278418 and "EpiHealthNet" PITN-GA-2012-317146 grants).

Keywords: embryonic stem cells, epiblast stem cells, iTRAQ

POS-03-199 Proteome Dynamics During Reprogramming of Fibroblasts to the Pluripotent State

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Reprogramming of somatic cells can be induced by the forced expression of the transcription factors Oct4, Sox2, Klf4 and c-Myc, causing cells to revert to the pluripotent state. These induced pluripotent stem cells (iPS cells) are functionally very similar to embryonic stem cells, offering great potential for clinical and medical applications, such as patient-specific regenerative medicine therapy and disease modeling. To fulfill these prospects, a better understanding of the underlying mechanisms that drive reprogramming to the induced pluripotent state is required. Here, we applied in-depth quantitative proteomics to monitor proteome changes during reprogramming of fibroblasts to iPS cells, quantifying 8000 proteins over 6 time points at 3-day intervals. We uncovered a 2-step resetting of the proteome during the first and last three days of reprogramming, where multiple functionally related proteins change in expression in a highly synchronous fashion. This comprised several biological processes with a previously unknown role in reprogramming, including changes in the stoichiometry of electron transport-chain complexes, repressed vesicle-mediated transport during the intermediate stage and an EMT-like process in the late phase. We found that components of multiple protein complexes changed in abundance co-ordinately, suggesting that their expression is regulated in a highly orchestrated manner. In addition, we demonstrate that the nucleoporin Nup210 is essential for reprogramming by permitting rapid cellular proliferation. Along with the identification of proteins expressed in a stage-specific manner, this study provides a rich resource towards an enhanced mechanistic understanding of cellular reprogramming.

Keywords: quantitative proteomics, reprogramming, nuclear pore complex

POS-03-200 Sox2, Oct4 and Nanog, Key Stem Cell Transcriptional Factors, Contribute to Different Aspects of Cancer Stem-Like Properties of HCC

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The importance of cancer stem cells in tumor initiation, progression and metastasis has been widely documented. In recent years, Sox2, Oct4 and Nanog, which are key transcriptional factors necessary for embryonic stem cell pluripotency, have been reported to be upregulated in many types of human cancers. It will help understand the mechanisms by which cancer stem cells arise and self-renew to identify roles of these three factors in tumor malignancy. In this study, we firstly analyzed the expression of Sox2, Oct4 and Nanog in HCC cells and patient tissue samples by western blot and immunohistochemistry. All of the three factors were found overexpressed in HCC. And furthermore, their high expressions were all associated with metastasis or bad prognosis of HCC. However, their expressions in HCC samples were not completely overlapped. We then examined the functions of them in promoting HCC cell malignancy. Results showed overexpression of each of the factors in Huh7 HCC cells could enhance cell transwell invasion, soft agar clone formation and retard cell proliferation. When we analyzed their expressions in tumor sphere, and in sorafenib-treated Huh7 cells, we found that only Oct4 was upregulated in tumor sphere compared with maternal Huh7 cells, and only Nanog expression increased in sorafenib-treated cells compared with untreated cells, indicating that the three factors may contribute to different aspects of cancer stem-like properties of HCC. Our results suggest potential prognostic value of Sox2, Oct4 and Nanog for HCC, and meanwhile provide mechanistic clues of cancer stem cell in HCC.

Keywords: Sox2, Oct4 and Nanog, HCC, cancer stem cell

POS-03-201 Transgelin Regulates Metastatic Ability of Cancer Stem Cells

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Recent reports have suggested that tumors are organized in heterogeneous cell populations and that the capability to initiate malignancies resides in only a small subset of cancer cells called cancer stem cells which have ability of self-renewal that drives tumorigenesis and differentiation that contributes to cellular heterogeneity. The number of these cells is very small, and is tightly controlled by the self-renewal pathway and the signals of their environment. These cells express CD133 which is normally used as a marker for cancer stem cells. In this study, CD133+ cells were separated from a Huh-7 HCC cell line using MACS. FACS was carried out to confirm purity of separated cell. The ability for self-renewal of the CD133+ cells was evaluated via colony-forming assay, and stemness was examined with spheres culture. The differences between CD133+ cells and CD133- cells were also analyzed using 2-DE. The differentially expressed proteins were analyzed and identified by ESI-Q-TOF MS/MS. As a result of the analysis, CD133+ cells highly expressed Transgelin which was expressed 25-fold higher in CD133+ cells than CD133- cells related to cell migration. Also up-regulated levels of Transgelin highly increased the migration of tumorigenic cells, whereas down-regulated levels decreased the invasive ability. Furthermore, Transgelin was co-expressed with CXCR4, which is related with tumor invasion. As a result of this study, the metastatic potential of CSCs arises from highly expressed Transgelin.

Keywords: Transgelin, Cancer Stem Cells, 2-DE

POS-03-202 Generating a Proteomic Profile on Neurogenesis, Throught the Comparision of Differentiating Human Foetal Neural Stem Cells

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Traditionally neural stem cells were cultured as neurospheres, a heterogenous agglomeration of neural cells at various stages of differentiation. This heterogeneity prevented accurate quantiative analysis. In 2008 Sun et all produced the first non-immortalised human foetal neural stem (NS) cell line from 9 week old human foetal brain tissue. These cells are cultured as homogenous monolayers, have a radial glia like appearance, self renew and form all three neural cell types, neurons, astrocytes and oligodendrocytes upon differentiation. More recenlty human foetal neuroepithelial like (NES) stem cells have been produced from 5 week old human foetal hindbrain, they resemble neural plate epithelial, with characteristic rosettes, upon differentiation they form a pure population of neurons. These homogeneous monolayer cultures allows detailed quantiatitive analysis of these cells.

Using SILAC we are comparing 4406 proteins from proliferating NES and NS cells, cultured under identical conditions, to better understand the intrinsic differences within the cells. Interesting findings include changes in Integrins and Signal Transducer and Activator of Transcription (STAT) proteins, affecting how they interact with their environment. Using iTRAQ a timecourse study is underway to compare the changes occurring in these cells as they differentiate.This work represent a thorough characterisation of non immortalised human foetal neural stem cells, providing a detailed account of the proteins being expressed by these cells as well as the molecular changes occurring as they differentiate, it will serve as a useful catalog to better understand brain development, aid in regenerative therapies, and the use of these cells as model systems for better understanding diseases affecting the brain.

Keywords: neurogenesis, human foetal neural stem cells, quantitative phosphoproteomics

POS-03-203 Comparative Nuclear Proteomic Analysis of Spermatogonial Stem Cells and Differentiating Spermatogonia Induced by Retinoic Acid

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Spermatogenesis is one of the most productive cell-renewal systems in the body. Spermatogonial stem cells (SSCs) form the foundation for normal spermatogenesis by both self-renewal and the production of spermatogonia that commit to differentiation into functional sperm. However, the molecular mechanism underlying the self-renewal and differentiation of SSCs remains largely unknown. In this study, to identify novel intrinsic factors regulating these processes in mouse SSCs, comparative analysis for the nuclear proteome was performed between SSCs and differentiating spermatogonia. Nuclear extracts from SSCs, which were expanded in culture, and differentiating spermatogonia, which were prepared by treating SSCs with retinoic acid, a differentiation inducer, were subjected to nano-LC-MS/MS analysis. We identified 1111 and 1050 proteins from SSCs and differentiating spermatogonia, respectively. From these proteins, 243 were found only in SSCs, while 212 were found only in differentiating spermatogonia. To further analyze the proteomic differences, the proteins differentially identified were functionally classified with Gene Ontology (GO) terms. According to the functional annotation, the major categories of GO terms for the proteins identified only in differentiating spermatogonia were RNA processing/splicing, transcription, protein transport, and proteolysis. These results suggest the potential importance of proteins involved in the regulation of gene expression and protein turnover in spermatogonial differentiation. This is the first large-scale nuclear proteomic study of SSCs and differentiating spermatogonia, which provides critical information for elucidating spermatogonial fate determination.

Keywords: spermatogonial stem cells

POS-03-204 Integrated Proteomics Identified the Differentiation Niche Induced by Glioma Stem Cells

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Glioma stem cells (GSCs) are considered responsible for the therapeutic resistance and recurrence of malignant glioma. To clarify the molecular mechanism of GSC differentiation, we established GSC clones having the potential to differentiate into glioblastoma, and subjected to DNA microarray/iTRAQ based integrated proteomics. We identified 21,857 mRNAs and 8,471 proteins which were integrated into an gene/proteomic expression analysis chart (*iPEACH: PTC/JP2011/58366, MCP 2013*), and the upregulated 469 mRNAs and 196 proteins in the differentiating GSCs were subjected to the GO analysis. The results revealed that the expression of cell adhesion molecules including integrin subfamilies and extracellular matrices (ECMs) were significantly upregulated during serum-induced GSC differentiation. This differentiation process was also accompanied by the upregulation of glioma specific proteins, such as EGFR, MAPKs, KRAS, vimentin, VEGF, Musashi etc., as well as CD44, GFAP, and downregulation of neural stem cell markers, such as CD133 and SOX2. The induction of ECMs dramatically accelerated the GSC differentiation and proliferation, which were suppressed by integrin αV blocking antibody or RGD peptide. In addition, the expression of integrin αV and its ligand FN was prominently increased in glioblastomas developed from mouse brain GSC xenografts. The combination treatments of anti-cancer drug temozolomide (TMZ) and RGD inhibited glioma progressions, induced their apoptosis, and lead the longer survival of mouse GSC xenograft models. These results indicate that GSCs induce/secrete ECMs to develop microenvironments with serum factors, namely differentiation niches that further stimulate GSC differentiation and proliferation via the RGD motif. A combination of RGD treatment with TMZ could have the higher inhibitory potential against the glioma recurrence that may be regulated by the GSCs in the differentiation niche. *PLoS ONE* in press

Keywords: Integrated Proteomics, glioma stem cell, differentiation niche

POS-03-205 High-Resolution Accurate Mass (HRAM) and Intelligent Acquisition-Enabled Global Discovery and Quantification of Histones, Histone PTMs and Histone Modification Enzymes in Mesenchymal Stem Cells

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Histone post translational modifications (PTMs) are a central theme in the regulation of gene expression. A rapidly growing list of modifications confirms that they play fundamental roles in chromatin modeling processes. These processes are also thought to play a role in cellular development and senescence. To date, most studies in this area have been carried out by genomic analysis, immunostaining, or top-down LC-MS/MS analysis, and as such are not fully quantitative. We have developed workflows for quantitative global profiling and targeted analysis of histones, histone PTMs and histone modification enzymes using a combination of HRAM and intelligent acquisition. Initial discovery experiments were performed to help drive targeted quantitative experiments. The combined results from the discovery experiments were used to build a local spectral library consisting of precursor and product ion m/z values and relative abundance distribution as well as relative retention time values. A set of peptides from the discovery data was selected based on known and novel PTMs. The spectral library information for the targeted peptides was used to create a targeted inclusion list and reference information to perform qual/quan determination real-time. The real-time feedback facilitated optimization of instrument parameters to maximize instrument duty cycle and detection capabilities resulting in significantly increasing the number of peptides quantified per experiment. The final assay performed qual/quan studies on 237 histone peptides and modified analogs. In addition, the samples were spiked with heavy labeled analogs and absolute quantitation was performed. The combined approach enabled quantitation of previously identified modified peptides as well as novel targets across different samples and were correlated to somatic or stem cell aging (replicative or genotoxic stress-induced senescence).

Keywords: stem cells, histone, quantitatve

POS-03-206 Marked Expression of MSY Genes During Differentiation of Human Embryonic Stem Cells Into Dopaminergic Precursor Cells

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A primary goal of the human Y chromosome proteome project is to identify and characterize the proteins of male specific region of Y chromosome (MSY). However, this goal may be hindered by the lack of quality observations of given proteins due to very low abundance, absence of expression in a given tissue, and expression only in rare samples. Human embryonic stem cells (hESCs) may play a vital role in human proteome project due to their unlimited potential to differentiate into any of the over 200 cell types within the body. Here, we analyzed the expression of 21 Y chromosome genes and 9 of their X homologues during hESC differentiation to dopaminergic precursor cells. The transcript and protein levels of target genes were analyzed at 5, 11, 15 days after neural differentiation of hESCs. Differentiated cells were characterized for expression of specific dopaminergic markers such as LMX1a, LMX1b, PITX3, TH and NURR1 using qRT-PCR and immunofluorescence staining. Of 30 genes analyzed in this study, 12 showed very low or no expression in hESC or differentiated cells. Furthermore, the expression level of 12 Y and X genes including, UTY, SRY, TMSB4Y, SMCY, DAZ, NLGN4Y PRY, SMCX, DDX3X, HSFX, RBMX, VCX Increased in arrange of 1.5 to 3 folds in differentiated cells compared to hESC. Interestingly, we observed more than 50 folds increase in transcript levels of MSY genes such as DDX3Y, RBMY1, PCDH11Y, BPY2, CDY1, and HSFY1 compared to their X counterparts in dopaminergic precursor cells. Western blot analyses revealed that the protein level of DDX3Y, PCDH11Y, RBMY also increase during differentiation. Our results highlight the importance of utilizing hESCs and their derivatives to enhance the quality of observations for given proteins.

Keywords: Chromosome Centric Human Proteome Project (C-HPP), human y chromosome proteome project, embryonic stem cells, dopaminergic neurons, Y chromosome, gene expression

POS-03-207 Investigating the Group A *Streptococcus* Surface Proteome Dynamic Using a Combination of Mass Spectrometry Techniques

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Streptococcus pyogenes, or group A *Streptococcus* (GAS), is a human-adapted pathogen that causes a diverse array of symptoms. GAS normally causes mild, non-invasive infections such as superficial throat and skin infections, however since the mid 1980s the incidence of severe, invasive infections has unexplainably increased. Pathogenic bacteria can rapidly alter the composition of their surface proteome, a process critical for bacterial virulence, to accomplish host environment adaptation, immune response evasion and binding of host proteins. Using cell fractionation methods we isolated the surface proteins, from several invasive and non-invasive strains of GAS, which were subsequently analyzed using LC-MS/MS and selected reaction monitoring (SRM). We show that strains of GAS characterized as invasive, have a higher proportion of proteins, and maybe more importantly higher levels of virulence factors on their surface compared to non-invasive strains. Furthermore we demonstrate that surface proteome composition correlated to how invasive and non-invasive strains interact with distinct repertories of human host proteins. The invasive strains ability to interact with specific human host proteins is expected to play a key role in bacterial pathogenesis. By characterizing the dynamics of the bacterial surface and their specific human protein interaction profiles we can not only gain a deeper understanding of the events that leads to bacterial dissemination but we may also identify key proteins on the bacterial surface that can be targeted for future drug development.

Keywords: group A Streptococcus, Bacterial virulence, host-pathogen interactions

POS-03-208 Comparative Analyses of Cellular Responses to Different Aggregation-Prone Proteins

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The intracellular accumulation of aggregation-prone proteins has been linked to neurodegenerative diseases. Each disease is characterized by the aggregation of specific proteins, leading to neuronal cell death. Despite many efforts to understand the mechanisms of proteotoxicity, little is known about the cellular networks that are affected by protein oligomerization and aggregation. Moreover, recent genetic screens revealed that genetic modifiers of toxicity are only partly overlapping across different aggregation-prone proteins (APPs) indicating that APP-specific responses might play a role in the development of toxicity. Our study aims to investigate which pathways are affected by the expression of a set of disease-related APPs and which responses are common or specific amongst the APPs. Using unbiased and targeted proteomic and phosphoproteomic analyses we compared the cellular responses to five APPs in *S. cerevisiae*. We quantified ~3200 proteins and ~11000 phosphosites across the strains expressing the APPs and validated selected module responses by selected reaction monitoring. The data analysis revealed cellular modules that are consistently affected by the overexpression of all APPs and that are therefore conserved responses to proteotoxicity. We also identified APP-specific responses, which suggests that each APP is involved in characteristic, aberrant interactions in a cell. Our study provides the first comparison of the cellular responses to different proteotoxic proteins using the same methodological approach in one model organism. Our results will guide the identification of disease-relevant targets in higher organisms and shed light on processes regulated in a cell challenged by proteotoxic proteins.

Keywords: proteotoxicity, *Saccharyomyces cerevisiae*, unbiased and targeted proteomics

POS-03-209 An Integrative Systems Analysis of Glioma and Medulloblastoma for the Identification of Diagnostic and Therapeutic Targets

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Brain tumors are the most dreadful and lethal diseases. High-grade gliomas and medulloblastomas (MB), which affect adults and children respectively, are the most common, highly aggressive brain tumors associated with limited therapeutics, poor survival and mostly show recurrence. A better understanding of molecular pathways responsible for disease progression is needed to improve therapeutics and patient outcomes. Systems analysis of glioma and MB represents a potential platform to gain knowledge of the molecules associated with this pathogenesis, which may assist for the therapeutics improvement. In this study, molecular information was curated from published research articles and data repositories on high-grade gliomas and MB cell lines, and bioinformatics tools were used to find out the significant signaling pathways, networks and modules associated with curated molecules. We identified TLR, IL-8, Netrin, STATs and Wnt mediated signaling as potential pathways for therapeutic interventions, particularly of malignant gliomas. GLIPR1 and MDH1 were identified as possible candidates for further studies. Similarly, analysis performed on medulloblastoma revealed P53, PI3K/AKT, Notch, Hedgehog and HGF mediated signaling as significant pathways and HNRNPK, PDE4A, HES5, GSTP1, SLC16A2, PPARs, Notch receptors and GADD45A appeared repeatedly in networks and modules suggest their involvement in MB pathogenesis. These studies provide molecular signature of high-grade gliomas and medulloblastomas and validation of identified molecules may help in therapeutic interventions.

Keywords: Glioma, Medulloblastoma

POS-03-210 Expanding the Known Substrate Degradome of Snake Venom Metalloproteinases by Mass Spectrometric Analysis Using PICS and TAILS

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Snake venom metalloproteinases play important roles in the pathological effects of viperid venoms including local tissue damage, hemorrhage and coagulopathy. Hemorrhagic Factor 3 (HF3), a metalloproteinase isolated from the venom of the snake *Bothrops jararaca*, induces severe local hemorrhage by synergistic effects upon plasma, extracellular matrix and platelets. Previous proteomic studies have shown that HF3 targets important components of extracellular matrix, such as collagens and proteoglycans, and some plasma proteins. However the full substrate repertoire of this metalloproteinase is unknown. Using PICS (proteomic identification of cleavage sites) a proteome-derived peptide library was used as substrate for identifying protease cleavage sites by liquid chromatography coupled to high-resolution mass spectrometry (LC-MS/MS) and bioinformatic analysis. Thereby we determined over 2000 cleavage sites and analyzed sequence preferences within the full P6 to P6′ range. Hydrophobic residues were preferentially found at the P1′ site with leucine, isoleucine and phenylalanine accounting for 40%, 11% and 7%, respectively, of all residues at this position. In addition, terminal amine isotopic labeling of substrates (TAILS) derived from the incubation of HF3 with mouse embryonic fibroblasts secretome followed by LC-MS/MS analysis resulted in the identification of more than 500 cleavage sites in native proteins. Various novel substrates were detected for HF3 after peptide isotopic quantification and bioinformatic analysis including the cysteine proteinase inhibitor, cystatin-C, and the apoptosis inducer, galectin-1. Taken together, these results greatly expand the known substrate degradome of HF3, and reveals new targets which may serve as basis to better elucidate the complex pathophysiology of viperid snake envenomation.

Financial support: FAPESP grants 11/08514-8 and 98/14307-9.

Keywords: *Bothrops jararaca*, snake venom metalloproteinases, degradomics

POS-03-211 Integrative Transcriptomic and Proteomic Data Analysis to Identify OSCC-Relevant Protein Targets

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In Taiwan, oral cavity squamous cell carcinoma (OSCC) is the fourth most common cancer type in men. Despite significant advances in treatment over the recent decades, 40-50% of OSCC patients die within 5 years of diagnosis, mostly due to metastasis and/or local recurrence. Identification of biomarkers for early detection of OSCC is urgently needed. Toward this end, data from omics approach were combined to identify OSCC-related proteins based on biological network analyses in this study. To identify potential protein markers for non-invasive OSCC detection, genes highly elevated in OSCC microarray data and proteins up-regulated in primary OSCC secretome were intersected to generate OSCC-relevant protein candidates. Several novel protein candidates were selected for further verification. In addition to gene-level analysis, the transcriptomic and primary OSCC secretome data were also integrated into pathway level data and analyzed with gene/protein set enrichment analysis (GSEA/PSEA) to identify OSCC-relevant protein sets. Most pathways altered in OSCC show similar trends in both GSEA and PSEA analysis. Several pathways were significantly altered in cancer and may have prognostic implication in tumorigenesis. After carefully examining the enriched pathways, we discovered one cytoskeleton protein and its associated complex which may play important roles in OSCC. Preliminary immunohistochemistry study confirmed the elevated expression of this candidate protein in human OSCC compared to normal tissue. Taken together, we established a pipeline to integrate transcriptomic and proteomic data and discovered several OSCC-relevant proteins. Utility of these protein candidates in OSCC detection deserves further investigation.

Keywords: gene set enrichment analysis (GSEA), protein set enrichment analysis (PSEA), oral cavity squamous cell carcinoma (OSCC)

POS-03-212 Multi-parameter Systematic Strategy Opinion that Predicts, Prevents and Personalized Treats a Cancer

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Caner is a complex whole-body disease that alters in the levels of gene, protein, and metabolite, and that involves multi-factors, multi-processes, and multi-consequences. Individual variation is involved in each stage of prediction/prevention, early-stage diagnosis/therapy, and late-stage diagnosis/therapy. The development of systems biology and omics such as genomics/transcriptomics/proteomics has promoted one to gradually change paradigms in oncology from traditional single factor strategy to multi-parameter systematic strategy. The therapeutic model of cancer has changed from the general radiotherapy and chemotherapy to personalized strategy. The development of predictive, preventive and personalized medicine will substantially change the understanding, prediction, prevention, and therapeutic model of cancer from a systematical and comprehensive point of view in the future. Patients will be treated according to the specific molecular profiles that are found in the individual tumor tissue/body-fluid and preferentially with targeted substances, if available.

Keywords: predictive preventive and personalized medicine (PPPM), omics, multi-parameter system strategy

POS-03-213 An Integrated View on the Exploitation of Affinity Purification-Mass Spectrometry Data

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Affinity purification-mass spectrometry (AP-MS) has emerged as a successful technique to map and understand biological pathways and their constituents that are protein complexes. Nonetheless, the data generated contain a large proportion of non-specific interactions and are complex to interpret due to the presence of single in multiple complexes.
In recent and continuing work we have developed techniques and accumulated observations which we will present as an integrated approach to AP-MS data analysis and processing. Starting with the necessity for data filtering, we have showed in a cross laboratory study with the Gstaiger and Aebersold groups that robust protein interaction maps can be obtained and we characterized the respective contributions of biochemistry and MS variability (Varjosalo et al., Nat Methods, 2013). In this study a simple and neutral filter was applied for transparency purpose but higher performance can be achieved with new methods under development in our group, which we will present and compare with existing solutions.
Depending on the aim of research, filtered AP-MS data can be used to infer protein complexes. We introduced a new method of unprecedented accuracy for this purpose (Stukalov et al., JPR, 2012) and we will illustrate its application on a large and small datasets.
To finish, we will discuss how new AP-MS data can be exploited to unravel biological functions in protein interaction networks, which we will exemplify on virus-host protein interactions (Pichlmair et al., Nature, 2012).

Keywords: systems biology, protein interactions, bioinformatics

POS-03-214 Prediction of Phosphorylated Residues Based on Machine Learning Technique and Large Scale Phosphoproteomics Experiment

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Protein phosphorylation is the most ubiquitous post-translational modification that plays important roles during various biological processes in cells. In human proteome, particular serine(S), threonine(T), and tyrosine(Y) residues are recognized and phosphorylated by their responsible kinases. While recent phosphoproteomics approaches using nanoLC-MS/MS have enabled generating the information on the phosphorylated residues at particular state, not all of the phosphorylated residues present in the sample can be uncovered yet. Therefore, computational prediction is essential to complement the biological experiments. In this study, we have developed a predictor for potential phosphorylated residues based on 198,536 kinase-substrate pairs, which were obtained by *in vitro* kinase reaction using 347 recombinant kinases and dephosphorylated human cell lysates. Position specific scoring matrix (PSSM) of entire human proteome was calculated by using the PSI-BLAST program against NCBI non redundant protein database. Then, PSSM around the phosphorylated residue of each substrate was collected, and was used for the training of support vector machine. The performance of the classifier was vaulted by testing it against all the known phosphorylated S/T/Y recorded in the UniProt Knowledge Base. We were able to predict the potential phosphorylated residue in accuracy of 89.7% (S), 92.5% (T), 96.5% (Y).. In this presentation, we will further show the kinase family specific predictor and kinase specific predictor based on our approach.

Keywords: phosphoproteomics, machine learning, kinase/substrate

POS-03-215 Integration of 'Omics and Phenotypic Data to Elucidate Mechanisms of Social Immunity

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We identified protein biomarkers and elucidated mechanisms of social immunity in *Apis mellifera*, the European honey bee. *A. mellifera* is a novel model system of innate immunity, aging, and behavior, and it plays a crucial role in agriculture as a pollinator of a variety of fruits and crops -the estimated contribution of honey bees to Canadian agriculture exceeds $1 billion. Honey bee pathogens trigger individual and colony level immune responses that vary between populations. To facilitate the selection of bees with high level of immunity, we identified protein biomarkers and pathways involved in social immunity. Selected bees with increase immunity can then be used in a breeding program to increase resistance to diseases and reduce the use of chemical treatments in beekeeping operations. The response to *Varroa destructor*, the most damaging threat of honey bees, was linked to proteins involved in innate immunity, odorant recognition and neurological processes. Combining our proteomics results with earlier published data, we developed a model that illustrates the mechanisms involved in social immunity. This model describes the release of metabolic signatures by the affected host larvae and the events these metabolites trigger when they reach the antennae of nurse bees. The metabolites are transported by odorant binding proteins to specific receptors, which in turn trigger signaling events and ultimately a neuronal/ behavioral response that stops the disease cycle. The integration of data from different methodologies increased our understanding of social immune mechanisms that have evolved in response to diseases.

Keywords: immunity, Apis mellifera, proteome profiling

POS-03-216 Proteogenomics Framework to Elucidate TGFB Induced Epithelial to Mesenchymal Transition (EMT)

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Epithelial-mesenchymal transition (EMT) plays a key role in pancreatic cancer progression. Despite the accumulation of a significant amount of data on EMT at the molecular level, this knowledge is yet to translate into therapeutic interventions. This is possibly because the majority of the studies are carried in 'isolation' and complex processes such as EMT can only be fully characterized using an integrated systems approach that incorporates genomics, transcriptomics and proteomics in a 'single' analysis.

We first induced EMT in PANC1 cells (human pancreatic carcinoma, epithelial cell line) using transforming growth factor beta (TGF-β) in a time dependent manner (0-72hrs). EMT was confirmed after 72 hours by analysis of well-established EMT markers such as E-cadherin and Vimentin by immunofluorescence, western blotting and RT-PCR. Simultaneous whole genome sequencing of PANC1 cells, and an EMT time course experiment through RNA-Seq (HiSeq2500) and quantitative proteomics analysis (SILAC) using high resolution mass spectrometer (AB SCIEX5600) is underway.

In parallel, we developed an informatics framework for the comprehensive analysis of proteogenomic data. The software consists of a suite of programs for efficient analysis and management of high-throughput 'omics' datasets. Using our automated software, proteomic data procured from any mass-spectrometer can be directly compared with genomic and transcriptomic data generated by next-generation sequencing. The software is statistically robust and user friendly. To our knowledge, this is the first global proteogenomics study of EMT. By adopting a systems approach and looking for similarities and differences across the three 'omics' contexts, we anticipate that critical new genes, pathways and regulatory networks will be identified, offering novel directions for EMT research with the potential for therapeutic opportunities in pancreatic cancer.

Keywords: epithelial to mesenchymal transition, proteogenomics, next generation sequencing

POS-03-217 Hemorrhagic Activity of HF3, A Snake Venom Metalloproteinase: Insights from the Peptidomic Analysis of Muscle Cells

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Manifestations of local tissue damage, such as hemorrhage and myonecrosis, are among the most dramatic effects of envenomation by snake species of the Viperidae family. Snake venom metalloproteinases (SVMPs) of the P-III class are main players of the hemorrhagic effect due to their activities in blood vessel disruption and inhibition of platelet aggregation. Hemorrhagic Factor 3 (HF3), a P-III class SVMP from *Bothrops jararaca*, shows a minimum hemorrhagic dose of 240 fmol on the rabbit skin. A recombinant protein composed of non-catalytic domains of HF3 (disintegrin-like and cysteine-rich domains; DC-HF3) inhibited collagen-induced platelet-aggregation and increased leukocyte rolling in the microcirculation. In this study, differentiated murine C2C12 skeletal muscle cells were incubated with native HF3 (50 nM) and the DC-HF3 protein (1 uM), for 2h at 37°C. The peptide fraction of the culture media was concentrated by solid phase extraction and peptidomic analysis was carried out by liquid-chromatography coupled to high resolution tandem mass spectrometry (LC-MS/MS) using a LTQ Orbitrap Velos. Spectra were analyzed using the software package MaxQuant and the SwissProt database. The peptidome of the culture medium of cells treated with DC-HF3 showed low complexity, however, treatment of C2C12 cells with HF3 revealed expressive proteolysis and pointed out potential new substrates of HF3 including collagen XVIII, transgelin, and nestin. These peptides were likely generated by proteolysis by HF3, however, activation of cell proteases/ networks cannot be ruled out. This work shows for the first time the targets of HF3 on muscle cells, and can contribute to future studies aimed at explaining the inflammation process and hemorrhage caused by HF3.

Financial support: CAPES (1214/2011) and FAPESP (98/14307-9)

Keywords: snake venom metalloproteinase, C2C12 muscle cells, secretome

POS-03-218 Comparative Analysis of Platelets Stimulated with Thrombin, TRAP and PA-BJ: A Proteomics Approach

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Platelets are anucleate fragments derived from precursor megakaryocytes and play essential roles in normal thrombus formation, thrombosis, inflammation and atherosclerosis. Following activation, platelets undergo release of the soluble α-granule, dense body, and lysosomal contents. We analyzed the secretome of platelets after activation with the agonists thrombin (2 nM), thrombin receptor activating peptide (TRAP; 10 μM) and PA-BJ (100 nM), a serine proteinase isolated from *Bothrops jararaca* venom that induces platelet-aggregation by cleavage of thrombin receptor PAR1. After treatment with each agonist, secreted proteins were separated from peptides by acetone precipitation and both fractions (proteins and peptides) were evaluated by liquid chromatography coupled to high-resolution tandem mass spectrometry (LC-MS/MS) and bioinformatics analysis. Treatment of platelets with all agonists resulted in the identification of components of inflammatory and hemostatic processes, including fibrinogen, von Willebrand factor, thrombospondin-1, coagulation factor XIII, multimerin-1, BM-40, platelet basic protein and platelet factor 4, among the proteins present in the secretome. The peptidomic analysis showed partial degradation of some of these proteins in the secretome of platelets activated by PA-BJ and thrombin. Notably, peptides from fibrinogen, a major protein involved in hemostasis, were abundant in the secretome of activated by PA-BJ and thrombin. Although a number of peptides seemed to be generated by direct proteolytic activity, indirect proteolysis, triggered by lysosomal enzymes, cannot be ruled out. Our results provide insights into the possible participation of peptides generated upon activation of platelets by serine proteinases in the modulation of thrombus formation.

Financial support: FAPESP grants 98/14307-9 and 2010/17328-0

Keywords: platelets, secretome, hemostasis

POS-03-219 Assessment of the Activation Profile of Multiple Small GTPase Isoforms Using Targeted Proteomics

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Small GTPases are a family of key signaling molecules that modulate various biological functions. Mutations of small GTPases and their regulators have been implicated in various types of cancer. Therefore, assessment of the activation profile of multiple small GTPase isoforms could provide insight into a unique aspect of cell signaling networks. In this study, we employed a multiplexed active small GTPase pull-down assay using four different binding domains simultaneously, i.e. GST-Raf1-RBD (for HRas, KRas and NRas), GST-PAK1-PBD (for Rac1, Rac2, Rac3 and Cdc42), GST-RalGDS-RBD (for Rap1A, Rap1B and Rap2B) and GST-Rhotekin-RBD (for RhoA, RhoB, RhoC and Ran). This multiplexed approach allows the binding of up to 14 active small GTPase isoforms in a single experiment. To identify and quantify the small GTPases, we used a targeted proteomic approach, i.e. multiple reaction monitoring (MRM). The best proteotypic peptide of each small GTPase was identified, and the top three transitions were optimized on a QQQ instrument. In addition, heavy isotopically labelled form of these peptides were synthesized as internal standards. Using this approach, we were able to identify 13 out of the 14 active small GTPases from platelet and THP1 cell lysate treated with positive control (GTPγs). Furthermore, our preliminary results showed distinct time-resolved activation profiles of seven small GTPase isoforms when platelets were stimulated with thrombin. We are currently employing this method to further investigate the differential activation profile of small GTPase isoforms in platelets and THP1 cells treated with different agonists.

Keywords: small GTPase, multiple reaction monitoring, targeted proteomics

POS-03-220 Sentinel Protein Assays Take System-Wide Snapshots of Alpha-Synuclein Induced Cytotoxicity

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Currently, proteomics is not well matched with screening applications where hundreds of conditions are measured for their overall effect on cell status (e.g. drugs or disease modifier genes). To address this limitation, the development of tools that simultaneously probe the activation status of multiple cellular pathways in a fast (< 1 hour per sample), non-redundant and information-rich manner is highly desired. Through rigorous experimental validation, a large number of proteins and phosphorylation sites have been previously documented as characteristic markers for the activity of different cellular pathways. Our aim was to develop a multiplexed assay for these indicators of pathway activity, or "sentinels", so that their measurement could provide a system-wide snapshot of cellular status.

Based on bioinformatic analysis and searching published literature, we have defined criteria and scored all yeast ORFs and their protein phosphorylation sites for suitability as sentinel (phospho) proteins. We have developed selected reaction monitoring (SRM) coordinates for these markers, including more than 500 phosphopeptides, in order to enable a multi-pathway readout of cellular status. We used the multiplexed assay to quantitatively compare the sentinel profiles of cells exposed to different levels of the cytotoxic protein alpha-synuclein as well as cells rescued by genetic modulators of alpha-synuclein toxicity. By probing the activity of multiple pathways simultaneously with a quick assay, our approach should enable screening hundreds of conditions for their effect on cell status.

Keywords: phospho-SRM, pathways, alpha-synuclein

POS-03-221 The Methylproteome Network of *Saccharomyces cerevisiae*

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Methylation of proteins occurs predominantly on arginine and lysine residues in the eukaryotic cell. Until recently, its predominance was unknown and its role obscure. This presentation outlines our efforts to construct the first 'methylproteome network' for a eukaryotic cell and presents evidence that arginine methylation modulates protein-protein interactions in this network.

We analysed the yeast methylproteome to identify methylated proteins and precise modification sites. Immonium ion-based scanning and targeted data acquisition - electron transfer dissociation MS/MS was used, as were yeast proteome arrays (containing 4,400 chips spotted onto microscope slides). This showed that protein methylation is widespread in the eukaryotic cell. To build the intracellular methylation network, all known and putative methyltransferases in yeast were knocked out and the methylproteome re-analysed to determine which enzyme was responsible for which methylation event. This led to the discovery of a new lysine methyltransferase, we named Efm2. Enzyme-substrate links were further investigated by the analysis of recombinant substrate proteins methylated by recombinant enzymes, by *in vivo* methylation assays and/or the incubation of proteome arrays with recombinant enzymes. Validated enzyme-substrate links were integrated with the yeast protein-protein interaction network to generate the first 'methylproteome network'. Interestingly, this suggested that many protein-protein interactions could be controlled by protein methylation. To test this, we constructed a new 'conditional' two-hybrid (C2H) system. Interactions of proteins were tested in the presence of a methyltransferase or in the presence of the same enzyme with active site knocked out. Of the protein-protein pairs involving arginine methylated proteins, half of those tested to date have shown increases in interaction in association with methylation.

Keywords: methylation, methylproteome network, protein-protein interactions

POS-03-222 Protein Quantitative Trait Locus (pQTL) Analysis in Mouse by Targeted Proteomics

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The hallmark of targeted proteomics is the quantification of a set of predefined peptides in a complex sample by the acquisition of specific precursor ion to fragment ion transitions over time. As the main implementation of this concept, selected reaction monitoring (SRM) has become a technology that ideally complements some limitations of shotgun strategies by its unique potential of SRM for reliable and reproducible quantification of proteins of low abundance in complex mixtures. To correlate protein abundance with genetic variations, a protein quantitative trait locus (pQTL) analysis relies on consistent and precise quantification of a set of peptides throughout a large number of samples. Here we applied SRM to a pQTL analysis across 58 liver samples of 29 diverse mice strains (BXD type derived from C57BL/6J and DBA/2J strains) to study the genetic control of 155 metabolic proteins. In total, we found 130 QTLs under chow diet or high fat diet, among which DHTKD1 and 1433B map significantly to the same QTL under both conditions. DHTKD1 is one component of the 2-oxoglutarate dehydrogenase complex, and its mutations cause 2-Aminoadipic and 2-Oxoadipic Aciduria in human. We found a strong correlations between DHTKD1 protein levels in the liver, α-aminoadipic acid levels in plasma, and α-ketoadipic acid in the urine. These modulated metabolite levels, which are also observed in humans with malfunctioning DHTKD1, appear to be dependent on sequence variants in the Dhtkd1 gene between the two parent strains. Furthermore, most proteins were not cis-regulated (i.e. by their own gene) and were often differentially regulated between the two dietary conditions. Our results suggest targeted proteomics-based QTL analysis as a powerful strategy to study correlation among genetic variance, protein abundance and phenotypes in complex systems and to reveal regulatory networks of metabolic diseases.

Keywords: protein quantitative trait locus(pQTL), targeted proteomics, murine genetic reference populations

POS-03-223 Platelet Secretory Granules Proteome Characterization: Association with Platelet Reactivity in Stable Cardiovascular Patients Treated with Aspiri

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Platelets arecell fragments playing a key role in haemostasis and atherothrombosis. They are activated by several agonists upon vessel damage, leading to the formation of a platelet plug. They contain 4 types of secretory granules that are essential to potentiate activation and promote platelet aggregation.Antiplatelet agents such as aspirin are a cornerstone in the treatment of atherothrombosis by decreasing the ability of platelets to be activated (i.e. platelet reactivity (PR)).However, a significant proportion of patients displays a preserved PR, despite aspirin treatment and an adequate inhibition of its target, the cyclooxygenase-1.Since platelet granules play a major role in the activation and aggregation process, they represent a potential candidate for PR modulation. The proteome of platelet granules was investigated after sucrose gradient fractionation. Granule proteins were identified by gas-phase fractionation mass spectrometry. This approach allowed identifying more than 800 proteins. We identified new proteins in theseorganelles, such as Syk and Lyn that were confirmed by western blot analysis, whereas MHC1 was confirmed in granules by confocal microscopy. We applied this strategy usingisobaric tagging mass spectrometry and platelets from stable aspirin-treated cardiovascular patients selected according to their extreme high or low PR (3vs.3).Fourteenproteins were differentially expressed between high and low PR patients. These data were further integrated with whole cells and releasates proteomes, transcripts andSNPs by network biology. Interpretation of the network showed an enrichment of genes involved in platelet activation, signal transduction and glucose metabolism. To our knowledge, this work constitutes the largest characterization of the platelet secretory granule proteome and illustrates the benefits of applying subcellular fractionation to patient samples.

Keywords: platelet, network biology, translational research

POS-03-224 Top-Down Analysis of Intact Antibodies Using Orbitrap Mass Spectrometry

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Therapeutic monoclonal antibodies (mAbs) have gained considerable importance over the past years due to their use to treat cancer and autoimmune diseases. Mass spectrometry plays a significant role among the analytical tools used for the analysis of therapeutic mAbs, being able to provide valuable information on antibody properties such as intact mass, amino acid sequence, disulfide bridges and post-translational modifications including glycosylation. Usually mass spectrometric analysis is performed at the peptide level which requires several sample preparation steps prior to analysis, including denaturation, reduction, alkylation, digestion, and release of glycan chains. Here we present a more straightforward, top-down approach which uses recent advances in Orbitrap mass spectrometry for the analysis of intact mAbs in native and denatured forms. Top-down analysis of the intact Herceptin® mAb (Genentech) was carried out on modified Exactive Plus, Q Exactive and Orbitrap Elite instruments (Thermo Fisher Scientific) in direct infusion electrospray or static nanospray mode. HCD was employed for the first two instruments, and ETD, HCD, and EThcD were used on the Orbitrap Elite instrument. Data analysis was performed using Protein Deconvolution 2.0 and ProSightPC 2.0. Different glycoforms of the antibody were baseline-resolved, allowing their accurate assignment. Comparison of HCD data acquired in native vs. denatured conditions showed very high similarity in terms of location of the assigned cleavage sites and total number of b and y fragment ions. In both cases most of the assigned cleavage sites were located in the disulfide-bond free regions. To further improve the sequence coverage, ETD and a combination of ETD and HCD (EThcD) were performed on the intact Herceptin antibody in denatured conditions.

Keywords: Orbitrap mass spectrometry, intact mAb, HCD, ETD, EThcD

POS-03-225 Top-Down and Flexible Analysis of Protein Using MALDI In-Source Decay

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Mass spectrometry (MS) provided fascinating ionization tools such as ESI and MALDI methods. Recent topics relating to radical ion chemistry in MS are MALDI-ISD and ESI-ECD/ETD, resulting in c'- and z'-ions originated from the specific cleavage at N-Ca bond on the peptide backbone. MALDI-ISD is used for identifying intact proteins as a tool of top-down proteomics. MALDI-ISD uses the hydrogen radicals to form the protein radicals. Hydrogen radicals are produced from matrix activated with UV laser photons. The resulting hydrogen radicals bind to carbonyl oxygen on the backbone and result in protein radicals. Recent advance of MALDI-ISD gives information about susceptible amino acid residues to the N-Ca bond cleavage. The susceptible residues Xxx-Asp/Asn and Gly-Xxx which give relatively intense c'-ion peaks can be rationalized from a criterion that those residues are preferred in flexible backbone structures free from intramolecular hydrogen-bonded structures such as a-helix and b-sheet. This presence of more susceptible amino acid residues than the rest in protein is of interest from the standpoints of interaction(s) between peptide backbone and matrix molecules and of protein flexibility. Protein flexibility is relating to the interactions with drugs, nucleic acids, peptides and proteins. Such a so-called "intrinsically disordered protein [6]" is relating to wide variety of biological functions. Here I describe a relationship between ISD flexible residues and protein backbone flexibility which can be estimated by using hydrogen/deuterium exchange (HDX) by NMR spectroscopy and the B-factor by X-ray crystallography.

Keywords: MALDI, in-source decay, flexible amino acid, Asp, Asn, Gly

POS-03-226 Global Proteomic Analysis of Intracellular and Extracellular Amastigote Forms of *Trypanosoma cruzi* Reveals Key Differences in Morphologically Similar Parasites

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Trypanosoma cruzi, the causative agent of Chagas disease, infects 8-10 million individuals throughout Latin America. Lately, however, the disease has gone global with reports of thousands of cases in the U.S., Europe, Japan, and other nonendemic regions. The intracellular amastigote (ICA) stage of *T. cruzi* is the least studied developmental form of this deadly pathogen despite its relevance for the chronic human infection. The main reason for this shortcoming is the hurdle to obtain large quantities of ICA. Nevertheless, a morphologically similar form can be obtained artificially by incubating mammalian tissue culture-derived trypomastigote (TCT) in axenic cultures. To date, therefore, most of the studies have been carried out using extracellular amastigotes (ECA) obtained by artificial differentiation of infective TCT in axenic culture. However, there is scarce information about biochemical differences between ICA and ECA to confirm that these stages are similar at the molecular level. Here, we developed a method to obtain large quantities of highly enriched ICA and ECA by differential centrifugation and anion-exchange chromatography. Next, we employed a proteome-based systems biology approach to compare these two amastigote forms. An in-depth proteomic analysis by 2D LC-MS/MS and spectral count analysis led to the identification and relative quantification of 2,077 protein groups. Functional enrichment analysis followed by pathway network mapping suggested striking differences between ICA and ECA, mainly in metabolic and surface proteins, which are often the major targets for chemo- and immunotherapies. Our findings may have significant implications for the research on biomarkers, drugs, and vaccines for Chagas disease.

Keywords: trypanosoma cruzi, amastigote forms, proteomics

POS-03-227 Investigation of Effects of AngiotensinII (ATII) on Human Articular Chondrocytes by Proteomic Approach

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Objective

Our previous study showed human articular chondrocytes express angiotensin II type 1/2 receptors (AT1/2R). Thus, we investigated whether ATII affected protein profiles of chondrocytes and whether the effect of ATII was cancelled by losartan, an AT1R- selective antagonist and its metabolite of Exp3174.

Methods

Human chondrocytes were obtained from 5 patients with osteoarthritis. Chondrocytes were cultured in media containing 10mM ATII, both 10mM ATII and 3mM losartan (caymon, MI, USA), and both 10mM ATII and 3mM Exp3174 for 24 hours. As a negative control, chondrocytes were cultured without the reagents for 24 hours. Then the chondrocytes were harvested and cellular proteins were extracted into lysis buffer. Next, the protein samples were subjected to 2-dimentional fluorescence difference gel electrophoresis and the protein profiles of each of the samples were obtained. Proteins which were significantly increased/decreased by ATII and the changes were cancelled by losartan or Exp3174 were identified by mass spectrometry.

Results

The above-mentioned dose of reagents had no cytotoxic effect. We found multiple protein spots, which were significantly increased/decreased by ATII and the changes were cancelled by losartan or Exp3174. These proteins were thought to be affected by ATII via AT1R. We identified 8 out of these proteins.

Conclusion

Multiple proteins were found to be affected by ATII via AT1R, indicating physiological or pathological roles of ATII/AT1R in osteoarthritic chondrocytes. Detailed investigation of the identified proteins may clarify the pathophysiological functions of ATII/AT1R in human articular osteoarthritic chondrocytes.

Keywords: human articular chondrocytes, angiotensin II

POS-03-228 Investigation of Effects of Edaravone on Human Brain Microvascular Endothelial Cells

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Background

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is widely used for treatment of acute brain ischemic stroke in Japan. Edaravone is reported to work as a free radical scavenger to inhibit oxidation like lipid peroxidation. A recent study showed that use of edaravone reduced the risk of bleeding after t-PA treatment by decreasing levels of MMP-9. This may indicate the potential of edaravone other than that of a free radical scavenger. Thereby, we here tried to elucidate novel drug actions of edaravone on human brain microvascular endothelial cells (HBMECs) by a comprehensive analysis of proteomics.

Methods

HBMECs were cultured in a fibronectin-coated dish. Next day, the HBMECs were treated with or without 10mM Edaravone for 4 hours. Then, proteins were extracted from the HBMECs and were subjected to 2-Dimensional Fluorescence Difference Gel Electrophoresis. Next, we tried to identify proteins the intensity of which was altered more or less than 1.3 folds on average with statistical significance (t-test, $p<0.05$) by the stimulation of Edaravone.

Results

We detected 1003 protein spots in total. Among the 1003 protein spots, the intensity of 38 protein spots was found to be changed by the above criteria. Among the 38 protein spots, 17 proteins were successfully identified. The identified proteins were cytoskeleton components, glycometabolic enzymes, antioxidant proteins, and translation regulators.

Conclusion

We detected and identified the proteins altered by the stimulation of Edaravone in HBMECs. These proteins might participate in the mechanism for the brain protection by Edaravone. We now plan to investigate roles of the individual identified proteins in the function of Edravone.

Keywords: edaravone

POS-03-229 Production and Characterization of Monoclonal Antibodies to Hepatitis B Virus X (HBx) Protein

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Hepatitis B virus (HBV) encodes the regulatory HBx protein, which is involved in both carcinogenesis and virus replication. However, mechanisms underlying HBx-mediated oncogenesis and virus propagation remain unclear. In order to reveal the role of HBx protein in HBV infection, purified HBx protein and an antibody specific to the HBx protein should be necessary. In our current study, we attempted to produce full-length HBx protein and monoclonal antibodies against HBx protein. Due to the difficulties in purifying HBx protein by conventional cell-based methods, the wheat-germ cell-free protein production system was used to synthesize HBx protein. A recombinant full-length HBx was successfully synthesized by the system. After immunization and cell fusion, fourty-eight mouse hybridomas producing MAbs to HBx were established. The MAbs obtained were fully characterized using immunoblot and immunofluorescent analyses. Some of tested MAbs were found to be applicable in both immunofluorescent and immunoprecipitation analyses. Using the antibodies, we revealed that SAM domain and HD domain-containing protein 1 is a HBx binding protein. Our newly-developed MAbs could thus provide a valuable means to study the HBV infection in human cells.

Keywords: wheat-germ cell-free protein production system

POS-03-230 Lipidomic Profiling Using a Prototype Microfluidic MS Platform

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Lipidomics is the comprehensive analysis of lipid species in biological samples. Lipids play prominent roles in the physiological regulation of biological processes associated with many diseases including cardiovascular diseases, obesity and neurodegenerative disorders. The ability to measure the wide array of lipid species could help our understanding of their roles in health and disease. The need for fast, comprehensive and sensitive lipid analysis challenges both separation and mass measurement.

Microfluidic devices optimized for lipid LC-MS analysis were used that are fabricated from ceramic materials and permit operation at high pressure with sub 2 micron particles. Integrating microscale LC components in a single platform avoids problems associated with connections, blockages and excessive dead volume. Lipids were separated using 150 μm x 100 mm devices packed with 1.7 μm C18 particles at flow rates of 2 μl/min. Data were collected using different MS platforms operated in negative and positive mode.

Small sample volume experiments were performed using standards and lipid extracts from biological samples. Lipids were separated according to acyl chain length and saturation degree. In positive mode, major lipid classes were identified and in negative mode fatty acids. Untargeted experiments were conducted using a Q-Tof platform with an alternate low and elevated CID method to acquire precursor and product ion information in a single run. Lipids profiles were processed using multivariate and pattern-recognition tools to group the observed changes and identified using compound database searches. Targeted experiments used MRM based methods for quantification. A microfuidic-based approach lead to equivalent results to using analytical columns, with a considerable reduction in solvent consumption. Demonstrated applications include large-scale lipid profiling and low-abundance lipids analyses in biological materials.

Keywords: micro fluidics, lipidomics

POS-03-231 The Anticancer Curcumin Induces the Unfolded Protein Response in Prostate Cancer Cells

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Curcumin an extract from *Curcuma Longa* has been known to possess anti-inflammatory, antioxidant and antitumor properties. In this study we have examined the effect of Curcumin in PC3 highly metastatic and RWPE normal Prostate Cancer Cell lines. We demonstrated that Curcumin concentration dependently inhibits the cell proliferation of PC3 Prostate Cancer Cell lines. By a quantitative proteomic approach through Tandem Mass Tag (TMT) combined with mass spectrometry (MS), we could identify proteins showing altered expression mainly in ER stress, protein folding, proliferation, apoptosis, anti-apoptosis and unfolded Protein response. Proteins of relevance were further identified with RT-PCR. Curcumin significantly inhibited colony formation in PC3 cells and upregulated both the mRNA and protein expression levels of 78kDa Glucose regulated protein (GRP78), Protein Disulfide Isomerase protein (PDI), Calreticulin and Elongation Factor 2 (elF2) indicating that endoplasmic reticulum (ER) stress is induced. These findings provide a basis for the further study of the anticancer effects *in vivo* of curcumin and how its use in chemoprevention will contribute to the deeper understanding of ER stress underlying mechanisms during prostate cancer prevention.

Supported by the NIH, National Institute on Minority Health and Health Disparities (8G12 MD 007583-27) to the Universidad Central del Caribe (UCC) Biomedical Proteomics Facility.

Keywords: PROSTATE CANCER, CURCUMIN, ER-stress/Unfolded protein response (UPR)

POS-03-233 Moss Proteomics and Peptidomics. Peptides in the Stress Adaptation Process

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The moss Physcomitrella patens is a new model system in plant science, that offers several advantages for studying of plant physiology, biochemistry and genetics. We utilized the P. patens protoplast as a model to explore the mechanisms involved in stress adaptation in plant. We used proteomic analysis to measure changes in the protein composition of freshly isolated protoplasts from the moss protonema. For this purpose, we compared results of 2D electrophoregrams of proteins from protoplasts and protonema using specific fluorescent dyes (DIGE) for identification of proteins specific to different living forms of P. patens. Besides, using a combination of high performance mass spectrometry with a bioinformatic analysis we described peptidome of the moss protoplast and green tissue. The DIGE demonstrate difference in the protein compositions of protoplasts and protonema. The analyses of peptidome showed that the amount of peptides identified in protoplasts is almost six times greater than in the protonemata from which they are isolated and five times greater than in gametophores.The isolation of moss protoplasts is accompanied by the degradation of proteins most of which are the proteins that belongs to the system of photosynthesis. Those processes of protein degradation lead to the generation of endogenous peptides, which is peculiar to stress responses of higher plants.

Keywords: peptidomics, Physcomitrella patens

POS-03-232 Use Specific Proteins of Taiwan Tea as Biomarkers for the Certification of Origin

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Oolong is a traditional Chinese tea (*Camellia sinensis*), one of the major teas in Taiwan, processing with partially fermentation, the major components are tea polyphenols and caffeine. Many studies have proofed that drinking of tea appropriately can prevent cardiovascular disease, obesity and cancer. Recently, the cheap cost and the poor-quality of the foreign tea have made an enormous impact on Taiwan teas. The blend of low-quality foreign teas with Taiwan teas resulted in the low competitiveness of Taiwan teas exportation. Furthermore, consumers are worried about the unclear place of production and indistinct safety of import foreign teas. In this study, we focus on finding the key biomarkers to differentiate between import foreign teas and Taiwan teas. The sources of Taiwan oolong teas come from Nantou, Chiayi, Taitung, New Taipei City, Taichung, Ilan, Hualien, Yushan, Lishan, Alishan, Deer Valley, Lushan, Dong Ding Mountain, and ones of foreign oolong teas come from Thailand, Indonesia, and South Vietnam. Until now, several studies have showed that the proteome expression profiles of plant leaves altered with the variant environment, including harvest time, composition of the soil, insect damage, disease, cultivation, and the pollution of pesticide and heavy metals, it have been carried out on different kinds of stress in plants and biomarker discovery. Herein, 2D-PAGE (two-dimensional polyacrylamide gel electrophoresis) was applied to the comparative proteomics analysis of the teas with different production place. These specific proteins could be used to distinguish Taiwan teas from the foreign teas.

Keywords: oolong tea, biomarker, MS

POS-03-234 Analysis of p53 Transcriptional Activity Toward the Promoters of Target Genes in Living Cells

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The tumor suppressor protein p53 is activated in response to genotoxic stresses and induces cell cycle arrest, apoptosis and DNA repair. p53 regulates the transcription of hundreds target genes wherein the regulation of p53 transcriptional activity for each target genes allows particular cellular responses in accordance to each stress. In order to understand the mechanisms of p53 transcriptional activity regulation, it is strongly required to establish an experimental system that can easily evaluate endogenous p53 transcriptional activity in living cells.

We report here an analysis system that can easily evaluate endogenous p53 transcriptional activity of p53 target genes in response to various cellular stresses. In order to normalize the amount of transcription due to the different insertion position of the reporter genes in the genomic DNA, we use FLP-FRT recombination which can induce plasmids into target site of cells. We established stable host cell lines which were transfected a FRT site targeted by FLP recombinase into HCT116 cells expressing the p53 wild type. Then we transfected Venus expression vectors having various p53-targeted promoters at the FRT site by FLP-FRT recombination into host cells and then we established stable reporter cell lines. We further analyzed p53 responses to UV, Etoposide, or Adriamycin stimulations using these reporter cell lines. As a result we observed difference in p53 transcriptional activation time, intensity, and increasing ratio of cell number among combination of promoters and stimulations. It is suggest that p53 response was fastest under UV stimulation compared with other stimulations. In addition, p53 transcriptional activity to *mdm2* promoter whose protein regulates p53 in a negative feedback manner does not significantly change when UV intensity increased. This system will give us to important information about regulation of p53 transcriptional activity together with proteomic analysis.

Keywords: tumor suppressor protein p53, transcriptional activity, living cell

POS-03-235 Phosphoproteomic Analysis of *Rhodopseudomonas palustris* Reveals the Role of Pyruvate Phosphate Dikinase Phosphorylation in Lipid Production

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Rhodopseudomonas palustris (*R. palustris*) is a purple nonsulfur anoxygenic phototrophic bacterium with metabolic versatility and able to grow under photoheterotrophic and chemoheterotrophic states. It has the ability in carbon management, carbon recycling, hydrogen generation, and lipid production; therefore it has the potential for bioenergy production and biodegradation. This study is the first to identify the phosphoproteome of *R. palustris* including 100 phosphopeptides from 54 phosphoproteins and 74 phosphopeptides from 42 phosphoproteins in chemoheterotrophic and photoheterotrophic growth conditions, respectively. Among the identified phosphoproteome, phosphorylation at the threonine residue, Thr487, of pyruvate phosphate dikinase (PPDK, RPA1051) was found to participate in the regulation of carbon metabolism. Here, we show that PPDK enzyme activity is higher in photoheterotrophic growth, with Thr487 phosphorylation as a possible mediator. Under the same photoheterotrophic condition, *R. palustris* with overexpressed wild-type PPDK showed an enhanced accumulation of total lipids than those with mutant PPDK (T487V) form. This study reveals the role of the PPDK in the production of biodiesel material, lipid content, with threonyl-phosphorylation as one of the possible regulatory events during photoheterotrophic growth in *R. palustris*.
Keywords: phosphoproteome, *Rhodopseudomonas palustris*, lipid production

POS-03-236 Y Chromosome-Located Gene, Lysine-Specific Demethylase 5D, Associated with Prostate Cancer Progression

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The objective of Human Y Chromosome Proteome Project is to map and annotate all proteins encoded by genes on the MSY sequences. Lysine (K)-specific demethylase 5D (KDM5D) is located on the AZFb region of Y chromosome and encode for a JmjC-domain-containing protein. Changes in KDM5D transcript level in prostate cancer have shown in various studies. To investigation the function of KDM5D in prostate cancer, we knocked down the expression of KDM5D in human prostate cancer cell (DU-145) using siRNA approach. Down-regulation of KDM5D was confirmed by qRT-PCR and western-blot analyses. Cell cycle analysis and MTT assay revealed that down-regulation of KDM5D reduces cell proliferation. Furthermore, we observed that KDM5D down-regulation could effectively increase apoptosis, as measured by the propidium iodide flow cytometric assay. In addition, colony formation significantly diminished in prostate cancer cell line following administration of KDM5D siRNA. To further our study, we are currently in the process of investigating the dynamic of KDM5D protein network using shotgun label free quantitative proteomics approach in knocked down and control cell line. In summary, we report for the first time that KDM5D promote cancer progression and might be a potential therapeutic target in prostate cancer.
Keywords: KDM5D, siRNA, prostate cancer progression

POS-03-237 Generation and Utilization of Full-Length Recombinant Proteins of Human T-cell Leukemia Virus Type 1

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Human T cell lymphotropic virus type 1 (HTLV-1) is the causal agent of adult T cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). In this study, we attempted the production of soluble full-length HTLV-1-encoding proteins, Gag, Env, TAX-1, HBZ, p27REX, p27I and p30II, using wheat germ cell-free protein production system. We initially synthesized the synthetic codon-optimized cDNA encoding HTLV-1 proteins. These cDNAs were introduced into pEU-bls-S1 vector. Although the expression of HTLV-1 proteins was favorable by this system, some viral proteins such as HBX, Env and Tax were found to be insoluble. By adding the Brij35 detergent and/or ZnCl2 in translational mixtures, we succeed to produce of the soluble proteins. By utilizing these soluble proteins, we developed a screening system for detecting specific antibodies targeting HTLV-1 proteins with the luminescent proximity homogeneous assay AlphaScreen. Our current system for the measurement of HTLV-1 antibodies can be applicable as a diagnostic and prognostic tool in ATL and HAM/TSP.
Keywords: wheat germ cell-free protein production system, Human T cell lymphotropic virus type 1

POS-03-238 Wheat Germ Cell-Free Protein Production of Human Immunodeficiency Virus Accessory Protein Vif

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Viral infectivity factor (Vif) is one of the human immunodeficiency virus (HIV) accessory proteins and is conserved in HIV-1, HIV-2 and Simian immunodeficiency viruses. Vif is an essential regulatory protein for efficient viral replication *in vivo*, for which Vif counteracts the antiretroviral cellular factors, namely APOBEC3 enzymes, by targeting them for ubiquitination and proteasome-dependent degradation. Vif protein tends to aggregate and becomes insoluble, therefore these molecular property of this protein has limited its structural characterization and functional analysis. In our current study, we evaluated the potential of the wheat germ cell-free protein production system for synthesizing full-length HIV-1 Vif protein. A synthetic codon-optimized cDNA of HIV-1 Vif was generated and then cloned into the cell-free expression vector pEU-E01-His. We found that full-length Vif protein was successfully synthesized by this system. By using the recombinant protein, we developed monoclonal antibodies (MAbs) targeting Vif protein. Finally, we established three hybridomas generating Vif MAbs that can be used for immunoblotting and immunoprecipitation analyses. These results indicate the feasibility of using the wheat germ cell-free system to produce insoluble and toxic virus proteins.
Keywords: pathogens

POS-03-239 Influence of Arginine, Lysine, Phenylalanine Residues and Phosphorylation on the Positive- and Negative-Ion Yields of Peptides in ESI-MS

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Liquid chromatography (LC) mass spectrometer equipped with an electrospray ionization (ESI) source also has become an indispensable tool for identification of proteins. It has been reported that an enhancement effect of basic and aromatic amino acids arginine (Arg), lysine (Lys) and phenylalanine (Phe) and a suppression effect of phosphorylation on the ion yields of $[M+H]^+$ were observed in ESI. However, the effects originating from an Arg residue and phosphorylation on the ESI ion yields have not yet been evaluated in detail. In this study, the influence of Arg, Lys and Phe residues and phosphorylation on the ion yields of model peptides have been evaluated using ESI mass spectrometry in both positive- and negative-ion modes. The results are explained by considering the total ionization process to be governed by two different factors, namely ionization efficiency of analyte molecules and the rate of desorption or vaporization of molecules which are related to the physicochemical properties of constituent amino acids of the model peptides. The presence of basic residues such as Arg and Lys enhances the ion yields of protonated molecules. The presence of the aromatic hydrophobic amino acid Phe residue results in an increase of the both positive- and negative-ion yields. In contrast, the presence of phosphate groups contributes to the suppression of the both positive- and negative-ion yields due to the low hydrophobicity of phosphorylated peptide. The enhancement effect of hydrophobicity on the ion yields was higher than that of basicity and acidity of the peptides.

Keywords: ESI-MS, phosphorylated peptide, ion yield

POS-03-240 Bioinformatics Analysis of Protein Dynamics in Urine of Healthy Volunteers Exposed 105-Day Isolation

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The study was conducted during the experiment with 105-day isolation of healthy volunteers in experimental complex. We collected urine samples from these persons, whose physical activity, diurnal rhythm, temperature parameters in object and level of oxygen and carbon dioxide were controlled during the experiment. The samples were analyzed using mass ioni cyclotron resonance mass spectrometer with Fourier transform LTQ FT MS on the basis of the AMTtags approach. It were identified about 600 urine proteins. For physiological interpretation of the proteomic data we used ANDSystem tool. Application of ANDSystem revealed proteins that are most closely associated with the regime of sodium intake as well as build the network of their interactions. Using cluster analysis (SOM) we identified clusters of proteins with similar dynamics of appearance and disappearance in the urine of volunteers in the experiment. It was revealed that there were 2 experimental period in which the protein composition of the urine significantly altered: 7 and 13 weeks, which may be associated with the transition of volunteers to reduced sodium consumption; in the 1st case, from high to medium and the 2nd, from the middle to low. It was suggested that from 4 to 6 weeks of experiment the activity in gluconeogenesis were elevated in the organism of subjects. During the same period some proteins are getting over-expressed in the urine, among them transmembrane protein tyrosine kinase signaling pathway mediating organ morphogenesis, the binding of cytoskeletal proteins in the apical cell space, as well as proteins carrying out the cellular response to Ca+ mediated stimulation and increases the activity of transporters, indicates an increase in angiogenesis and induce the activity of endopeptidase. Thus, this bioinformatics approach allowed a comprehensive analysis of proteomic data with access to the physiological processes of the human body with the assessment of their dynamics during the experiment.

Keywords: urine proteome analysis, associative protein networks, claster analysis

POS-03-241 Process Optimization for Shake Flask Bio-Treatment of Disperse Yellow 9 Textile Dye with White-Rot Fungi and Their Enzymes

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Industries that release highly colored effluents are textiles, paper and pulp mills, dye-making industries, alcohol distilleries and leather industries. Effluents from these industries contain chromophoric compounds and can be mutagenic and inhibitory to aquatic biosystems. Bioremediation utilizes metabolic potential of microorganisms in order to clean up the environmental pollutants to less hazardous or non-hazardous forms. White-rot fungi and their lignin degrading enzymes; laccase, manganese peroxidase and lignin peroxidases are useful in the treatment of colored industrial effluents and other xenobiotics. This study was designed to investigate the oxidation (decolorization/degradation) of three selected synthetic dyes; Bromophenol Blue (BB), Acid Violet 7 (AV7) and DisperseYellow 9 (DY9), by white-rot fungus *Trametes versicolor*. The best decolorized dye DY9 was selected for subsequent optimization studies. After the step by step applications, the highest color removal yield was 93% in DY9 sample after 120 h of incubation at 35˚C, pH 4.5 in krik medium with added 1% starch and 0.01% ammonium sulphate as carbon and nitrogen source respectively. Ligninolytic enzyme activities were correlated to dye decolorization and maximum manganese peroxidase activity 416.33 U/ml was also noted in the maximally decolorized medium. The result indicated that *T. versicolor* was obviously able to breakdown synthetic dyes and manganese peroxidase was considered as a major lignin-degradation enzyme in this reaction. Manganese peroxidase enzyme play an important role in the bioremediation of these dyes and its activity is induced by dyes. The effects of dye concentration, fungal inoculum size as well as pH were studied. Samples were periodically collected for the measurement of color unit, laccase, manganese peroxidase and lignin peroxidase activity.

Keywords: decolorization, bioremediation, microbial enzyme system

POS-03-242 Identification of *HAMP* Transcript Variant Coding for an Abnormal Polypeptide in Human Hepatoma-Derived Cell Line HLF

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Hepcidin is a small peptide mainly produced by the liver and thought to be the key regulator in iron homeostasis. Hepcidin is coded by *HAMP* gene, which is composed of 3 exons, and codes 84 aa preprohepcidin containing a secretory signal peptide in the N-terminal and the typical motif of proprotein convertases to create a mature hepcidin-25. We identified a new alternatively spliced *HAMP* transcript in hepatoma-derived cell line HLF, although other cell lines such as HepG2, Hep3B, Huh-7, HLE and WRL68 did not have that. There was no any other mutation on the *HAMP* gene in HLF. The cloning and sequencing analyses showed that the alternative transcript was identical to preprohepcidin sequence, but lacked an internal 60 bp, corresponding to the exon 2. Then, whole exome sequencing of the above six cell lines was performed, which revealed the frameshift-causing deletion of the *SF1* gene, coding splicing factor 1 protein, one of multiple components of the RNA splicing machinery, in HLF only. There were some mutations in other component genes, but these mutations were not specifically in HLF. The data suggest that the *SF1* gene mutation may relate to the alternative splicing of *HAMP* gene.

Keywords: hepcidin, HAMP gene, splicing factor 1

POS-03-243 Venom Proteomics of the Brazilian Spider *Acanthoscurria gomesiana*

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Spiders are among the most diverse and successful predators on Earth. One of the reasons is the production of a toxic venom refined through millions of years of evolution. Spider venoms are composed mainly of peptides and proteins and present a variety of biological activities. However, few of these components have been fully characterized. Recent studies indicated the presence of components with antimicrobial activity in the venoms of the Brazilian spider *Acanthoscurria gomesiana*. In order to explore the antimicrobial and other potentially active molecules, we conducted a proteomic study of *Acanthoscurria gomesiana* venom combining in-solution digestion of crude venoms and RP-HPLC fractionation of proteins and peptides, high resolution LC-MS/MS analysis and automated *de novo* sequencing of peptides followed by database and homology search. We found new proteins with homology to cysteine knot toxins, chitinases, alpha-amylases and actins. Antimicrobial activities were observed in RP-HPLC fractions and new peptide structures could be determined by *de novo* sequencing of MS/MS spectra.

Keywords: *Acanthoscurria gomesiana*, De novo sequencing, venom proteomics

POS-03-244 Proteomic Analysis of Two Transcriptional Start Sites of the *argC* Gene and Its Implication on the *Sinorhizobium meliloti* Physiology

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The *argC* gene encodes an N-acetyl-gamma-glutamyl phosphate reductase, which is necessary for arginine biosynthesis. Arginine is an essential amino acid in bacteria, it is the most common nitrogen storage compound, and its synthesis demands a huge amount of energy and reducing power. In *Sinorhizobium meliloti*, a gram-negative alfa-proteobacteria which is able to fix atmospheric nitrogen in symbiosis with alfalfa (Medicago sativa), the *argC* coding sequence has two different start codons. The first start codon (TTG) is located near to a putative -10 box at 60 bp upstream of +1, and the second start codon (ATG) is situated to 125 bp upstream of +1 (Diaz et.al., J.Bacteriol. 193:460,2011). Our results showed that the *argC* protein in the wild type strain 1021 was mainly transcribed from the ATG start site; however, the TTG start site was also functional under certain conditions. When we evaluated strains containing plasmids with a constitutive promoter having both sites (TTG, ATG) or only one site (ATG), the protein transcribed from the ATG start codon was postranslationally modified in the cell, showing an increased number of different electrophoretic identities detected by proteomic analysis (using 2D-gels). These spots were identified by LC-MS/MS with phosphorylations in several residues, some of them specific of each electrophoretic entity. These modifications have not been previously reported in the *argC* protein. The induced constitutive transcription of *argC* from the ATG start site has metabolic implications because such strain was impaired in nitrogen fixation when in symbiosis with alfalfa. We conclude that when *argC* is only constitutively synthesized through the ATG start codon, the organism undergoes a metabolic imbalance, and the cell has to contend with this, modifying the *argC* postranslationally, through a phosphorylation mechanism which results in an inactive protein. Part of this work was supported by PAPIIT-UNAM grant IN205113.

Keywords: bacteria, regulation, phosphorylation

POS-03-245 Proteomic Analysis of Hepatocarcinoma Cell Line SMMC7721 in Response to Over Expression of the RING Finger Protein, HSPC238

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Proteins with the RING-type zinc-finger domain are present in all eukaryotes. HSPC238 with a C3HC4 type RING zinc finger is found to play a possible role in various signal transduction pathways and regulatory. However, the precise molecular mechanisms of these events remain poorly elucidated. In this study, a two-dimensional gel electrophoresis (2DE)-mass spectrometry (MALDI-TOF/TOF) proteomic approach was used to investigate the protein expression pattern in hepatocarcinoma cell line SMMC7721 which exogenously expressed HSPC238 (SMMC7721/HSPC238). We have identified 35 protein spots that changed in expression (≥2-fold) in SMMC7721/HSPC238. These proteins are involved in metabolism, signal transduction, apoptosis, mitosis, cell cycle, cell motility or gene regulation. In particular we found down regulation of 22 genes that modulate actin remodeling and cell migration, cell proliferation and invasion, and up regulation of 9 genes which are involved in apoptosis, metabolism and signal transduction. In addition, SMMC7721/HSPC238 cells can significantly inhibit cell proliferation, invasion, soft agar colony-forming ability and tumorigenicity in nude mice. In summary, this study provided new clues for understanding the mechanisms of HSPC238. The inhibition of SMMC7721 cell proliferation, invasion, soft agar colony-forming ability and tumorigenicity might give evidences of HSPC238 for developing new therapeutic approaches against hepatic carcinoma.

Keywords: HSPC238, proteomics, SMMC7721

POS-03-246 Biomarkers of Social Immunity in Honey Bees: Real-World Application of Biomarkers in a Marker-Assisted Selective Breeding Program

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We report the successful identification protein biomarkers of social immunity in honey bees and applied them in a selective breeding program to improve honey bee stocks. Honey bees play a crucial role as pollinators for the agricultural industry and the environment but, worryingly, colony losses attributed to bee-specific infectious diseases have increased dramatically in the last few years. Although honey bees have evolved defensive innate and social immunity mechanisms against pathogens, the degree of immunity and therefore the ability to fight disease differ among honey bee lines. In a multidisciplinary and multi-center project, we are developing proteomics-based diagnostic tests for social immunity to select stocks with natural disease resistance that should allow selective breeding that will ultimately reduce the need for chemical treatments in beekeeper operations. In the initial phase of the project, we used quantitative proteomics to identify biomarkers by correlating social immunity traits with the levels of proteins in antennal samples collected over a period of 3 years. We are now validating these biomarkers using MRMs and applying them to identify and breed selected stocks in a marker-assisted selection (MAS) breeding program. MRM development, data acquisition and analysis, the statistical development of the predictive models, and the results of ROC sensitivity and specificity curves will be presented. Our results show that we have confirmed the diagnostic value of the protein biomarkers, and used them in marker-assisted selection. To our knowledge, this is the first time that protein based biomarkers were used to guide a selective breeding program in any organism.

Keywords: animal, agriculture, MRM

POS-03-247 Depth and Width of Human Proteome

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The international Human Proteome Project (HPP), a logical continuation of the Human Genome Project, was launched on 23 September 2010 in Sydney, Australia. Despite the fact that unofficially project starts from 2007, two basic questions not currently addressed yet: first, it is not clear the depth of human proteome - what is the minimum copy number of a certain protein which could to be detected in blood plasma or cells? Secondly, what is the total number of protein species in human body? At the moment, these problems remain unsolved. Of course, these two parameters are related: the higher sensitivity of proteomic technologies allow us to detect the more different protein species. Based on the results obtained with existing experimental techniques and data-analysis, we tried to evaluate both parameters in human body as a function of the sensitivity limit. We assume, that in case applying SRM for detecting protein complement in plasma, liver and HepG2 cells, the sensitivity of 10^{-18}M (which corresponds to one protein molecule per 10^7 HepG2 cells, 10^8 liver cells or 1 μL of plasma), will be enough. For estimation the number of protein species in human body, data from UniProt KB (www.uniprot.org) were used. Information about heterogeneity of protein species was expressed as number of single amino-acid polymorphisms (nsSNP/SAP), alternative splicing (AS) and post-translational modifications (PTMs) per gene. Multiplying the average number of variations per gene for all human genes (coded on all 24 different Chrs), we have estimated the maximum number of protein species (without combinatorial variants) as 1,9 mln. Interestingly, that number of SAPs, AS are almost continuously since 2011 according UniProtKB data. Experimental verification was performed using 2DE with various dyes allow to identify protein species with a sensitivity of 10^{-4}M to 10^{-12}M.

Keywords: Human Proteome Project, protein species

POS-03-248 The Utility of ETD in Peptidomics for Endogenous Secretory Peptides

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A peptidomic survey of endogenous secretory peptides provides information for predicting potentially bioactive peptides (Mol Cell Proteomics 8: 1638-47, 2009). Following functional assays, we have described novel bioactive peptides that had escaped genomic or proteomic identification (J Biol Chem 282: 26354-60, 2007; J Proteome Res 9: 5047-52, 2010; J Proteome Res 10: 1870-80, 2011; Mol Cell Proteomics 12: 700-9, 2013). However, MS/MS sequencing of peptides in their native form remains challenging. While widely used in the proteomics community, ETD has not been fully investigated on endogenous peptides. We used ESI-Orbitrap-ETD, in parallel with CID, to analyze peptides derived from secretory granules of a human pancreatic neuroendocrine cell line. A total of 967 unique peptides were identified with an overlap of 397, ranging from 1000 to 15000 Da (charge states 2 to 14). About half of the identified peptides were larger than 3000 Da. For a subset of peptides not reached by CID, ETD produced better fragment ion ladders to provide confident identification. A major drawback of ETD was poor performance for ions beyond m/z 1000 regardless of charge state. Nonetheless, ETD's advantages were noted in identifying a new C-terminally amidated peptide with antimicrobial activity, or in differentiating nearly isobaric peptides (less than 2 ppm) that arise from alternatively spliced exons of a neuropeptide precursor gene. Both fragmentation methods complemented each other to localize phosphorylation sites. These findings point to the utility of ETD in peptidomics. Our study also heralds problems that need to be overcome in top-down proteomics.

Keywords: peptidomics, ETD, bioactive peptide

POS-03-249 Pyrococcus Furiosus: The Perfect Standard for Proteomics?

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In proteomics there is an ever-present need for tailored standards which allow for assessing the efficiency and quality of ideally all steps of the analytical workflow - from sample preparation to protein identification and quantitation. The hyperthermophilic Archaea bacterium *Pyrococcus furiosus* (Pfu) has a huge evolutionary distance to other organisms such as yeast or humans. Indeed, only seven tryptic peptides can be found in common between human and Pfu protein databases, rendering it a potentially interesting candidate as a universal proteomics standard. We systematically investigated the possibilities to utilize this uniquely complex standard in proteomic workflows, on the theoretical level (database searches and estimation of false discovery rates), and on the experimental level (assessing reporter ion interference, LC-MS quality control). Based on our result we conclude that Pfu represents an ideal and complex standard in proteomics, designed by evolution and ready to use.

Keywords: standards, reporter ion inteference, identification results

POS-03-250 Alcohol Induced ER Stress Markers in Human Microglia Cells

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Microglia cells (MC) are the line of defense after the blood-brain barrier (BBB). There is evidence that alcohol can exert deleterious effects on the nervous system. Nevertheless the specific effects of alcohol on the ER stress responses in the central nervous system (CNS) remain poorly understood. We hypothesize that these responses occur by modulating changes in proteins triggering a process known as unfolded protein response (UPR). This process typically protects cells from the toxic effects of accumulated misfolded proteins causing ER-stress. Although much is known about ER stress, less is understood about the consequences of the disruption of these interactions due to alcohol treatment of MC. We investigated alterations in MC viability using MTT cytotoxic assay. Proteins were analyzed with 2D gel electrophoresis and identified using peptide mass fingerprinting. Confirmation at the gene expression level was performed by qRT-PCR. Results show that microglia treated with 0.5% alcohol did not show a significant decrease on cell viability when compared to control, leading to an acute ER stress response as previously shown by our lab, whereas microglia treated with 3% alcohol had a decrease cell viability of almost 50%. MC's proteome induced with alcohol, demonstrated 23 differentially expressed proteins. Alcohol changed the expression of key components of the UPR-ER stress induced pathway that includes chaperones, ER stress markers, antioxidant enzymes, protein degradation enzymes, and enzymes related to alcohol metabolism. qRT-PCR highlighted enhanced expression of UPR and antioxidant genes that increased with alcohol treatment. Results of these analyses provide insights into alcohol mechanisms of regulating MC, and may suggest that alcohol induced a UPR transcriptional program in MC. We speculate that activation of an ER stress response by alcohol will induce an oxidant neuroinflammation leading to the development of alcohol-related diseases in the MC.

Keywords: ER-stress, unfolded protein response (UPR), microglia

POS-03-251 Identification of Cell Polarity Target Phospho-Protein by Using Inducible Gene Knock-Out Strategy

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Regulation of cell polarity is essential and useful to understand the mechanism underlined cell differentiation and morphogenesis, which is observed during embryogenesis and carcinogenesis. We have been studying cell polarity regulation machinery through aPKC-PAR (atypical Protein Kinase C and Partitioning defective protein) protein complex for more than 15 years. Our studies suggest that aPKC-PAR protein contributes to proper maintenance of epithelial cell polarity, carcinogenesis and embryogenesis. To identify the target proteins, that are phosphorylated through aPKC pathway, is very informative to understand cell polarity regulation mechanism and to develop anti-cancer drugs. We have been established inducible gene knock-out experimental system to identify molecular targets by using Cre-loxP system. In this presentation, we propose novel approach to identify phospho-protains that regulates cell polarity though aPKC-PAR signaling pathway.
Keywords: cell polarity

POS-03-252 Analysis of Structural Changes in Erythropoietin by LC/MS Induced by Different pH Conditions

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In this study**,** erythropoietin (EPO) was analyzed under different pH conditions (pH3 and pH9) for 4 weeks. The purpose of stability testing is to verify how the quality of drug varies, with time under the influence of a variety of environmental factors. Intact EPO and deglycosylated EPO were confirmed via SDS-PAGE followed by induction of high-pH condition to induce aggregation of EPO. Aggregation analysis of proteins can be formally classified SEC-HPLC. Analysis of changed modification site (glycosylation, deamidation and oxidation) performed by UPLC-ESI-Q TOF-MS/MS. The samples showed that there is no changed modification site identified by Biopharmalynx 1.2. HPAEC-PAD was performed to identity carbohydrate compositions and NP-HPLC has been performed to identify changing of glycan structure. All samples showed to have the same glycan composition, and identified to have similar contents of neutral and amino sugar. In this analysis, stability testing of the drug can help identify the degradation products and it helps set storage conditions.
Keywords: erythropoietin, SEC-HPLC, UPLC-ESI- Q TOF-MS/MS

POS-03-LB-001 On-Line Chip-Based Strategy for 2D Fractionation - Comparing Peptides Found between 1D and 2D Proteomic Analysis

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On-line two dimensional (2D) liquid chromatography is widely used for proteome identification/quantification using the advantage of increasing peak capacity. A recently developed strategy for this involves a first dimension high pH reverse phase (RP) separation, followed by a second dimensional analytical RP chromatography separation at low pH. We have developed a simplified on-line chip-based 2D-LC approach that uses the cHiPLC system couled directly to the MS for proteomic analysis of complex samples. *E. coli* lysate samples were tested with this approach. Samples were first loaded onto a 200 μm ID cHiPLC column at pH 9.8 at a flow rate of 1 uL/min. Step gradients of acetonitrile were used to elute the peptide fractions, which were diluted to pH 2.5 before being captured by a 200 μm ID cHiPLC trap column. Each fraction was then separated using a standard 75 μm ID cHiPLC analytical column at 300 nL/min online with a TripleTOF 5600 system. Data was processed with ProteinPilot Software and a peptide alignment and comparison tool.
A variety of workflow comparisons were performed to assess impact on proteomic workflows (1D, 2D-6 fractions and 2D-10 fractions, as well as multiple sample loads). Using approximately 1 ug of *E. coli* digested cell lysates, we achieved 1.8x and a 2.1x increase in peptide identification numbers (5% local FDR) for the 2D-6 and 2D-10 fractions versus the 1D configuration. The other advantage of the 2D workflow is the larger sample loading capacity on the column. When the loading amount was increased 10x, the number of detected peptides increased by 3.3x and 4x for the 2D 6 and 10 fraction workflows, respectively, over the 1D workflow. The measured retention time of peptides detected in both the 1D 1 ug and 2D 10 ug experiments showed good correlation (r2 0.99, slope 1.0). Analysis of peptide overlap between fractions in the 6 step workflow showed that only about 10% of the peptides were found in multiple fractions.
Keywords: 2D fractionation, RP/RP, on-line 2D

POS-03-LB-002 Shedding Light on Placental Infection and Inflammation in Preterm Birth by Terminomics - A Comprehensive Protein Termini Orientated Genome Wide Analysis of Human Placental Tissue

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Preterm birth affects approx. 12% of all deliveries, and represents a major risk factor for impaired growth and development. Since prematurity imparts huge emotional cost to the family and burdens the health care system, it's vital to understand and diagnose early the initiation and progression of infection and inflammation leading to premature labor. With subclinical infections being beyond the main causes, clinical criteria are poor predictors, leading to frequent misdiagnosis. Especially, the roles of inflammatory pathways and inflammatory mediators, and the roles of proteases and their targets in preterm birth are currently unclear. Hence, there's a pressing need for in-depth proteomic analysis of human placenta and preterm placental inflammation. We propose to establish a system-wide understanding of infection-induced inflammation in preterm labor. Preliminary analysis of term placentas from elective caesarean sections identified >15,000 peptides and >4,000 proteins by our combined shotgun proteomics and terminomics approach (preTAILS & TAILS). We identified >5,000 protein N-termini, including termini of a myriad of rare proteins which are usually not identifiable by a shotgun approach alone, highlighting the power of our combined strategy, and the feasibility of our study. Furthermore, our results represent highly valuable information for the human proteome project.
Keywords: terminomics, placental inflammation and infection

POS-03-LB-003 Glycosylation Profiling of Cancer Biomarkers by A Nanoprobe-Based Strategy Combined with Lectin-Based Enrichment

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Glycosylation is the most prevalent protein modification and 50% of proteins are suggested to be glycosylated based on the genomic information. Increasing evidences have shown that the significant change in protein glycosylation pattern is an important feature during oncogenesis. To faciltate sensitive glycopeptide characterization from serum, here, we present a sequential enrichment method by antibody functionalized nanoprobe and lectin-based enrichment followed by LC-MS/MS analysis. Based on the sensitivity of nanoprobe for serum marker purification and the selectivity of lectin for glycopeptides enrichment, we aim to establish a simple protocol for the analysis of low-abundant serum glycoprotein bypassing sample fractionation, immuno-depletion of abundant serum proteins with minimum sample loss. To demonstrate the general availability of this approach, prostate-specific antigen (PSA) and alpha-fetoprotein (AFP), the clinically used glycoprotein marker for prostate cancer and liver cancer diagnosis, respectively, were selected as model systems. Taking advantage of efficient affinity extraction by surface-functionalized magnetic nanoparticles (MNPs), we present a successful nanoprobe-based immunoassay for enrichment of low abundant targeted glycoproteins, PSA and AFP, from human serum. This two-step workflow has shown the detection of multi-glycosylation of purified glycoproteins from human serum. Analysis of the glycosylation pattern for the standard PSA and AFP showed five glycoforms from PSA and six glycoforms from AFP. Currently, the practicability of this approach is being demonstrated to qualitatively compare the glycosylation profiles in normal controls and cancer patients. Furthermore, we expect to quantify the glycoforms in cancer patient in comparison with normal control group to evaluate the level of specific glycosylation and its correlation to the progression of cancer.

Keywords: N-linked glycopeptides, nanoprobe, mass spectrometry

POS-03-LB-004 The Role of Collapsin Response Mediator Protein 2 in Amyloid-β25-35 Induces Impairment of Cognitive Function and Long-Term Potentiation

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Alzheimer's disease (AD) is characterized by amyloid-β (Aβ) protein and tau deposition in the brain. Numerous studies have reported a central role of Aβ in the development of AD, but the pathogenesis is not well understood. Collapsin response mediator protein 2 (CRMP2) is an intracellular protein mediating a repulsive axon guidance molecule, Semaphorin3A. In the Sema3A signaling cascade, CRMP2 is phosphorylated by cyclin-dependent kinase 5 at Ser522, a prerequisite to its subsequent phosphorylation by glycogen synthase kinase-3β at Ser518, Thr514, and Thr509. In AD brain, a hyperphosphorylated form of CRMP2 is a component of the paired helical filaments. To gain insight into the role of CRMP2 phosphorylation in AD pathogenesis, we investigated the effects of Aβ in CRMP2 phosphorylation-deficient knock-in (*crmp2$^{ki/ki}$*) mice, in which the Ser residue at 522 was replaced with Ala. Intracerebroventricular (i.c.v.) injection of $A\beta_{25-35}$ peptide, a neurotoxic fragment of Aβ protein, to *wild-type* (*wt*) mice increased hippocampal phosphorylation of CRMP2. Behavioral assessment revealed that i.c.v. injection of $A\beta_{25-35}$ peptide caused impairment of novel object recognition in *wt* mice, while the same peptide did not in *crmp2$^{ki/ki}$* mice. In electrophysiological recording, *wt* and *crmp2$^{ki/ki}$* mice have similar input-output basal synaptic transmission and paired-pulse ratios. However, long-term potentiation was impaired in hippocampal slices of $A\beta_{25-35}$ peptide-treated *wt* but not those of *crmp2$^{ki/ki}$*. Our findings indicate that CRMP2 phosphorylation is required for Aβ-induced impairment of cognitive memory and synaptic plasticity. We are now performing proteomic analysis of brains from *wt* and *crmp2$^{ki/ki}$* mice treated with or without $A\beta_{25-35}$ peptide.

Keywords: Alzheimer's disease, CRMP, phosphorylation

POS-03-LB-005 Insights into UV-A & Dark Incubation Induced Paclitaxel in *Taxus chinensis* by Genomic and Proteomic Analysis

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Paclitaxel is a well-known anti-cancer drug and its output was restricted by the limited barks of *Taxus*. It is desiderated to find a simple and efficient method to improve paclitaxel production and avoid the over-exploitation of *Taxus*. We found that under certain conditions the content of paclitaxel in *Taxus chinensis* leaves was significantly increased 56% exposure to UV-A radiation coupled with dark incubation (UV-A & dark), which may represent a new thought for industrial production of paclitaxel. RT-qPCR analysis of the key genes in paclitaxel biosynthesis pathway reveals that genes were unexpectedly induced during the dark incubation instead of UV-A irradiation. Comparative proteomics studies displayed several UV-A & dark regulated key enzymes involved in paclitaxel pathways and the global cellular UV-A & dark responsive protein network.

Keywords: paclitaxel, taxus chinensis, UV-A & dark incubation

POS-03-LB-006 Enzymatic Approach to Reduce the Complexity of Proteome Samples by Depleting Glutamine-Containing Peptides

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Shotgun proteomics is a widely used approach for biological discovery of proteins, and a lot of chemical-labeling strategies have been introduced to identify or quantify the peptides and proteins, they were divided into three basic types: sulfhydryl group-directed, amine-directed and carboxyl- directed approaches. There is no labeling strategy to label the specific amino acid except the cysteine, and the broad dynamic range of high abundance proteins among different sample and cell types present a challenge in the analysis of low abundance proteins. Transglutaminase (TGase) is calcium-dependent enzyme catalyzing the cross-link formation between glutamine and lysine side chains, leading to an intrachain or interchain isopeptide bond. We describe here a novel enzymatic approach, utilizing TGase to catalyze the Q-containing peptides crosslink to the BSA proteins, to reduce the complexity of proteome samples by depleting the protein complex formed by Q-containing peptides and BSA proteins. The Q-containing peptides decreased markedly due to the depletion by TGase. We also determined that more new peptides and proteins were obtained by dataset searches for TGase-mediated site-specific labeling and deplete reactions after LC-MS/MS analysis.

Keywords: complexity, transglutaminase, glutamine-containing peptides

POS-03-LB-007 Systematic Evaluation of Ultrahigh Resolution MS Instrument Parameter to Optimize Topdown Analysis

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The combination of ultrahigh resolution together with the complementary dissociation techniques of collision induced dissociation and electron transfer dissociation enables thorough topdown protein characterization. Compared to peptides, intact proteins have a much higher number of fragmentation channels and therefore fragment ion intensities are usually lower compared to peptide fragment ions. This requires averaging of MS/MS spectra to increase the data quality but also makes it more difficult to do topdown analysis on LC timescale. With the introduction of the compact highfield Orbitrap mass analyzer topdown experiments can be done at LC time scale if the experimental parameters are chosen appropriately. This study evaluates the different instrument parameters to increase both spectral quality and speed of analysis.

Proteins in the range of 8 kDa to 30 kDa have been used to study the effect of various experimental parameters on the speed and spectral quality for topdown MS/MS. Amongst those were precursor ion charge state, AGC target value for precursor cations and ETD anions, resolution, ETD reaction time, HCD fragmentation energy, Orbitrap vacuum quality, number of averaged spectra or transients etc. Consistently across the studied proteins, the precursor ion charges states with the highest number of charges gave best ETD fragmentation efficiencies. They all had their maximum fragmentation efficiency at around 5 ms for ETD reaction time. In contrast to ETD, lower charged precursor ions gave best results for HCD fragmentation. AGC target value, resolution and number of averaged spectra have a direct influence on the speed of analysis. FTMS^n target values ranging from 5E5 to 1E6, a resolution of 120,000 or 240,000 FWHM at *m/z* 400, and averaging 5-10 MS^n scan events are giving optimal results. Finally, recommendations can be deduced from the systematic evaluation of those parameters for the best compromise between spectral quality and speed of analysis.

Keywords: top down proteomics, instrumentation

POS-03-LB-008 Characterization of Monoclonal Antibodies with LC-MS by Integration of *De Novo* Sequencing and Database Search

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Introduction

Charactering monoclonal antibodies (mAbs) remains a challenge due to incomplete sequence coverage for complementarity determining regions in protein databases. In addition, mAbs can be heterogeneous due to modifications that can occur during expression, purification and storage. Multiple enzyme digestion coupled with high resolution LC-MS/MS is a common practice to determine the primary sequences and modifications of monoclonal antibodies. In this work, an algorithm integrates database search and *de novo* sequencing for data analysis, resulting in full length sequence characterization of antibodies.

Methods

1. Perform a database search and PTM search against a public antibody database to find sequence candidates.
2. Perform *de novo* sequencing. Peptide-spectrum matches (PSMs) identified in database search at 0.5% FDR are used to estimate the accuracy of *de novo* sequences. Highly confident *de novo* sequence tags are used for homology matching.
3. Reconstruct sequences using homology matches and intact antibody masses.

Results

The algorithm was implemented in software PEAKS and tested with a human antibody and a mouse antibody. The samples were digested with AspN, chymotrypsin, GluC, LysC, pepsin and trypsin, and analyzed with LTQ-Ortritrap.

All MS/MS spectra were searched against a public antibody database. At 0.5% false discovery rate (FDR) at PSM level, the majority (>95%) of the sequences in constant region of heavy chain and light chain can be covered. However, the variable domains containing CDRs only cover of <70% for the heavy chain and <50% for the light chain. By finding homology matches between *de novo* sequences and candidate sequences and recontructing candidates iteratively, full sequences of the antibodies and the sites of modifications were determined with 0.5% of FDR at PSM level.

Conclusion

An novel algorithm of LC-MS data analysis for characterization of monoclonal antibodies

Keywords: bioinformatics, antibody, proteomics

POS-03-LB-009 Proteomic Investigation of Breast Cancer Biomarkers Using Human Plasma and Serum Samples

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Aim: Early diagnosis of breast cancer (BC) and monitoring its progression is still a major challenge. Our objective was to identify the proteins differentially expressed from plasma and serum samples at different stages of BC, using different blood collection tubes and a label-free, ion quantitation liquid-chromatography/tandem-mass spectrometry (LC-MS/MS) approach for BC diagnosis. **Methods**: The study cohort consisted of 26 BC patients including women with ductal carcinoma in situ (DCIS, n=6), invasive BC (IBC, n=8), benign breast disease (BBD, n=6), metastatic BC (MBC, n=6) and healthy controls (n=8). The blood was collected in 4 different blood tubes (2 serum/2 plasma). Quantitative analysis of blood proteins and peptides was performed using LC/MS/MS. The findings were statistically analysed using the Progenesis-LCMS software. **Results**: Over 100 differentially abundant proteins were identified. Results from each individual tube were normalized against controls and statistically compared. A greater number of proteins were identified from Gold-top serum tubes. The significance criteria applied was $p<0.05$, false discovery rate $q<0.02$, fold change >3. This information has been used to further assess differential proteins as potential for diagnosis in the stages of BC. **Conclusions**: LC-MS/MS is a highly specific and sensitive method for screening of proteins in blood of BC patients. Our findings indicate that the low-mass (<3kDa) component gave a very rich source of proteins and that the serum profile is a better source for biomarker identification. Several proteins identified have strong potential to define the different stages of BC and warrant future validation studies.

Keywords: Breast Cancer; LC-MS/MS; biomarkers; Serum; Plasma

POS-03-LB-010 Ero1alpha and PDIs Constitute a Hierarchical Electron Transfer Network of Endoplasmic Reticulum Oxidoreductases

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Ero1α and endoplasmic reticulum (ER) oxidoreductases of the protein disulfide isomerase (PDI) family promote the efficient introduction of disulfide bonds into nascent polypeptides in the ER. However, the hierarchy of electron transfer among them is poorly understood. Here, Ero1α-associated oxidoreductases were identified by proteomic analysis and further confirmed by surface plasmon resonance. Ero1α and PDI were found to constitute a regulatory hub, whereby PDI induced conformational flexibility in an Ero1 α shuttle cysteine (Cys99) facilitated intra-molecular electron transfer to the active-site. In isolation Ero1α also oxidized ERp46, ERp57 and P5, however, kinetic measurements and redox equilibrium analysis revealed that PDI preferentially oxidized other oxidoreductases accepting electrons from the oxidoreductases via its a' domain bypassing the a domain, which serves as the electron acceptor from reduced glutathione. These observations provide an integrated picture of the hierarchy of cooperative redox interactions among ER oxidoreductases in mammalian cells.

Keywords: redox, Endoplasmic reticulum, Protein disulfide-isomerase

POS-03-LB-011 Zebrafish Scube1 [Signal Peptide-CUB (Complement Protein C1r/C1s, Uegf, and Bmp1)-EGF Domain-Containing Protein 1] Is Involved in Primitive Hematopoiesis

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Scube1 (signal peptide-CUB-EGF domain-containing protein 1), the founding member of a novel secreted and cell-surface SCUBE protein family, is expressed predominantly in various developing tissues in mice. However, its function in primitive hematopoiesis remains unknown. In this study, we identified and characterized zebrafish scube1 and analyzed its function by injecting antisense morpholino oligonucleotide (MO) into embryos. Whole-mount in situ hybridization revealed that zebrafish scube1 mRNA is maternally expressed and widely distributed during early embryonic development. Knockdown of scube1 by MO downregulated the expression of marker genes associated with early primitive hematopoietic precursors (scl) and erythroid (gata1 and hbbe1), as well as early (pu.1) and late (mpo and l-plastin) myelomonocytic lineages. However, the expression of an early endothelial marker fli1a and vascular morphogenesis appeared normal in scube1 morphants. Overexpression of bone morphogenetic protein (bmp) rescued the expression of scl in the posterior lateral mesoderm during early primitive hematopoiesis in scube1 morphants. Biochemical and molecular analysis revealed that Scube1 could be a BMP co-receptor to augment BMP signaling. Our results suggest that scube1 is critical for and functions at the top of the regulatory hierarchy of primitive hematopoiesis by modulating BMP activity during zebrafish embryogenesis.

Keywords: zebrafish, primitive hematopoiesis, bone morphogenetic protein

POS-03-LB-012 Serum *N*-Glycans Profiling for the Discovery of Potential Oral Cancer Tumor Markers

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Oral cancer is the tumor grows on the lips, tongue, cheeks, floor of the mouth, hard and soft palate, sinuses, and pharynx. The high prevalence and mortality rate of oral cancer in Taiwan makes it important to investigate new biomarkers for the surveillance of high-risk population. In our previous study, we have identified several potential carbohydrate and glycoprotein tumor markers from oral cancer cell lines. Here, we further investigated the serum *N*-glycomes of normal (21, healthy volunteers) and oral cancer patients (61, obtained from tissue bank of NCKUH) by mass-spectrometric (MS) glycomic measurements. The mass spectrum obtained from 20 μl of serum sample and the primary structures of serum *N*-glycans could be predicted by GlycoWorkbench software. The relative intensities of different *N*-glycan subclasses were calculated by expressing the intensity of each glycan ion as percent of the total intensity of all glycan ions. We found that the proportions of bi-, tri- and tetra-antennary *N*-glycans were significantly increased in oral cancer patients. In addition, these *N*-glycan structures also showed high sensitivity and specificity for cancer patients. Furthermore, high AUC values (>0.8) were also obtained from ROC curves of specific *N*-glycans. We are now verifying our findings in more oral cancer patients. Our results should be valuable information for the diagnosis and prognosis of oral cancer.

Keywords: oral cancer, serum N-glycomes, tumor marker

POS-03-LB-013 Proteomic Alterations of *Plasmodium falciparum* by Dihydroartemisinin, Mefloquine and Chloroquine

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Malaria is a life-threatening parasitic disease which annually claims almost 1 million deaths globally. While no effective vaccine against malaria parasite, Artemisinin in combination with mefloquine (MQ) or piparaquine has been used as first line drugs in many endemic areas while chloroquine (CQ) in early season of some areas. Despite poor understanding on antimalaria action of artemisinin, increased tolerance to artemisinin combination therapies has been reported in That-Cambodia border. Dihydroartemisinin (DHA), an active metabolite of artemisinin, has been employed for exploring parasite response by proteomic approach. *P.falciparum* parasite was treated with IC_{50} concentration of DHA, MQ or CQ. Since these antimalarials have been reported to interfere host protein ingestion of malaria parasite at different steps, sample preparation has been optimized to ensure effective removal of host cytosolic proteins from intact parasite. The 2-DE map with 96% success rate in MS identification was created. 2D-DIGE, Western Blotting and MS analysis were also employed to study proteins that are differentially expressed by the drug effect. An averaged number of 1,500 spots were detected in each gel. Of which, 256 spots were significantly changed upon exposure to DHA. Reduction of host proteins (e.g. catalase, carbonic anhydrase I, peroxiredoxin II, and globin chains) of approximately 1.16-4.07 folds was observed. Similar pattern was also scrutinized in the case of MQ but not for the globin chain. By contrarily, CQ caused the increase of these proteins while decrease of enzymes in digestive vacuole such as falcipain 2 was observed. The effects of DHA show relationship with time and the dosage and early detection within 30 minutes. It is worth to mention that these host proteins are derived from the digestive vesicles of parasites. Therefore, our findings lend support to the conclusion that DHA and MQ inhibits the transportation of host cytosol proteins into the parasite, which consequently leads to not only the deprivation of nutrition of parasite but also causing a defect in its oxidant-defensive mechanism against the antimalarial drugs. CQ, on the other hand, accumulates undigested endocytosed vesicles in the parasites.

Keywords: Plasmodium falciparum, dihydroartemisinin, proteomics

POS-03-LB-014 Proteomic Analysis of Urine from HIV Co-Infected Tuberculosis Patients

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Tuberculosis (TB) is a leading cause of death among people who are HIV-positive because HIV weakens the immune system. The currently available TB diagnostic tests are highly variable in sensitivity and specificity. It is very difficult to diagnose TB in HIV-positive patients. In this present study, we investigated protein profile in urine of TB/HIV patients that could be used to find new potential diagnostic markers of TB infection. The urine samples were pooled into 3 different groups including TB/HIV co-infected, TB and healthy control groups. Shotgun proteomic analysis was used to study *Mycobacterium tuberculosis* protein profile using 1-D SDS-PAGE, protein bands were identified by excision then in-gel digested with trypsin and peptide mixtures were analyzed using liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS). Mascot search engine was used to identify proteins from NCBI protein database. As a result, overall 282 bacterial proteins were found by this proteomic study. Only 3 of these bacterial proteins expressed in pooled urines of active TB in HIV-negative patients and 5 expressed in pooled urines of HIV co-infected TB patients. In addition, 28 proteins expressed both in TB and TB/HIV pooled urines. However, 50S ribosomal protein L2 that only expressed in urinary of TB/HIV co-infected patients and fatty acid CoA ligase FadD21 that was TB and TB/HIV shared proteins were select for validation. They may serve as useful markers in the urinary diagnosis of HIV co-infected Tuberculosis. In addition, this data set will provide interesting insights into the mechanism of TB infection in HIV-infected individuals.

Keywords: proteomic analysis, HIV, tuberculosis, diagnostic markers

POS-03-LB-015 Targeted Quantification of TMPRSS2-ERG Fusion Protein Products in Prostate Cancer Cell Lines and Tumors Using an Antibody-Independent PRISM-SRM Approach

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Fusions between the transmembrane protease serine 2 (TMPRSS2) and ETS related gene (ERG) have been identified in ~50% of prostate cancer cases and represent one of the most specific biomarkers that define a distinct molecular subtype of prostate cancer. The studies on TMPRSS2-ERG fusions have seldom been performed at the protein level, primarily due to the lack of high-quality antibodies or an antibody-independent method that is sufficiently sensitive for detecting the truncated ERG protein products resulting from TMPRSS2-ERG fusions. To address this issue, we applied a recently developed PRISM (high-pressure high-resolution separations with intelligent selection and multiplexing)-SRM (selected reaction monitoring) strategy for quantifying truncated ERG protein in prostate cancer cell lines and tumor tissues. PRISM-SRM assays for 16 peptides covering various fusion protein products were developed. Analyses of the prostate cancer cell line and tumor samples applying these assays led to the highly confident detection of 6 unique ERG peptides in both TMPRSS2-ERG positive cell lines and tissues, and none in the TMPRSS2-ERG negative samples, indicating that ERG expression is highly correlated with TMPRSS2-ERG gene rearrangements. Significantly, the concomitant detection of two mutually exclusive peptides also demonstrated that at least two ERG protein isoforms are simultaneously expressed in TMPRSS2-ERG positive samples, at levels ranging from 20 - 1200 amol/µg of total protein in cell lines, and 10 - 1200 amol/µg of total protein in tumors. Three peptides shared across almost all possible TMPRSS2-ERG fusion protein products were determined to be the most abundant peptides in both cell lines and tumor tissues, and hence, can be used as "signature" peptides for detecting ERG over-expression resulting from TMPRSS2-ERG fusion. These PRISM-SRM assays provide a valuable resource for studying TMPRSS2-ERG fusion protein products in cells and clinical specimens, thus improving our understanding of the TMPRSS2-ERG fusion event in the biology of prostate cancer.

Keywords: TMPRSS2-ERG fusion, prostate cancer cell, PRISM-SRM approach

POS-03-LB-016 An Experimental Platform for the Analysis of Mammalian Protein Interaction Networks and Ligand Receptor Interactions

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Cells receive clues about their extracellular environment trough specific cell surface receptors that can interact with peptides, proteins, drugs or even whole pathogens. These receptors then propagate their respective signal trough protein-protein interactions to trigger and modulate intracellular signaling pathways. Identifying the corresponding ligand-receptor interactions and understanding the organization of protein complexes and their associated protein interaction networks is becoming increasingly important to get new functional insight on cellular processes. Here, we describe Captivate, an integrated workflow to characterize mammalian protein complexes and LRC, a platform to elucidate target receptors of peptide and protein ligands. We use these two platforms to study ligand receptor and receptor protein interactions in the BMP signaling pathway. In particular we focus on ACVR1/Alk2 receptor kinase and FKBP12; two proteins intimately linked to Fibrodysplasia Ossificans Progressiva (FOP) a catastrophic human disorder leading to heterotopic ossification (HO) throughout the body. Our strategy is well suited to identifying protein interaction partners that can subsequently be used as (1) entry points for focused research projects and (2) to globally map out whole signaling systems linked to important biological processes.

Keywords: protein-protein interaction, ligand receptor capturing, PTM

POS-03-LB-017 Bottom-Up Mass Spectrometry Reveals Biomarker Candidate Proteins in Cerebrospinal Fluid of Patients Treated with Electric Spinal Cord Stimulation for Severe Chronic Pain

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Chronic neuropathic pain causes significant suffering for patients globally and large costs for societies**.** Spinal cord stimulation (SCS) gives pain relief for 60-70% of patients with otherwise treatment-resistant neuropathic pain. For these SCS responders, SCS is in many ways an ideal treatment. However, SCS typically only relieves pain partially, and is invasive, initially costly, not globally available and laborious (trial stimulation, surgical implantation, battery changes and sometimes lead migration correction surgery). Though successfully used since the 1960s, the SCS pain relieving mechanism in neuropathic pain remains unknown. It is plausible that the cerebrospinal fluid (CSF) mirrors mechanism relevant molecular changes taking place in the brain/spinal cord during SCS. Knowing the mechanism could lead to novel ideas for pharmacological interventions against neuropathic pain, and improve current SCS treatment and patient selection. CSF samples were collected from SCS-responsive neuropathic pain patients (n=12) at two separate occasions. 1) when the SCS had been off for 48 h 2) when the SCS had been used normally for three weeks. The proteomes of these off- and on-state samples from each patient were relatively quantified using mass spectrometry in a bottom-up proteomic approach. Seven proteins, 5 up-regulated and 2 down-regulated, were found to be significantly altered by SCS ($P \leq 0.01$). This result shows that assumption-free longitudinal proteomic investigation of patient CSF samples can generate novel leads and bring understanding of therapeutic mechanisms in humans.

Keywords: neuropathic pain treatment, mechanistic protein biomarkers, mass spectrometry

POS-03-LB-018 Analysis of the Liver Non-Parenchymal Cells Proteome in Response to Ethanol: Novel Molecular Targets of Disease

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The development of alcoholic liver disease (ALD) is a complex process involving both the parenchymal and non-parenchymal cells in the liver. The knowledge about the liver Non-parenchymal Cells (NPC) proteome in response to ethanol is very limited. Herein, we imployed comparative proteomics to analyze the regulation of alcohol to Non-parenchymal Cells during liver fibrosis development in rat model. In this study, rats were treated with alcohol to build a liver fibrosis model. Liver fibrogenesis was detected by pathological section staining by Masson staining. NPCs were enriched by percoll density gradient centrifugation. Proteins were separated by two-dimensional electrophoresis (2DE) gels stained by Coomassie Brilliant Blue (CBB) G-250, and the differentially expressed proteins were detected and identified by mass spectrometry. The expression level of some differential expressed proteins were verified by real time RT-PCR and Western Blot analysis. 26 protein spots with more than 2-fold difference were detected and identified by mass spectrometry. In which, 7 proteins were selected for real time RT-PCR analysis. NDUV2 was further verified by western blot in the alcoholic liver fibrosis rat model. This study detected and identified a group of differential proteins relate to alcoholic liver fibrosis, and might offer some new clues in understanding the mechanisms of alcohol-induced fibrosis.

Keywords: ethanol, non-parenchymal cells, liver fibrosis

POS-03-LB-019 Dissection of the Human GalNAc *O*-Glycoproteome: Mapping Specific Functions of Individual Polypeptide GalNAc-transferase Isoforms by Zinc-Finger Gene Engineering of Human Cells

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Posttranslational modifications (PTMs) greatly expand the function and regulation of proteins, and glycosylation is the most abundant and diverse PTM. Deficiencies in protein glycosylation lead to a number of severe diseases and multisystemic disorders. Recently we have begun to uncover more subtle disease phenotypes associated with deficiencies in glycosyltransferase genes that are members of large homologous gene families with partial redundancies. Thus deficiencies in protein *O*-GalNAc glycosylation, where the first initiation step is regulated by 20 distinct GalNAc-transferases, produce cell and protein specific effects and subtle distinct phenotypes such as hyperphosphatemia with hyperostosis and dysregulated high density lipoprotein cholesterol (HDL-C) and lipid metabolism. We uncovered a possible mechanism by which one of the 20 GalNAc-transferases, GalNAc-T2, co-regulates proprotein convertase (PC) processing and activation of the lipase inhibitor ANGPTL3, which can affect HDL-C and lipid plasma levels, both of which are heritable risk factors for coronary artery disease (CAD). More recently we have used Zinc-finger nuclease (ZFN) gene targeting of the human C1GalT1 chaperone *COSMC* to generate stable HepG2 "SimpleCells" with homogenous truncated GalNAc O-glycans in order to characterize the simplified O-glycoproteome. We have now applied this strategy to show non-redundant O-glycosylation performed by a single polypeptide GalNAc-T using differential analysis of O-glycoproteomes produced in an isogenic cell model with and without knock-out or knock-in of GalNAc-transferases. We have found several isoform specific substrates for GalNAc-T1, -T2 and -T3 that serves as potential biomarkers for disease caused by dysfunctional O-GalNAc-glycosylation and demonstrate that the human O-glycoproteome is differential and dynamic.

Keywords: glycoproteomics, GalNAc, O-glycosylation

POS-03-LB-020 Approaching Nanoflow Level Sensitivity Using Microflow Rates for Peptide Quantitation

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There has been a significant amount of research focused on discovering proteins/peptides that are differentially expressed in specific cell and disease conditions, increasing the need for better quantification strategies to confirm or refute their ultimate utility. Also required is increased throughput and robustness which means accelerated chromatography and/or higher flow rates. There has been increased interest in working in the microflow regime (10-20 uL/min) to obtain a good balance between throughput, robustness and sensitivity. Coupled with recent advances in QQQ technology that provides higher sensitivity detection, microflow chromatography could provide a step forward in both productivity and ease of use. Standard concentration curves were performed on a set of peptides to evaluate impact of separation time and flow rates on lower limits of quantitation (LLOQ) on two different hybrid triple quadrupole linear ion trap systems. Microflow LC (10 uL/min, 50mm 0.3mm i.d column) was performed on the QTRAP® 6500 system using a standard highflow source. The nanoflow LC experiments (300 nL/min, 15cm 75 uM i.d. column) were run on the QTRAP 5500 system using the nanoflow source and precut capillary spray tips. All concentration points were quantified using MultiQuant™ software platform.The average difference in sensitivity across the peptides analyzed between the nano flow and microflow experiments was within ~2x, while the increase in throughput was ~3x faster moving from nanoflow to microflow. This preliminary data demonstrates that using a more sensitive MS system allows higher throughput, more robust chromatography to be used for quantification with little loss in sensitivity. Further optimization of these low flow methods will be performed and more peptides and matrices will be explored to confirm how broadly this observation can apply.

POS-03-LB-021 Using Variable Widths in Q1 Selection Windows to Improve Data Quality in Data Independent Acquisition

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AB SCIEX

Recently, there has been resurgence in interest in data independent workflow (DIA) due to recent technological innovations in both MS hardware and software. This acquisition strategy can now be routinely applied to proteomic samples to collect high quality quantitative data on high numbers of peptides/proteins. In DIA, fixed-width Q1 windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution composite MS/MS spectra are acquired. The complexity of the MS/MS spectra depends on the number of peptides eluting off the column at the same time within the same m/z window. Here the impact of varied Q1 window width on quantitative data quality will be assessed. As a benchmark, data was acquired with standard SWATH acquisition (25Da window) and MRM workflow (1Da window). Reproducibility of replicates across a range of signal intensities was used as a measure of quantitation quality. The m/z density histograms were assessed for a number of proteomes and showed some variation. Acquisition methods for both fixed and variable window sizes were built for a range of window numbers (24, 30, 40 and 60). The cycle time was held constant, which means that the accumulation time spent on each window decreased with increasing window numbers. For variable window methods, we maintained constant complexity in each window by adjusting the Q1 width to maintain a constant MS1 sum in each window. As the window size decreased, more peptides were detected at a 1% false discovery rate due to improved S/N, especially for the lower abundant peptides. Variable windows improve reproducibility in quantification, due to improved signal/noise and correct peak detection. Further work will be done on multiple proteomes to fully characterize the impact and generate workflow guidelines.

POS-03-LB-022 Phosphoproteomic Analysis of Pancreatic Cancer Signaling Identifies Drug Targets for Individualized Patient Treatment

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LC-MS phospho-proteomics is fast becoming an essential technology to unravel the complex molecular events that lead to and propagate cancer. We have developed a global phospho-proteomic workflow to determine activity of signaling pathways and drug targets in pancreatic cancer tissue.

Peptides resulting from tryptic digestion of proteins extracted from frozen tissue of pancreatic ductal adenocarcinoma and background pancreas (twelve cases), were labelled with tandem mass tags (TMT 8-plex), separated by strong cation exchange chromatography, then were analysed by LC-MS/MS directly or first enriched for phosphopeptides using IMAC and TiO_2 columns, prior to analysis. In-house, commercial, and freeware bioinformatic platforms were used to identify relevant biological events from the complex dataset.

Of the 2,101 proteins identified, 150 demonstrated significant difference in abundance between tumor and non-tumor tissue. They included proteins that are known to be up-regulated in pancreatic cancer (e.g. Mucin-1), but the majority were new markers. Of the 6,543 unique phosphopeptides identified (6,284 unique phosphorylation sites), 635 showed significant regulation, particularly those on proteins involved in tight junction signaling. Hierarchal clustering of phosphopeptide relative abundance showed three patient groups, two of which separated based on recurrence. Signaling pathways associated with epithelial to mesenchymal transition and migration were significantly modulated. Activator phosphorylation sites on drug targets including; Fyn, Akt1, ERK2, HDAC1, GSK3-alpha, and Casein Kinase I epsilon, were found to be highly modulated (≥ 2 fold) differentially case by case.

Here we identify significantly modulated protein expression as well as determine activity of signaling pathways and drug targets in pancreatic cancer tissue. These phosphopeptide measurments separated cases based on time of recurrence and may help stratify patients into different treatment regimens.

Keywords: phosphoproteomics, pancreatic cancer, drug target activity

POS-03-LB-023 EDEM2 and OS-9 are Required for the ER-Associated Degradation of Nonglycosylated Sonic Hedgehog Proteins

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Misfolded secretory pathway proteins are detected and eliminated by the ER-associated degradation (ERAD). ER-resident lectins mediate substrate recognition in *S. cerevisiae* through bipartite signals consisting of unfolded local structure and adjacent glycans. Trimming of the glycans is essential for the directional delivery of the substrates. Whether similar recognition and delivery mechanism exists in mammalian cells is unconfirmed. Here we show that human sonic hedgehog (SHH) protein is degraded efficiently by ERAD requiring HRD1, SEL1L and p97, in a process independent of the presence of glycans. Moreover, the ER lectins EDEM2 and OS-9 are required for ERAD of both glycosylated and non-glycosylated SHH, while EDEM3 is only required for ERAD of glycosylated SHH. Interaction of EDEMs and OS-9 with SHH occurs independently of either the substrate glycan or the lectin-like domain, which is different from their counterpart in *S. cerevisiae.* Our study shows that EDEM2 is required for ERAD of both glycosylated and non-glycosylated SHH protein, and it has the ability to interact with these substrates independent of the glycan on the substrates or its glycan-binding domain. The study reveals distinct property of these mammalian ER lectins in ERAD through recognition of misfolded polypeptide segment, and the key roles EDEM2 and EDEM3 play in ERAD of SHH proteins.

Keywords: ER-associated degradation, EDEM, sonic hedgehog

POS-03-LB-024 Derlin2 Facilitates HRD1-Mediated Retro-Translocation of Sonic Hedgehog at the Endoplasmic Reticulum

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Endoplasmic reticulum-associated degradation (ERAD) is an important system that eliminates misfolded proteins from the ER. Three derlins have been implicated in this process, but their precise function remains unknown. In this study, we report that although both derlin1 and derlin2 are capable of binding the ERAD specific ubiquitin ligase HRD1 complex, they associate with the HRD1 complex with different affinity. Accordingly, these derlins have divergent and non-redundant functions in ERAD with derlin2 being the primary functional partner for HRD1. We show that derlin2, but not derlin1 or derlin3, is essential for ERAD of both glycosylated and non-glycosylated SHH-C, as well as NHK. Derlin2 appears to act at a post-targeting step for HRD1-dependent retro-translocation. Without derlin2, the assembly of HRD1 into a functional retro-translocation homo-oligomer proceeds normally, and substrate targeting to the HRD1 complex also occurs. However, the ERAD substrate SHH-C is largely trapped inside the ER lumen. These observations raise the possibility that derlin2 may regulate the movement of substrates through the HRD1-containing 'retro-translocon'. Our study is the first to report that derlin2 functions specifically with HRD1 in ERAD of certain substrates independent of their glycosylation status. The mammalian ERAD system may require multiple derlins that each functions with a distinct E3 partner to eliminate a specific subset of substrates. This is different from the model in *S. cerevisiae*, in which Hrd1p alone is sufficient for retro-translocation.

Keywords: derlin, ER-associated degradation, sonic hedgehog

POS-03-LB-025 AGSY: Automatic Glycopeptide Sequencing by Y1 Ion

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The major challenge in glycopeptide identification is the lack of peptide fragments when glycopeptides are subjected to the collision type of tandem mass spectra detection, such as CID or HCD. Although the ETD technique can fragment glycopeptide; however, its poor fragmentation efficiency on glycopeptide results in the difficulty for amino acid sequence identification. To improve upon this issue, a new strategy, automatic glycopeptide sequencing by Y1 ion (AGSY) has developed. AGSY is based on a three-stage mass spectra acquisition for enzyme-digested glycopeptides and capable to reveal the glycopeptide structure by sequential mass detections which include the automatic assignment of glycopeptide Y1 ion detection followed by the Y1 ion fragmentation in order to determine amino acid sequence of glycopeptides. The first stage is a full range mass scan to determine the molecular weight of ions which will be subjected for the second stage mass spectrum acquisition, the Trap-HCD induced MS^2 detection. The third stage acquisition is the CID-induced MS^3 detection with the precursor ion selected from the automatically determined glycopeptide Y1 ion in the MS^2 spectrum. The glycosylated residues as well as the amino acid sequences of glycopeptides are identified from MS^3 results. Bovine fetuin was utilized as a model glycoprotein for AGSY analysis. We have demonstrated the identification of N-type and O-linked sites of fetuin with a newly found O-linked glycosylation site T295. Glycan compositions were determined by GlycoWorkBench based on the molecular weight of each glycan calculated from the precursor ion signal (MS) and glycopeptide Y1 ion signal (MS^2). Moreover, the extracted ion chromatogram of each glycopeptide provides the quantitation results in AGSY. In summary, AGSY has demonstrated to be a novel platform for comprehensive glycopeptide analysis including simultaneously glycosylation site identification as well as amino acid sequence and glycan composition determination for each glycopeptide.

Keywords: glycopeptide identification

POS-03-LB-026 Quantitative Proteomic Analysis of Human Lung Tumor Xenografts Treated with the Ectopic ATP Synthase Inhibitor Citreoviridin

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ATP synthase is present on the plasma membrane of several types of cancer cells. Citreoviridin, an ATP synthase inhibitor, selectively suppresses the proliferation and growth of lung cancer without affecting normal cells. However, the global effects of targeting ectopic ATP synthase *in vivo* have not been well defined. In this study, we performed quantitative proteomic analysis using isobaric tags for relative and absolute quantitation (iTRAQ) and provided a comprehensive insight into the complicated regulation by citreoviridin in a lung cancer xenograft model. With high reproducibility of the quantitation, we obtained quantitative proteomic profiling with 2,659 proteins identified. Bioinformatics analysis of the 141 differentially expressed proteins selected by their relative abundance revealed that citreoviridin induces alterations in the expression of glucose metabolism-related enzymes in lung cancer. The up-regulation of enzymes involved in gluconeogenesis and storage of glucose indicated that citreoviridin may reduce the glycolytic intermediates for macromolecule synthesis and inhibit cell proliferation. Using comprehensive proteomics, the results identify metabolic aspects that help explain the antitumorigenic effect of citreoviridin in lung cancer, which may lead to a better understanding of the links between metabolism and tumorigenesis in cancer therapy.

Keywords: quantitative proteomic analysis, ectopic ATP synthase, human lung tumor xenografts

POS-03-LB-027 Application of Survival Analysis Methodology to the Quantitative Analysis of LC-MS Proteomics Data

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Protein abundance in quantitative proteomics is often based on observed spectral features derived from LC-MS experiments. LC-MS data frequently have large proportions of missing peak intensities due to censoring mechanisms on low-abundance spectral features. Recognizing that the observed peak intensities detected with the LC-MS method are all positive, skewed and often left-censored, we propose using survival methodology to carry out differential expression analysis of proteins. Various standard statistical techniques including non-parametric tests such as the Kolmogorov-Smirnov and Wilcoxon-Mann-Whitney rank sum tests, as well as the parametric survival model, accelerated failure time model with the Lognormal,Weibull and Loglogistic distributions were used to detect any differentially expressed proteins. The statistical operating characteristics of each method are explored using both real and simulated data set.

Keywords: LC-MS proteomics, left censoring, missing data

POS-03-LB-029 Towards the Proteome Profiling and Signature of Glioblastoma

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High grade glioma (glioblastoma) is the most common brain tumor. Its malignancy makes it the fourth biggest cause of cancer death. In our experiments we used several glioblastoma cell lines generated in our laboratory to obtain proteomics information specific for this disease. This study starts our developing the complete 2DE map of glioblastoma proteins. 2DE separation with following imaging, immunochemistry, spot picking, and mass-spectrometry allowed us detecting and identifying more than 100 proteins. Several of them have prominent differences in their level between norm and cancer. Among them are alpha-enolase (ENOA_HUMAN), pyruvate kinase isozymes M1/M2 (KPYM_HUMAN), coffilin 1 (COFF1_HUMAN), translationally-controlled tumor protein TCTP_HUMAN, annexin 1 (ANXA1_HUMAN), PCNA (PCNA_HUMAN), p53 (TP53_HUMAN) and others. Most interesting results were obtained with protein p53. In all glioblastoma cell lines, its level was dramatically up regulated and enriched by multiple additional isoforms. What is more, exosomes purified from glioblastoma (not normal cells) cultural media also contain p53 and PCNA. This distribution is well correlated with presence of these proteins inside of cells themselves. At this initial step we suggest the panel of specific brain tumor exosomal markers (signature) to help creating noninvasive techniques to diagnose disease. These preliminary data point to these proteins as promising markers of glioblastoma.

Keywords: proteome, glioblastoma, signature

POS-03-LB-028 Y Chromosome Genes Expression Profiling During Differentiation of NT2 Cells to Dopaminergic Neurons Under Androgen Treatments

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We investigated the expression pattern of human Y chromosome genes during NT2 cells to neural cells using combination of Dihydroepianostrone (DHEA) as a neurostroied. Quantitative Real-Time PCR analysis showed that expression of neural progenitor markers such as Pax6 and TUJ1were significantly up-regulated under defined treatments. In particular, PTX3 and TH expressions as dopaminergic neurons markers significantly increased under DHEA+RA treatment. Several Y chromosome genes including RBMY,HSFY,DDX3Y,BPY2 and CDY were detected only during differentiation and in neural progenitor. Furthermore, marked increase of several Y chromosome genes at mRNA level including HSFY, RBMY, CDY, DDX3Y,BPY2 was also observed. Western blot analysis also confirmed the accumulation of Y chromosome proteins in neural progenitor compared to NT2. These include HSFY, RBMY, BPY2, DDX3Y. DHEA treatment also affected the expression of Y chromosome genes; for instance expression of SRY and ZFY decreased significantly in neural progenitor cells. Overall, our results suggest a possible contribution of Y chromosome genes in neural development. Studies to elucidate the roles of these genes during neural differentiations are in progress.

Keywords: chromosome Y, dopaminergic neurons, DHEA

POS-03-LB-030 Two Dimensional Electrophoresis (2DE) Based Approach for Detecting of Numbers of Protein Species in Cell

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After completion of the Human GenomeProject and obtaining information about all human genes, there is the similar task in proteomics - inventorying of all human proteins. Because of very wide distribution of protein copies inside cell (from millions to single copies) this is very challenging. Insufficient sensitivity of methods for detection of proteins at a single molecule level does not yet allow obtaining the whole image of human proteome. But to study the human proteome, we need at least to know its size, or how many different protein species compose this proteome.

Using the available technologies it is possible only to estimate not to calculate this size. This is the task that could be at least partially realized by the method described in this presentation. The size of cell proteome was estimated by the approach of protein staining in 2-D gels using dyes with different sensitivity. Using approximation to maximum sensitivity (detection of a single smallest polypeptide), it was estimated that the minimal numbers of protein species for model objects, *E. coli* and P.furiosus, were estimated to be 6000 and 3400, respectively. A single human cell (HepG2) we expect to contain minimum 70,000 protein species.

Keywords: 2DE, proteome, size

POS-03-LB-031 Prognostic Biomarkers for Predicting Metastatic Relapse versus Non-Relapse Hepatocellular Carcinoma

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The natural course of early HCC is unknown and its progression to intermediate and advanced HCC can be quite diverse. Some early stage HCC patients enjoy disease-free survival whilst others suffer aggressive relapse to Stage IV metastatic cancer within a year. Thus, prognosis determination and indication for treatment are critical in the clinical management of HCC patients. In this study, comparative proteomics of HCC tumour tissues was carried out using 2D-DIGE and MALDI-TOF/TOF MS to identify proteins that can distinguish these two groups of Stage I HCC patients. Among the 148 differentially expressed protein spots, four proteins, namely heat shock 70 kDa protein 1 (HSP70), argininosuccinate synthase (ASS1), isoform 2 of UTP-glucose-1-phosphate uridylyltransferase (UGP2) and transketolase (TKT), were identified to have the potential to differentiate metastatic relapse (MR) from non-relapse (NR) HCC patients after validation by western blotting and immunohistochemical assays. TMA analysis showed that only HSP70, ASS1 and UGP2 were significant in the prediction of the two groups of HCC patients. HSP70 alone (AUC of 0.708; sensitivity = 80.5%; specificity = 63.0%) was shown to be the best prognostic biomarker, but its sensitivity increased to 90% when combined with ASS1 as a panel of 2 biomarkers. In addition, ASS1 and UGP2, as a pair has a higher specificity of 76.7% as compared to their use as a single biomarker. Hence, these two biomarker combinations when jointly used as prognostic biomarkers can offer greater sensitivity and specificity in identifying metastatic relapse (MR) from non-relapse (NR) HCC patients.

Keywords: hepatocellular carcinoma, relapse, prognostic biomarkers

POS-03-LB-032 A Novel Oncogenic Gene, L-FABP, Promotes Cancer Cell Migration and Invasion by Inducing the Expression of VEGF-A and MMP-2

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Hepatocellular carcinoma (HCC) is one of the most common human cancers and the second cause of cancer-related deaths in Taiwan. HCC is notoriously resistant to systemic therapies, and often recurs even after aggressive local therapies. HCCs rely on the formation of new blood vessels for growth, and VEGF is critical in this progress. Oncogenic gene, liver type fatty acid binding protein (L-FABP), was shown to be highly expressed in liver cancer. In the present study, we observed that L-FABP increased the migration and invasion level of HEK293 and immortalized human hepatocyte (Hus) cells by up-regulating the expression level of VEGF-A and MMP-2. Knockdown of L-FABP in HEK293/L-FABP stable clones or Huh7 cells resulted in reduced migration and invasion. L-FABP also promoted tumor growth and metastasis significantly by xenograft analysis. The mechanisms of enhancing tumor growth by L-FABP was found that L-FABP physically associated with VEGFR2 on membrane lipid rafts, and then activated Src/ PI3K/ Akt/ mTOR/ HIF-1 α pathway, leading to the up-regulation of MMP-2 and VEGF-A followed by promoting the tumor growth and metastasis significantly. The results of this study suggest that L-FABP could be a potential target for hepatocellular carcinoma chemotherapy.

Keywords: HCC, L-FABP, VEGF-A, migration, metastasis

POS-03-LB-033 iPhos: A Tool Kit to Streamline the Label-Free LC-MS-Based Tyrosine Phosphoproteome Investigation

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Protein phosphorylation is a vital post-translational modification in both normal and diseased cells. And the low abundance of tyrosine phosphorylated (P-Tyr) peptides among the total phosphopeptides in biological samples remains an challenge in mass spectrometry-based proteomic analysis. Herein, we have demonstrated an alternative analytic strategy to confidently identify tyrosine phosphorylated peptides by using the alkaline phosphatase treatment combined with high-resolution mass spectrometry. While the process is applicable, the key integration along the pipeline was mostly done by tedious manual work. Therefore, we developed a software package, iPhos, to facilitate and streamline the workflow for comprehesive tyrosine phosphoproteome characterization. The iPhos package includes three modules. iPhos Module-1 can process liquid chromatography mass spectrometry (LC-MS) analysis derived from phosphorylated and dephosphorylated samples prepared under different conditions to select the possible tyrosine phosphorylated peptide signals. iPhos Module-2 provides customized inclusion lists divided by peak retention times for subsequent targeted LC-MS/MS analysis. iPhos Module-3 combines the peptide identifications and quantification results from pattern-based label-free quantification tools. The utility of iPhos was demonstrated by analyzing the tyrosine phosphoproteome of CL1-0/CL1-5 lung cancer cells, resulting 302 p-Tyr peptides which corresponded to 335 p-Tyr sites. Among them, 36 p-Tyr proteins showed altered levels between CL1-0 and CL1-5 cells. From this list of proteins, 7 P-Tyr proteins have not been previously reported to be associated with lung cancer metastasis. The application of iPhos provided a comprehensive understanding the role of tyrosine phosphorylation in lung cancer metastasis.

Keywords: tyrosine phosphoproteome, targeted LC-MS/MS analysis, label-free quantitative proteomics analysis

POS-03-LB-034 Impact of Human Blood Specimen Collection, Manipulation, and Storage on Protein Integrity and Implications for Use in Clinical Research

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Caprion Proteomics US LLC, USA

Human plasma and serum proteins are a promising source of clinically relevant disease biomarkers. However, comprehensive guidelines for handling these specimens are lacking and their collection, manipulation and storage protocols are based mainly on accepted practices rather than careful comparative analysis and testing. We investigated the impact of collection tube types, incubation time and temperature before and after centrifugation, freeze/thaw cycles, and freezer storage time and temperature on protein integrity using proteomics approaches. Plasma and serum were collected from volunteers under well controlled conditions, subjected to MARS-14 depletion medium, digested with trypsin and analyzed by LC-MS for discovery studies. Verification was performed using a highly multiplexed MRM assay. Data show that incubation of blood was more deleterious than incubation of serum or plasma, suggesting that centrifugation of blood tubes after collection is critical for sample intergity. The P100 protease inhibitor tube resulted in the fewest changes over incubation time. Increasing number of freeze/thaw cycles affected specific proteins only and the P100 and serum tubes with mechanical separators were found to be most protective against freeze/thaw cycles. Some proteins show changes upon freezer storage for 6 months, with smaller changes after 18 months. Overall, there were more changes at -20 ˚C than at -80 ˚C. The results provide extensive information on pre-analytical variables and clear guidelines for sample handling for proteomic analysis. The information collected is being used to establish an MRM assay for the analysis of stored samples in order to determine sample quality and utility in clinical research.

Keywords: sample integrity, biomarker, clinical research

POS-03-LB-035 Utilized Mass Spectrometry-Based Protein Profiling System to Identify Potential Biomarkers of Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the most common malignant liver tumor. The purpose of this study is to characterize proteins secreted from the HepG2 cells, which may relate to cell differentiation and tumor metastasis. In the proteomic analysis, the secretome was identified by nano-HPLC/ESIMS/MS followed by peptide fragmentation pattern analysis. In this study, three proteins, p130Cas, TDP43 and TCTP, were identified and confirmed by Western blotting, which showed significant differential expression compared with the normal liver cells. Analyzing differential protein expressions of HepG2 cell by proteomic approaches identified p130Cas, TDP43 and TCTP as key proteins and may serve as biomarkers for HCC.

Keywords: hepatocellular carcinoma, proteomics, tumor maker

POS-03-LB-036 Is NAP an Additional Neuroprotection in Alzheimer's Disease?

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Alzheimer's disease (AD) is the most common cause of dementia of late life. The objective in utilizing proteomic techniques of this study was to identify protein biomarkers associated with NAP (NAPVSIPQ, amino acid sequence from 354 to 361) treated with human neuroblastoma cells SH-SY5Y. Experimental results suggested that stathmin was a protein marker for NAP-induced neuroprotective activity in neuroblastoma cells. Thus, the use of NAP is suggested for treatment as an additional protection in Alzheimer's patients.

Keywords: Alzheimer's disease, NAPVSIPQ, stathmin

POS-03-LB-037 Identification of Specific Epitopes for West Nile Virus Diagnostics

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The zoonotic West Nile Virus (WNV) belongs to the family Flaviviridae, similar to Yellow Fever Virus or Japanase Encephalitis Virus. Most WNV infections result in no or flu-like symptoms. In some cases, especially in older or immunocompromised persons, severe neurological disease (such as encephalitis or meningitis) can develop. The serologic detection of WNV infections is hampered by the cross-reactivity of antibodies against related flaviviruses. In the context of the EU-funded WINGS project, we studied the human antibody response to WNV. The entire WNV proteome (structural and non-structural proteins) were expressed as a series of overlapping fragments fused to a carrier-protein. Using this library we are performing a serologic screen by studying antibody binding of sera from humans infected with WNV and other flaviviruses. The results demonstrate that, although the humoral immune response to WNV in humans is heterogeneous, several dominant peptides are recognized. In addition, the data indicate that some peptide sequences can be used for the development of a specific serologic WNV test as they show only marginal cross-reactivity with antibodies against related flaviviruses.

Keywords: West Nile Virus proteom, flaviviruses

POS-03-LB-038 Elucidation of Structure-Activity Relationship of Recombinant Human Kinases by Quantitative Phosphoproteomics

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Protein kinase regulates a variety of cellular functions via protein phosphorylation-based signal transduction. It is known that kinase itself is modified with phosphorylation at multiple sites with different occupancy, resulting in the activation/inactivation in some cases. For most kinases, however, details of phosphorylation-activity relationship are unknown. In this study, we employed recombinant human kinases with different phosphorylation status, and analyzed their phosphorylation sites using mass spectrometry. In addition, the kinases were reacted with de-phosphorylated HeLa cell lysates, and the *in vitro* substrates were analyzed by quantitative shotgun phosphoproteomics based on phosphopeptide enrichment followed by nanoLC-MS/MS.

At first, we employed two tyrosine kinases, Bruton's tyrosine kinase (BTK) and anaplastic lymphoma kinase (ALK). ATP treated and non-treated samples were prepared for each kinase. LC-MS/MS analyses following trypsin digestion were carried out to examine the phosphorylation status of these kinases. As a result, 48 and 9 phosphorylation sites were totally identified for BTK and ALK samples, respectively. Among them, 11 and 7 phosphorylation sites were exclusively identified for the ATP-treated samples of BTK and ALK, respectively. Next, *in vitro* kinase assay with HeLa cell extracts was performed. Consequently, approximately 800 phosphopeptides for each ATP-treated kinase sample were successfully identified. On the other hand, 42 and 128 phosphopeptides were identified for non-treated BTK and ALK samples, respectively, indicating that ATP-induced phosphorylation activated both kinases. The obtained information would be useful to investigate the phosphorylation-activity relationship both *in vitro* and *in vivo*.

Keywords: phosphoproteomics, LC-MS/MS, recombinant human kinases

POS-03-LB-039 Antibodypedia - How to Find the Right Antibody for the Right Application

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Antibodies are useful tools to characterize the components of the human proteome and to validate potential protein biomarkers discovered through various clinical proteomics efforts. The lack of validation results across various applications for most antibodies often makes it necessary to perform cumbersome investigations to ensure specificity of a particular antibody. A need therefore exists for a standardized system for sharing validation data about publicly available antibodies and to allow antibody providers as well as users to contribute and edit experimental evidence data, including data also on the antigen. Here we describe a publicly available portal called Antibodypedia, originally developed within the 6th framework EU program Proteome Binders and now an integrated part part of the HUPO Antibody Initiative (HAI). The Antibodypedia initiative is hosted at the Science for Life Laboratory by the Royal Institute of Technology, Stockholm, Sweden. Antibodypedia is a searchable database containing annotated and scored affinity reagents. It aims to provide the research community with information to aid in selecting antibodies tailored to specific biological and biomedical assays. Today it holds almost 660 000 reviewed antibodies covering approximately 90% of all human genes, and the antibodies are supported by primary data from over 386 000 experiments and over 74 000 references. Based on the amount of supporting data available, e.g. validation experiments and references, antibodies in the database are ranked. In this way the researchers are presented with easily accessible quality control data and can make a competent selection of the right antibody for the right application.

Keywords: antibodypedia, antibody search, database

POS-03-LB-040 Membrane Phosphoproteomics Profiling for the Elucidation of Molecular Dynamics during Sperm Capacitation

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The spermatozoa are not capable fertilizing ability before entering the female genital tract. To active the fertilizing ability, the spermatozoa require to undergo activation process called capacitation. Although more than 50 years have passed since sperm capacitation was reported and the physiological functions in a variety of mammalian species were studied, it is noteworthy that the molecular basis of this process is still today far from completed.

In this study, to have a more understanding for the dynamics of sperm membrane proteome during capacitation, the quantitative phosphoproteomics study of the sperm membrane was performed. We have combined detergent based membrane protein extraction and IMAC to analyze the membrane proteome as well as the phosphoproteome in mouse sperm. For the quantitation purpose, we also develop a straightforward method to quantify the phosphorylation process during sperm capacitation that may provide some cue for understanding of the physiological mechanism. To our knowledge, this is the first report for the labeling free quantitation approach of the phosphoproteomics of sperm membrane during capacitation process. In this study, several sperm membrane protein which may associate with hyperactive motility, ion flux control, acrosome reaction and sperm-egg interaction were discovered. In addition by the quantitation of the protein phosphorylation before and after the capacitation process, several phosphorylation events were discovered to be differentially expressed. Those phosphorylation sites provide excellent candidates for the site direct mutagenesis for the study of sperm function.

This approach provides a site specific quantitation result for the protein phosphorylation during sperm cell capacitation. The results will help to improve our understanding of the protein phosphorylation that associates with hyperactivited motility and redistribution of the membrane component during capacitation.

Keywords: sperm, membrane proteomics, phosphoproteomics, capacitation

POS-03-LB-041 One-Shot Human Proteome Analysis by nanoLC-MS/MS with Meter-Scale Monolithic Silica Capillary Columns

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Despite the rapid progress in mass spectrometry-based shotgun proteomics, it is still difficult to uncover the entire human proteome. Recently, we reported the complete *E. coli* proteome analysis by a nanoLC-MS/MS system using a meter-scale C18 monolithic silica column under shallow gradient conditions without pre-fractionation [Iwasaki et al., Anal Chem 2010]. We further extended this 'one-shot proteomics' approach to human induced pluripotent stem cell (iPSC) proteome, and identified 98,977 non-redundant tryptic peptides from 9,510 proteins (corresponding to 8,712 genes), including low-abundance protein groups (such as 329 protein kinases) from triplicate measurements of five iPSCs and three fibroblasts within 10 days [Yamana et al., JPR 2013]. Here, we investigated the effects of the column length and the gradient time on the identification number and the total analysis time to maximize the efficiency of the one-shot proteomics workflow.

Tryptic peptides from HeLa cell were used as a test sample, and two MS instruments, Q-TOF (ABSciex TripleTOF 5600) and Q-orbitrap (Thermo Q-Exactive), were used through this study. In general, longer columns with shallower gradient provided higher identification numbers of peptides and proteins. Increasing the injected amount was also effective to increase the identification number. However, these effects were less significant when the identification number of proteins was over 6,000. In this presentation, we will propose the optimized conditions of this one-shot approach using 2 - 6 meter C18 monolithic silica capillary columns.

Keywords: monolith

POS-03-LB-042 On-Line Chip-Based Strategy for 2D Fractionation - Comparing Peptides Found between 1D and 2D Proteomic Analysis

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On-line two-dimensional (2D) liquid chromatography is widely used for protein identification and quantification using the advantage of increasing peak capacity. A common workflow is the combination of strong cation exchange (SCX) as an orthogonal first dimension followed by analytical reversed-phase (RP) chromatography. A more recently developed strategy has emerged where the first dimension is a high pH RP separation, which provides higher peak capacity. 2D workflows are essential for measuring lower abundant proteins in complex proteomes, however are complex to implement. A simplified chip based 2D-LC workflow has been developed using a high pH/RP first dimension and low pH RP secondary dimension coupled directly to the MS for proteomic analysis. Detailed analysis of detected peptides between all the separation strategies was performed.

E. coli cell lysates were reduced and alkylated with iodoacetamide, before being subjected to tryptic digestion. Online 2D-LC workflow was performed using ekspert™ nanoLC 425 system (Eksigent). Each fraction was then separated with a 75 μm x 15cm C18 cHiPLC column at 300 nL/min and analyzed with TripleTOF® 5600 system (AB SCIEX).

A chip based LC system was configured to simplify the on-line 2D RP-RP workflow. The workflow was applied to the analysis of *E. coli* cell lysates and a variety of workflow comparisons were performed to assess impact on proteomic workflows (1D, 2D-6 fractions and 2D-10 fractions and multiple sample loads). Using 1ug of *E. coli* digested cell lysates, there is a 1.8x and 2.1x increase in the identification numbers for the 2D-6 and 2D-10 fractions respectively, versus the 1D experiment at the peptide level (5% local FDR). When the loading amount was increased by 10x, the number of detected peptides increased by 3.3x and 4x for the 2D 6 and 10 fraction workflows, respectively, over the 1D workflow. Further optimization of both the first and second dimension is ongoing to further improve the peptide detection rates.

Keywords: proteomics, two-dimensional (2D) fractionation, reversed-phase chromatography

POS-03-LB-043 Proteomic Analysis of Proteins Involved in Apoptosis during Leukaemic Cells Treatment with HDACI BML-210

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The clinical benefits of histone deacetylase (HDAC) inhibitors in differentiation therapy are under very active investigation. The HDAC inhibitors inhibit proliferation and stimulate differentiation and apoptosis in transformed cells both *in vitro* and *in vivo*. In this study we did a proteomic analysis of how HDAC inhibitor BML-210 alone changes protein expression level, using the human promyelocytic leukemia cell line NB4 as a model. Indeed, in HDACI BML-20 significantly increase apoptotic cell number (up to 80 %) during the first two days of treatment. Also we detected activation of caspase 9 and caspase 3/8 pathways during apoptosis after 20 microM BML-210 treatment. The proteins for proteomic analysis were isolated from untreated and BML-210-treated cells, fractionated with 2DE and further trypsinized. The peptides were chromatographically separated using Agilent 1100 HPLC system with the flow splitter and analyzed by electrospray ionization MS in positive ionization mode using the ion trap "HCTultra PTM Discovery System". We identified that PCNA protein was downregulated after treatment with 20 microM BML-210 and can be involved in a DNA synthesis and reparation processes during cell proliferation and apoptosis. Also we found few proteins that were upregulated in BML-treated cells: LGUL protein (involved in regulation of NF-kappa-B activity), RANG protein is involved in cell cycle regulation and CLIC1, EFHD proteins are important in apoptosis.Taken together, our results suggest that the HDACI BML-210 can affect proteins that important for proliferation and apoptosis of the leukemic cells. This could be important to find new targets in cancer treatment.

Keywords: leukemia, HDAC inhibitor, BML-210

POS-03-LB-045 Glycopeptide and Glycan Analysis of Monoclonal Antibodies Using Capillary Electrophoresis Electrospray Ionization Mass Spectrometry

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Glycosylation of monoclonal antibodies (mAb) is an important post-translational modification and changes in glycosylation pattern have significant impact on biological activity and immunogenicity. Various analytical techniques have been widely used to profile glycans attached to mAb. Recently capillary electrophoresis - mass spectrometry (CE-MS) has gained much attention in analyzing the glycans. In the present work, CE-MS technique was employed for characterization of monoclonal antibody glycosylation pattern at both at glycopeptide and free glycan levels. Monoclonal antibody was subjected to tryptic digestion and deglyosylation treatment with trypsin and PNGase F respectively. The tryptic peptide map of the mAb was generated and the glycopeptide was assigned using accurate mass measurement on a Q-TOF mass spectrometer. In addition, CE-MS/MS analysis was performed to search for diagnostic oxonium ions generated from a glycan moiety to identify the glycopeptides. On the other hand, released glycans were labeled with 8- aminopyrene-1,3,6-trisulfonate (APTS) and analyzed by CE-MS. Analysis of total ion electropherogram reveals the presence of several N-glycan moieties present on mAb (uncharged N-linked glycan species G0, G0F, G1, G1F, G2 and G2F and mono-sialylated glycan moiety G2F+1NANA) were identified in replicate runs. In addition, the relative percentage of each glycan shows the major and minor forms of the glycan present on this mAb. The powerful data processing capabilities of Agilent MassHunter and BioConfirm suite enable in successful and detailed identification/profiling of the glycoforms of monoclonal antibody. These results clearly indicate that CE-MS can be used effectively as an alternative analytical tool to monitor N-glycan profiles of mAb.

Keywords: monoclonal antibodies, N-glycans, CE-MS

POS-03-LB-044 In-Depth 2-DE Reference Map of a Medically Important Fungus, *Aspergillus fumigatus*, and Proteomic Profiling on Exposure to Itraconazole

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Aspergillus fumigatus (*A. fumigatus*) is a saprophytic, ubiquitous and opportunistic fungus, which leads to allergic or invasive aspergillosis in humans depending on the immune status of the host. Proteomic profiling of this medically important fungus may lead to better understanding on the proteins and molecular pathways functional in *A. fumigatus*. Proteome analysis using 2-DE followed by mass spectrometric analysis (MS/MS) led to the identification of 259 unique proteins from 370 protein spots. In order to understand molecular targets of itraconazole (ITC), a standard antifungal drug, we analyzed proteomic profile of *A. fumigatus* on exposure to minimum inhibitory concentration (MIC_{50}) of ITC, 0.154 μg/ml. Proteomic profiles of *A. fumigatus* treated with ITC showed modulation of 175 proteins (66 upregulated and 109 downregulated) as compared to the control. Peptide mass fingerprinting led to the identification of 54 proteins- 12 up-regulated and 42 down-regulated proteins. The differentially expressed proteins belong to cell wall maintenance, cell stress, transport proteins, oxidative phosphorylation and others including hypothetical proteins. We compared the differentially expressed proteins on exposure to ITC with that of other standard antifungal drugs, AMB and CSF, and ART, an antimalarial compound with antifungal activity, to identify the common targets of these drugs. We found that the molecular effects of ITC and ART were more similar, which substantiates our earlier observation that ART has synergistic effect with ITC (Gautam *et al*., 2012). Some of the common targets of ITC, ART and AMB include phosphoglycerate kinase PgkA and methyltransferase SirN-like protein. These and other proteins targeted by ITC may serve as important leads for development of new antifungal drugs.

Keywords: *Aspergillus fumigatus*, Itraconazole, Proteomics

POS-03-LB-046 Novel Proteomic Biomarkers in the Evaluation of Childhood Asthma

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Rationale:Clinically we lack reliable tools to evaluate the severity of childhood asthma. **Aims:** To find asthma related biomarkers by proteomic methods. **Methods:** using two dimensional differential gel electrophoresis techniques plasma samples of 48 stable asthmatic children (4 groups divided by disease severity according to GINA) and 12 healthy control children were separated and screened for differential expression of proteins. The candidate proteins were identified by Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF-TOF). The candidate markers were further validated by ELISA methods in another larger pediatric population (93 stable asthmatic children and 28 healthy controls). **Results**: by using proteomic techniques, 36 proteins were found differentially expressed ($p<0.05$) between 4 asthmatic groups and healthy control group, 20 proteins were identified by Mass Spectrometry which represent set of 8 proteins. Further validation of these proteins indicated four biomarkers (AT-III, A2M, CD5L, C3) showed differential expressions between different groups. AT-III, C3, A2M were differentially expressed between asthmatic and healthy control group ($p<0.05$) ; among the different asthmatic groups, A-TIII showed a trend of raising up with disease severity (ANOVA $p<0.05$) , A2M and CD5L showed reversed trends with disease severity (ANOVA, $p<0.05$, $p<0.01$). AT-III had negative correlations with A2M (r=-0.252, $p<0.05$), CD5L (r=-0.212, $p<0.05$), and FEV1%/FVC% (-0.330, $p<0.01$), respectively; CD5L had positive correlations with A2M (r=0.243 , $p<0.05$) and FEV1%/FVC% (r=0.273, $p<0.01$). **Conclusions:** Proteomics is a useful method in identify asthma related biomarkers. A panel of four biomarkers (CD5L, AT-III, A2M, and C3) relates closely with childhood asthma disease severity. The combination of the four biomarkers can be a powerful tool in evaluating and monitoring childhood asthma progress.

Keywords: asthma, children, biological markers

POS-03-LB-047 Enrichment of Protein N-Terminal Peptides for Shotgun Proteogenomics

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The advent of next generation sequencers allows high throughput genome sequencing of various organisms. Despite the genome availability, it is still difficult to predict the gene structure owing to the lack of high-accuracy algorithms for in silico gene annotation. Therefore, re-annotation of the predicted gene model at protein level is essential to establish the protein databases based on genome information. Shotgun proteomics has been widely employed to identify the translated products of genes as digested peptides. In some cases, however, the information of protein N- and C-termini is missing since the peptide identification by proteomic LC-MS in data-dependent acquisition mode is based on random sampling. Therefore, in order to explore shotgun proteogenomics, enrichment of terminal peptides before LC-MS is inevitable to obtain the essential information for genome annotation. Here we introduce a new enrichment method for N-terminal peptides. In this method, reductive dimethyl labeling was applied to amino groups of protein N-termini and Lysine residues of standard proteins such as BSA and ovalbumin. After protein digestion, protein N-terminal peptides are enriched by subtracting internal peptides. Note that endogenous N-terminal acetylated peptide of ovalbumin was also enriched by this method. In this presentation, we will report the applicability of this method to whole cell lysates of several organisms.

Keywords: N-terminal peptides, enrichment, proteogenomics

POS-03-LB-048 Dynamics of Protein Expression Measured by SRM Reveals Direct Targets and Secondary Messengers of Estrogen Receptor in MCF-7 Breast Cancer Cells

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Estrogen receptor-regulated proliferation of breast cancer cells is mediated through the network of protein targets, some of which induce subsequent expression of secondary messenger proteins. Accurate measurement of protein expression dynamics allows discriminating between direct targets and secondary messengers. To facilitate measurement of dynamics of protein expression in MCF-7 breast cancer cells, we first developed a simple and reproducible proteomic sample preparation protocol based on mass spectrometry-compatible detergent. Second, using our protocol in combination with SILAC, we quantified more than 3,000 proteins and identified 150 differentially expressed proteins in 6 and 36 hours following estradiol stimulation. This is one of the largest sets of estrogen-regulated proteins measured by proteomics. Third, we developed SRM assays for 57 estradiol-regulated proteins and measured dynamics of their expression in time (3 to 72 hours) and in the presence of estrogen receptor antagonist 4-hydroxytamoxifen (0.1 to 1000 nM). Such measurements confirmed proteins induced through estrogen receptor and eliminated several false positive identifications. A sharp increase in the expression of 22 proteins including trefoil factor 1 and cyclin-dependent kinase 1 was observed at 3 hours following estradiol stimulation, while delayed expression at 24 hours was found for NGFI-A-binding protein 2 (NAB2). Further analysis and literature data revealed that NAB2 is a secondary messenger protein which expression is induced by the early growth response 3 protein (EGR3), a transcription factor and a direct target of estrogen receptor. To summarize, dynamics of protein expression measured by SRM assays facilitates identification of direct targets and secondary messengers of estrogen receptor-induced protein expression. Presented approach will complement experimental methods in functional proteomics, systems biology and signaling network analysis.

Keywords: SRM, SILAC, estrogen regulation, breast cancer cells

POS-03-LB-049 Proteome Profiling and Identification of *In Vivo* Substrates of Cysteine Cathepsins in a Murine Model of Pancreatic Cancer

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Recent studies have suggested a new regulatory role for cysteine cathepsin proteases in tumor development and progression. While a few known *in vitro* substrates of these proteases provide a first link to tumor biology, they fail to completely explain marked differences in tumor phenotypes observed *in vivo* when individual cysteine cathepsins are knocked out in the context of the RIP1-Tag2 (RT2) murine model of pancreatic neuroendocrine tumorigenesis. Therefore, we aimed to proteomically identify cathepsin substrates and to profile other protein differences that exist between individual cysteine cathepsin knockouts in this cancer model.
Specifically, we employed TAILS (Terminal Amine Isotopic Labeling of Substrates) a proteomic technology for protease substrate discovery and proteome characterization where an N-terminal enrichment strategy allows the identification of protein N-termini and protease-generated neo-N-termini. Labeling with iTRAQ (isobaric tags for relative and absolute quantitation) reagents allows the quantitative comparison of proteins present in different samples. 8-plex iTRAQ facilitated a multiplex approach, whereby tumors from 6 different cathepsin knockouts (null for cathepsins B, L, S, H or X and BS double knockouts) and 2 wild type RT2 mice were analyzed in a single experiment.
Among >1500 total proteins analyzed in 3 biological replicates we identified a number of potential substrates, with some overlap between different cathepsins. In addition, we detected changes in other proteolytic pathways, and in protein expression levels in general. Biological consequences of select substrate cleavages will be discussed in relation to the observed differences in tumor phenotypes.
* These authors contributed equally, # joint senior authors.

Keywords: proteolytic processing, positional proteomics, cancer

POS-03-LB-050 PrESTs as Reagents for Proteomics

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Antibody based enrichment of peptides coupled to mass spectrometry (MS) has been shown to be a valuable tool for targeted proteomics. Methods like Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA) enable high throughput protein quantification in very complex samples with high sensitivity, specificity and precision. However, this technique has so far been limited to the study of protein targets for which monoclonal anti-peptide antibodies are available.
Here we introduce a MS based strategy for targeted absolute protein quantification using an already existing vast resource of highly validated polyclonal antibodies together with heavy isotope labeled Protein Epitope Signature Tags (PrESTs) as internal standards, resources originating from the Human Protein Atlas (HPA) project. Highly purified and accurately quantified heavy PrESTs are spiked into cell lysates prior trypsin digestion, thereby minimizing the risk of differences arising between samples and standards during sample preparation. Antibodies coupled to magnetic solid-phase support enrich target peptides from the digested sample and the endogenous protein concentration is calculated from the ratio of heavy to light peptides detected by MS.
In this study, antibodies were used in a 41-plex setup to enrichment peptides from one HeLa cell lysate with corresponding spiked in heavy labeled PrESTs. The sample was analyzed in triplicates and 21 proteins were successfully quantified, which correlated with results from PrEST-SILAC experiments.
Today, over 35,000 PrESTs with corresponding antibody exists within the HPA resource, eliminating the time needed for antibody generation normally associated with assay development towards novel targets for peptide enrichment using anti-peptide antibodies.

Keywords: Targeted Proteomics, Protein Quantification, Polyclonal Antibodies

POS-03-LB-051 Deep Proteome Characterization as a Tool for Identification of Novel Intraamniotic Infection and Inflammation Biomarkers in Preterm Birth Patients

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Background: Intraamniotic infection and inflammation (IAI) has been demonstrated to be associated with a significantly increased rate of neonatal adverse outcome, even in the absence of demonstrable positive cultures. The biochemical and protein composition of amniotic fluid is altered during pregnancy and reflects both physiological as well as pathological changes in the fetomaternal compartment. Modern proteomic technologies are able to detect and characterize even the slightest changes in protein composition of various biological matrices. Thanks to the ability to both identify and quantify a large number of proteins, this approach seems to be a very promising one for the detection of changes in amniotic fluid protein composition and for the identification of possible biomarkers for the prediction pregnancy related complication.

Objectives: We employed advanced proteomics in identification of novel potential biomarkers of IAI. The study was performed on amniotic fluid samples from preterm birth patients with confirmed ($n=31$) and ruled out ($n=26$) IAI.

Results: We successfully identified and quantified 847 amniotic fluid proteins (5% FDR) and selected more than 50 candidates, which showed dysregulated abundance between the two patient groups. To illustrate, these include neutrophil defensin 3, a range of histone proteins (H2, H3, H4 etc), antileukoproteinase and other proteins known to be involved in host against pathogen response, tissue remodeling and cellular death.

Conclusion: We used multidimensional shotgun proteomics to describe the amniotic fluid proteome and characterize differences among individual patients group, which resulted in a rich group of novel biomarker candidates. These are currently being validated using complementary methods, using both antibody based techniques as well as targeted proteomics approaches.

Keywords: preterm birth, iTRAQ

POS-03-LB-052 Characterization of the Host and Microbiota Proteomes in Pediatric IBD

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Inflammatory bowel disease (IBD) is a chronic incurable gastrointestinal disease and is comprised of two main subtypes including Crohn's disease (CD) and ulcerative colitis (UC). IBD is increasing and very aggressive in children; for unknown reasons, IBD is more common in Canada than other parts of the world. In order to reduce suffering and complications from undiagnosed and so untreated disease, it's very important to develop an early and accurate diagnosis assay and find possible biomarkers for the kids. In this study, we first analyzed the pediatric tissue samples from 5 patients with CD (inflamed and non-inflamed colon tissue), and 4 healthy controls. This analysis was done using a shotgun mass-spectrometry approach in conjunction with a super-SILAC strategy for the comprehensive proteome quantification. A total of 4302 proteins were identified to be in common among all three groups, especially 342 proteins are significant changed. For example, the detection of lactoferrin is in agreement with previous reports indicating the presence of lactoferrin in fecal samples from IBD patients. In addition, several pathways such as antigen processing and presentation, mitochondrial dysfunction, caveolar-mediated endocytosis signaling, and glycolysis were also found to be significant enriched.

Keywords: Inflammatory bowel disease, super-SILAC

POS-03-LB-053 Targeted Phosphoproteomics to Analyze Kinase-Mediated Signaling Pathway

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It is well known that kinase-mediated phosphorylation signals are involved in many biological functions, such as cell growth, cell division, apoptosis and cell death. Shotgun phosphoproteomics based on phosphopeptide enrichment followed by nanoLC-MS/MS has been widely used to identify/quantify novel phosphorylation sites. This approach could be further extended to targeted analyses of a series of phosphorylated molecules to quantify the entire signaling pathways. For this purpose, selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) mode with triple quadrupole MS instruments has been used to increase sensitivity, selectivity and accuracy although the method development step is generally time-consuming, especially for optimizing the SRM transition conditions. Recently, with high-speed scanning Q-TOF MS and Q-orbitrap instruments, MRM-HR mode and data-independent acquisition mode were introduced. Furthermore, high-resolution single ion monitoring (SIM) mode becomes available for these high-end MS instruments. In this study, we focused on Her2 and its downstream molecules, and quantify the phosphorylated forms by these targeted phosphoproteomics approaches.

At first we employed the SIM mode by using Q-orbitrap MS, and successfully quantified C-terminal tyrosine phosphopeptides of HER2 from enriched HeLa cell lysate phosphoproteome at 1 fmol level. In contrast, this phosphopeptide was not observed by the same instrument in data dependent acquisition mode, implying that sensitivity was enhanced in SIM mode. In this presentation, we will report the comparison between various quantitation modes to quantify the HER2-mediated phosphorylation pathway.

Keywords: quantitative phosphoproteomics, HER2

POS-03-LB-054 iTRAQ-Based Plasma Protein Profiling of Mild Cognitive Impairment Across Two Independent Cohorts

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To unlock the full potential of disease modifying treatments, it is essential to develop early biomarkers of Alzheimer's disease. For practical reasons, plasma biomarkers that could provide a signal at the early prodromal stage of mild cognitive impairment (MCI) would be ideal. Recent findings indicating a variety of potential markers need replication in independent cohorts. To determine which biomarkers might be consistently dysregulated across cohorts, we examined and compared the plasma protein profiles of cognitively impaired and cognitively normal elderly participants of the Sydney Memory and Ageing Study (MAS; 411 cognitively normal subjects and 261 MCI patients) and the Hunter Community Study (HCS; 49 cognitively normal subjects and 187 MCI patients) utilising the proteomic approach of isobaric tagging for relative and absolute quantitation (iTRAQ). Plasma proteins representative of several functional groups were significantly dysregulated in MCI plasma relative to cognitively normal subjects. The functional groups included acute phase reactants, inflammatory markers, lipid transport and clotting factors. In particular, ceruloplasmin and vitamin D-binding protein were significantly ($p < 0.05$) down-regulated by >20% in plasma of MCI patients across both cohorts. Several apolipoproteins, including ApoB, ApoA and ApoE were also significantly ($p < 0.05$) down-regulated by varying levels across cohorts. Hence, the plasma protein profile in patients with MCI differed from that of cognitively normal elderly subjects, indicating the potential for plasma protein markers in the diagnosis of AD at its prodromal stage. MCI.

Keywords: biomarkers, mild cognitive impairment, iTRAQ

POS-03-LB-055 Mapping the Chromosome 10 Proteome: Objectives and Progress

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The Chromosome 10 Consortium is a member of the Chromosome-Centric Human Proteome Project (C-HPPP) initiative. It focuses on characterizing the proteome associated with this meta-centric chromosome which accounts for 4.5% of the genome. Chromosome 10 is most known for accommodating the *RET* proto-oncogene and the *PTEN* tumor suppressor genes. Pathway analysis showed that the chromosome 10 genes are enriched in subsets of signaling (*e.g.* Wnt signaling), vesicle transport (*e.g.* regulation of ARF proteins), and metabolic (*e.g.* oxidation/reduction) pathways. In addition, inferring molecular functions of individual genes, protein domain involved in intracellular signaling (*e.g.* ANX and ArfGap) and protein-protein interactions (*e.g.* ANK, LH2, and PDZ) are relatively enriched. 764 proteins have been reportedly associated with chromosome 10 and are linked to 155 diseases in the OMIM database (Online Mendelian Inheritance in Man), covering a wide spectrum, including papillary thyroid carcinoma, glaucoma, macular degeneration and epilepsy. Among the annotated 764 protein-coding genes on chromosome 10, 527 have protein-level evidence, while 210 have only transcript-level evidence. Thus, we decided to further explore protein existence of the 210 genes by first searching for peptide-level evidence in publically available mass-spectrometry databases such as peptideAtlas. In parallel, to take advantage of next-generation sequencing-based transcriptomic data, we are also seeking transcript-level evidence of known and novel isoforms from RNA-Seq data in public databases, such as TCGA and a cell line dataset published by Frida *et al*, as well as our own datasets on various human samples. Based on the information, we plan to select a few cell lines that are potentially expressing the missing 210 proteins for further mass spectrometry analysis to obtain experimental evidence on protein existence.

Keywords: chromosome, CHPPP

POS-03-LB-056 Improved Enrichment of S-Nitrosylated Peptides Using Iodoacetyl Tandem Mass Tag Reagents, Immbolized anti-TMT Antibody Resin and TMT Elution Buffer

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Thermo Scientific iodoacetyl Tandem Mass Tag (iodoTMT) Reagents are isobaric, cysteine-reactive reagents for irreversible labeling, enrichment, and multiplexed quantitation of cysteine-containing peptides. The iodoTMT reagents can be used to quantify cysteine modifications (e.g. S-nitrosylation, oxidation, and di-sulfide bridges), and enriched with an anti-TMT antibody resin. Here we report development of a selective TMT elution buffer using small molecule analogs of the TMT reagent reporter region. Cell lysates and purified proteins were denatured, reduced, and labeled with iodoTMT reagents prior to enzymatic digestion. For cysteine S-nitrosylation quantitation, samples were treated with a GSH-NO donor agent, alkylated with MMTS to block unmodified sulfhydryls, and desalted. S-nitrosyl groups were selectively reduced with ascorbate, alkylated with iodoTMT reagent, and enriched an anti-TMT antibody resin. Peptide samples were analyzed using a Thermo Scientific LTQ Orbitrap XL mass spectrometer and Thermo Scientific Proteome Discoverer 1.3 software. Elution buffers were screened by surface plasmon resonance (SPR) using a TMT-derivatized CM5 chip on a GE Healthcare Biacore 3000 instrument.

The iodoTMT-labeled peptides are enriched with an anti-TMT antibody raised against the reporter region of the TMT reagents. To improve the specificity of iodoTMT-labeled peptide elution, we scouted analogs of the TMT reagent reporter region, identified structural features of the antibody recognition site, and determined the relative affinity of each analog by SPR. Using total cysteine-labeled peptide samples and low pH or the new TMT elution buffer, labeled peptide enrichment specificity increased from 80% to 96%. When S-nitrosylated cysteines were labeled in a modified S-nitro switch assay, our results showed a 50% increase in iodoTMT-labeled peptide identification by LC-MS using the new TMT elution buffer.

Keywords: iodoTMT, S-nitrosylation, Cysteine oxidation

POS-03-LB-057 Customized Real-Time Control of Benchtop Orbitrap MS

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The novel Microsoft .NET® based implementation of an Instrument Application Programming Interface (API) allows a high performance access to bench-top Orbitrap mass spectrometers. The user can trace scan data streams, monitor and refine instrument parameters and place user defined scans at runtime.

An event driven architecture is providing the capability to synchronize application workflows very comfortable and straight forward. The user can be notified, whenever an instrument value or status is changing.

Without the loss of scan speed compared to a common instrument method, this interface supports customized analysis strategies with a flexible instrument behavior during online acquisitions. Individual applications for various research topics, daily service, and maintenance can make full use of the spectrometer's capabilities.

An example C# application was developed to acquire sets of high resolution fragment scans at varying collision energy in order to populate a MS2 spectral database. To ensure a high quality of data, the MS1 stream of an LC/MS experiment is monitored for specific quality criteria like signal/noise, ion flux and spectral purity of a target isolation window to prevent 'co-fragmentation'.

When a suitable data scenario is detected, the instrument control is taken over to activate data acquisition and to deploy customized scan definitions with varying collision energy. A synchronization of data acquisition with the execution of the customized scans helps to generate very compact .RAW files, exclusively containing the requested scans.

Incomplete sets of fragment scans are determined online for all analytes, in order to cycle the LC/MS experiment until all missing energy variations are acquired.

More complex and time consuming data analysis that keeps track of the presence and criteria status of all defined targets at once is possible, without a decrease in scan speed.

Keywords: real-Time, instrument control

POS-03-LB-058 Preliminary Verification of Lung Cancer Plasma-based Stratification Markers for Chemotherapy

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This study aims to screen 550 candidate biomarkers, discovered in a variety of proteomic and genomic datasets, for their ability to predict the benefit of chemotherapy treatment for late stage non-small cell lung cancer patients. To achieve quantifiable sensitivity for low abundance targets in plasma, nine pairs of human plasma samples (paired by contrasting response to chemotherapy and by similar history) were processed by IgY14-SuperMix affinity depletion (Sigma Aldrich, St. Louis, MO) followed by tryptic digestion, addition of the isotopically-labeled peptides, chromatographic separation by one and two-dimensional reverse phase liquid chromatography before targeted mass spectrometry with triple quadrupole mass spectrometry. A Wilcoxon rank test was applied to the LC-MS/MS data and revealed 14 candidates significantly differentially expressed with p-value < 0.01. To our knowledge, this study represents the first verification effort via targeted mass spectrometry quantitation for blood-based biomarkers that can predict the benefit of chemotherapy treatment.

POS-03-LB-059 Proteomic Analysis of Salivary Proteins in Patients with Oral Precancerous Lesions

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Many oral cancers have been documented to be associated with or preceded by precancerous lesions. Therefore, identification of salivary biomarkers may lead to development of effective early detection systems for oral cancer. The objective of this study was to identify potential salivary biomarkers for early diagnosis of oral cancer patients by proteomics. Saliva was collected by Navazech's method, centrifuged at 14,000 rpm for 10 minutes at 4°C and the supernatant was kept at -80°C until used. Whole salivary proteins of six oral cancer patients and six healthy controls were profiled using shotgun proteomics based on protein digestion and peptide sequencing by tandem mass spectrometry and automated database searching, together with two-dimensional electrophoresis. The results showed that protein patterns in saliva of each type of oral lesion: oral lichen planus, oral leukoplakia and oral squamous cell carcinoma were different and also differed from healthy subjects. Cystatin, a cysteine protease inhibitor, was downregulated in oral lichen planus group when compared to healthy patients while transformation/transcription domain-associated protein, isoform CRA_e, associated with tumor suppressor p53, was found to be upregulated in cancer patients. In conclusion, upregulation of structural proteins and downregulation of receptor proteins were found in precancerous patients and higher levels of signaling proteins and proteins associated with metabolism were found in cancer patients. This may lead to further investigations to identify useful tools for early detection of oral cancer in the future.
This project is supported by the Office of the Higher Education Commission and Mahidol University under the National Research Universities Initiative.

POS-03-LB-060 Deep Subcellular Proteome Profiling of Human Induced Pluripotent Stem Cell by One-shot nanoLC-MS/MS Analyses with Meter-scale Monolithic Silica Columns

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The molecular mechanisms for self-renewal and pluripotency of human induced pluripotent stem cell (hiPSC) still remain unclear. Previously, some researchers have been performed to characterize hiPSCs using high-resolution MS-based proteome analyses. However, the technology of proteome analysis is still immature to uncover the real proteome state in the cells including low expressing proteins. On the other hand, proteome profile in each subcellular location is a valuable knowledge of cellular functions. We thought fractionation of cellular organelle is effective approach to increase the proteome coverage and to get the knowledge of localization.
In this study, we improved the identification efficiency of low expressing proteins using subcellular fractionation technique combined with high sensitive one-shot nanoLC-MS/MS analyses. Cellular organelle of nucleus, cytoplasm and membrane were isolated and analyzed by nanoLC-MS/MS using meter-scale monolithic silica columns. Finally, we successfully identified 7,799 unique proteins from each subcellular fraction of iPSC and fibroblast cells using 100 ug of total injected samples. Importantly, we could identify 400 transcription factors which are known as very low expression levels. This deep subcellular proteome analysis data disclosed more detailed differences between iPSC and fibroblast cells and elucidated the key components governing the cellular functions of iPSC.
Keywords: iPSC, nanoLC-MS/MS, monolithic column

POS-03-LB-061 Changes in the Protein Profiles of *Beta vulgaris* Leaf Apoplastic Fluid with Iron Deficiency and Iron Resupply

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The aim of this work was to study the effects of Fe deficiency and resupply on the proteome profile of *Beta vulgaris* leaf apoplastic fluid. Plants were grown in Fe-sufficient nutrient solution [45 µM Fe (III)-EDTA] and Fe-deficient (0 µM Fe) nutrient solutions, and Fe resupply was carried out with 45 µM Fe (III)-EDTA for 24 hours. Apoplastic fluid was obtained by centrifugation as in López-Millán et al. (2001), and proteins were precipitated with 10% TCA. Proteins were analyzed by 2-DE IEF SDS-PAGE, using four biological replicates: 80 µg of protein were loaded in IPG strips (pI 3-10), and 8 cm 12% acrylamide gels were used for the second dimension. The 2-DE gels were analyzed using PDQuest v8.0, and 210 ± 12, 216 ± 11 and 211 ± 20 protein spots were found in control, Fe-deficient and Fe-resupplied (previously Fe-deficient) plants, respectively, with 231 spots being consistently detected. Only consistent spots (those occurring in three out of four gels), showing changes statistically significant (t-test, $p<0.05$) and having increases >1.5-fold or decreases >35% were considered for the differential analysis: in total, 33 proteins were found to change in relative abundance in the different classes. When compared to the control, the number of increases/decreases was 10/3 and 7/10 in –Fe and Fe-resupplied plants, respectively. In the Fe-resupplied plants, increases/decreases were found in 3/9 protein spots when compared to the -Fe plants. Twenty-eight spots (85% of the total 33) were identified using nLC-MS/MS.

POS-03-LB-062 Integrated -omic Analysis Uncovers a Fundamental Hypoxia-driven Mechanism of Breast Cancer Progression

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Although extensive studies have been conducted to understand breast cancer progression, we do not precisely know how hypoxia, a pivotal microenvironmental factor, is involved in this process. Addressing this question, more precisely identifying the critical genes involved, is of crucial importance to provide alternatives and more efficient anti-cancer therapies.
We used breast cancer cell lines with distinct aggressiveness properties as a model and developed a strategy based on a system-wide quantitative proteomics in combination with a migration screen and protein network analysis. We found that the less aggressive cell line temporally regulated cell migration under hypoxia and the underlying mechanism involved the PTPRG and TACSTD2 proteins. In a large-scale migration screen, we identified a novel migration component enriched in lysosomal genes (e.g. ASAH1, GBA, GLB1, GUSB) that suggested a mechanism of cell migration based on the cellular trafficking of proteins. We dissected the underlying mechanism by phosphoproteomic analysis. The regulation profile of stathmin (STMN1) at serine 16 remarkably discriminated the poorly invasive from the highly invasive cell line. Unexpectedly, stathmin knockdown dramatically enhanced cell migration of poorly invasive cells only under hypoxia.
Combined, these results led us to propose a model of cancer progression based on the hypoxia-dependent trafficking of vesicles along microtubules. In this context, the post-translational regulation of this trafficking machinery upon hypoxia constantly refines the balance between preventing and promoting migration forces. This study represents the first integrative-level attempt to understand the mechanism of cell migration under hypoxia and demonstrates that hypoxia might inhibit the early stages of cancer progression, however promotes progression at later stages. This study constitutes a paradigm shift in the cancer field and sheds light onto the fundamental role of the microenvironmental constraint.

POS-03-LB-063 Quantitative Proteomic Analysis of Oyster Larvae to Study the Effect of Multiple Climate Change Stressors by 8-plex iTRAQ Labeling

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Carbon dioxide has been found to be one of the major pollutants is not only the cause of global warming but also leads to a more severe problem to the oceans called ocean acidification, altering seawater carbonate chemistry, resulting in low pH (more H^+ ions) and decreased concentrations of carbonate (CO_3^{2-}) ions, which are used in building the calcium carbonate ($CaCO_3$) shells in marine invertebrates, including economically important oysters. Early life-stages of oysters are particularly vulnerable to climate change-driven low pH, because their shell is made of the highly soluble form of the calcium carbonate, aragonite. Shot gun proteomics using 8 PLEX iTRAQ was used to quantify and characterize the proteins in the oyster larvae exposed to climate change both at physiological and population levels. Using the information available from the oyster genome, 1350 proteins were identified in the oyster larvae in all multiple climate stressor conditions. Proteins involved in immune system process, catalytic activity, antioxidant activity and ion binding are down regulated during exposure to low pH in addition salinity stress, whereas cellular and metabolic processes, structural molecule and enzyme regulator activities are unregulated in temperature exposure in addition to salinity and pH. Protein interaction networks and pathway analysis of differentially expressed proteins exhibit wide range of biological pathways, showing higher regulations to the energy metabolism in most of the climate change stressors. Any individual stressor does not seem to affect the larvae but when multiple stressors are combined larvae show slow growth, decreased response to signals and might lead to confusion in making their preference for selecting a substrate for further settlement and metamorphosis. This study on larval growth coupled with proteome change is the first step towards the search for novel biomarkers which will help to understand the changes in the molecular mechanisms involved by climate change on marine invertebrate organisms.

POS-03-LB-064 Targeted Proteomic Absolute Quantification on Transporters of Human Pancreatic Cancer Cells with Gemcitabine-resistance

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The prognosis of pancreatic cancer remains very poor, partly due to the prominent resistance to chemoradiotherapy. However, the mechanism underlying resistance of pancreatic cancer has not been understood well. Recently, the cancer stem cells are considered to be responsible for not only tumor initiation but also therapy resistance. Previously, we found that the frequency of CD133+CD44+ cells and hypoxia-resistant cancer cells increased in pancreatic cancers when treated with the neoadjuvant chemoradiotherapy (CRT). Furthermore, in this study we investigated the expression of transporter proteins in gemcitabine resistant human pancreatic cancer cells after neoadjuvant CRT using quantitative targeted absolute proteomics (QTAP).
To quantify multiple transporter proteins of gemcitabine resistant human pancreatic cancer cells with high sensitivity, we developed QTAP analysis using liquid chromatography-tandem mass spectrometer (LC-MS/MS) with multiple reaction monitoring (MRM) in combination with probe peptides. When we performed QTAP and quantitative RT-PCR analysis of transporters in human pancreatic cancer cells, we found that mRNA expression was not correspond with the protein expression in many transporters. And the expressions of multiple transporters were up-regulated in neoadjuvant CRT samples. Next, with immunohistochemical staining analysis, it demonstrated that these transporters are expressed on epithelial cells in pancreatic cancers. Furthermore, to investigate the role of transporters in gemcitabine-resistant tissue, we examined the cells exposed to Verapamil, which an inhibitor of the ABC transporter. As expect, after inhibited ABC transporter, the sensitivity for gemcitabine in pancreatic cancer cells was increased.
Our finding suggested that these transporters have an important role in the resistance of pancreatic cancer to treatment. Specific inhibitors for the transporters should be promising drug of targeting therapy for pancreatic cancers.
Keywords: cancer stem cell, transporter, QTAP

POS-03-LB-065 Deciphering Human-Microbe Proteome Interactions using an *E. coli* Proteome Chip

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We developed a new high-throughput protein purification protocol that allows us to purify 4,256 *Escherichia coli* proteins and to spot them on glass slides to form an *E. coli* proteome microarray (chip). We detected the interactions between human serum antibodies and genome-wide *E. coli* proteins using this *E. coli* proteome chip. Thus, we identified new serological biomarkers for inflammatory bowel disease (IBD). Each protein array was screened using individual serum from healthy controls and clinically well-characterized patients with IBD [Crohn's disease (CD) and ulcerative colitis (UC)]. Surprisingly, SAM analysis identified a total of 417 *E. coli* proteins that were differentially recognized by serum antibodies between healthy controls and CD or UC. We also identified two sets of serum antibodies that were novel biomarkers for specifically distinguishing CD from healthy controls, and CD from UC, respectively. We also detected the interactions between a human antimicrobial peptide exiting in the gut (Lactoferricin B, Lfcin B) and *E. coli* proteome using this chip. The result showed that Lfcin B binds to bacterial response regulators, BasR and CreB of two-component system (TCS). The electrophoretic mobility shift assays and kinase assays indicate that Lfcin B inhibited the phosphorylation between response regulators (BasR and CreB) and their cognate sensor kinases (BasS and CreC). These results indicate that this *E. coli* proteome microarray is a powerful tool for the study of human-microbe proteome interactions.
Keywords: host-microbe interaction, Proteome microarray, antimicrobial peptide

POS-03-LB-066 Comparative Proteomics of Wild Type, Mutant and Overexpressing Strains of Diazotrophic Anabaena PCC7120 Unviels the Role of *ahpC* in Abiotic Stress Management

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The present study first time compares the proteome of wild type *Anabaena* PCC7120 with two transgenic lines- one with an extra copy of *ahpC* (AnFPNahpC) and another with mutated *ahpC* (ΔahpC) using 2DE coupled with MALDI-TOF MS. A total of 222 differentially expressed proteins corresponding to 157 unique gene products, were employed to figure out the proteins directly/indirectly linked with *ahpC*. Of these 48 and 19 unique protein spots encoding 19 and 15 proteins, respectively for overexpressed (AnFPNahpC) and the mutant (ΔahpC) strains appeared associated in proteome rearrangment. Protein expression and enzyme activity in the AnFPNahpC were respectively 3.0 and 3.2 fold higher than the wild type cells, while no such activity was observed in ΔahpC strain. While photosynthesis, respiration and nitrogen fixation were upregulated in AnFPNahpC the same were downregulated in ΔahpC. The multiple abiotic stress tolerance potential of AnFPNahpC was clearly visible in the transcript of the *ahpC* gene which was 2.15-6.0 fold higher than the wild type. A group of upregulated proteins that seemingly conferred abiotic stress tolerance in AnFPNahpC included those involved in photosystem protection as well as HSPJ, GroEL, TF, Dps, PPIB, Prx, MnCAT and ClpB. However, *ahpC* mutation resulted in downregulation of the SODA, Bfr, RBP, FDX, CBS, UroD and RibC thus rendering sensitivity in ΔahpC *Anabaena*. The observed physiological and proteomic data provide testimony to the pivotal role played by *ahpC* in stress management by *Anabaena*. In view of the above *ahpC* may be used for developing transgenic cyanobacteria suitable for field application.
Keywords: Proteomics, transformed Diazotrophic Anabaena

POS-03-LB-067 Diagnosis of Male Reproductive System Disorders with Proteomic Biomarkers Measured in Seminal Plasma

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Non-invasive methods for differential diagnosis constitute current unmet needs in the management of male reproductive system disorders. In our search for biomarkers, we focus on the proteome of seminal plasma (SP), a proximal fluid suitable for discovery of novel biomarkers and for development of non-invasive diagnostic tests. We previously identified more than 3,100 proteins in SP of healthy men and men with infertility, prostate cancer and prostatitis, and use this SP proteome as a foundation of our biomarker discovery platform. In our presentation, we will discuss in detail development of male infertility biomarkers and also suggest approaches for identification of biomarkers of prostate cancer and prostatitis.

To develop markers for differential diagnosis of male infertility, we selected 79 proteins based on proteomic profiling of SP from fertile men and men with obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). Using quantitative SRM assays, we verified 30 proteins and then validated 18 proteins in 148 SP samples. We identified two proteins, epididymis-specific ECM1 and testis-specific TEX101, which differentiated between OA, NOA and normal spermatogenesis with near absolute specificities and sensitivities. The performance of ECM1 was confirmed by ELISA in 188 samples, and a 2.3 μg/mL cut-off distinguished OA from normal spermatogenesis with 98% specificity, and OA from NOA with 74% specificity, at 100% sensitivity. Immunohistochemistry and immuno-SRM assays revealed differential expression of TEX101 in distinct histological subtypes of NOA. As a result, we proposed a simple 2-biomarker algorithm for differential diagnosis of OA and NOA, and, in addition, for the differentiation of NOA subtypes. Clinical assays for ECM1 and TEX101 will replace diagnostic testicular biopsies and improve the prediction of testicular sperm retrieval, thus increasing the reliability of assisted reproduction techniques.

Keywords: biomarkers, male infertility, differential diagnosis

POS-03-LB-068 Scalable Multiplexed and Sensitive Immunoassays by the Proximity Extension Assay

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Olink AB

Medical research is developing an ever greater need for comprehensive data generation to realize the promises of personalized health care based on molecular biomarkers. The evolution of immunoassay technologies to fulfill these needs has been much slower than for the more easily scalable genomics counterparts as exemplified by DNA microarrays and next generation sequencing. The nucleic acid proximity based methods, proximity ligation assay (PLA) and proximity extension assay (PEA), with their dual reporters have shown potential to relieve the shortcomings of antibodies and their inherit cross-reactivity in high capacity protein quantification. We now present further developments and illustrate scalable, specific, and multiplexed immunoassays detecting 92 proteins in minute sample amounts of 1 uL serum, fine needle biopsies, or even single cells, at sub pg/mL sensitivity. Several applications examples for biomarker research in cancer and cardiovascular disease will be presented. With the lack of antibody cross reactivity, the scalable multiplexed PEA platform technology will be used to build repertoires of immunoassays from all commercially available antibodies, estimated at around 5,000. These will constitute an novel resource of biomarker discovery and development.

Keywords: Immunoassay, Biomarker, Multiplexed