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

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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

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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

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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

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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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