

A proteomic approach to molecular mechanisms of aging and neurodegenerative diseases

Tosifusa Toda

Proteomics Collaboration Center, Tokyo Metropolitan Institute of Gerontology,
35-2 Sakaecho, Itabashiku, Tokyo 173-0015, JAPAN
ttoda@tmig.or.jp

Decline in brain functions and increase of neurodegenerative disorders with aging have been thought to be associated with increased oxidative stress in aged brain tissues. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) may cause damages on both DNA and protein. Damaged DNA likely alters gene expression, and altered proteins must get susceptibility to misfolding and aggregation of proteins. However, we have not had enough evidences to prove the above working hypothesis up to the present. The application of proteomics to studies on brain aging provides a novel window to look at the abnormal protein metabolism in neurodegenerative disorders in aged animals.

Age-related decline in cellular functions is observed universally in all cell types including mitotic fibroblasts and post-mitotic neuronal cells. Although the phenotype of aged cells depends on the state of differentiation, there must be a common pathway of aging in all cell types, if the accumulation of oxidative damages on DNA and protein is the major cause of the cellular aging. Thus we decided to carry out the proteomic analysis, in which age-related protein alterations were compared in both human fibroblast cultures and in mouse brain tissues to figure out the universal mechanism of aging and cellular background of age-related diseases.

Proteomic analysis of replicative cell aging

Proteomic alterations in process of cellular senescence of normal human diploid fibroblast line TIG-3 was analyzed as a model of replicative cell aging. TIG-3 showed the maximum life span around 70-80 PDL in our optimized culture condition. Various phenotypes of aged cells such as decline in cell motility, accumulation of auto-fluorescent pigments and slowing down the cell cycle were observed at the late

passages of the population doubling. The shortening of telomere had been believed to be the major cause of the cellular senescence, but not all phenotypes of aged cells could not be ascribed to the length of telomere.

In our proteomic analysis, we detected many spot proteins, of which relative abundance altered in the course of cellular aging. A major part of the results of our proteomic analysis are summarized in our proteome database on the web site (<http://proteome.tmig.or.jp/2D>). From the data, we found that age-related protein alteration in relative abundance is categorized into 5 groups, (A) increase straight, (B) decrease straight, (C) increase after transitional decrease, (D) decrease after transitional increase, and (E) irregular or insignificant variation. In group (A), molecular chaperon HSP-60 showed the most significant increase. In group (B), phosphoprotein vimentin, which may play an important role in actin filament stabilization and UCH-L1, which works in the final step of polyubiquitination-dependent protein turnover, showed significant decrease. Stathmin, which may regulated microtubule formation,

and SOD-1, which is a superoxide radical scavenger, shows transitional increase at the border of phase 3 and phase 4, and then decrease in the last phase of cellular senescence in turn (Fig. 1). These behavior of proteins indicated that oxidative stress increased in the aged cells. Both radical scavenger SOD-1 and stress protein HSP-60 were induced in the course of cellular aging. On the other hand, decrease of UCH-L1 suggested that the overall activity of polyubiquitination-dependent protein turnover system might be impaired in the aged cells.

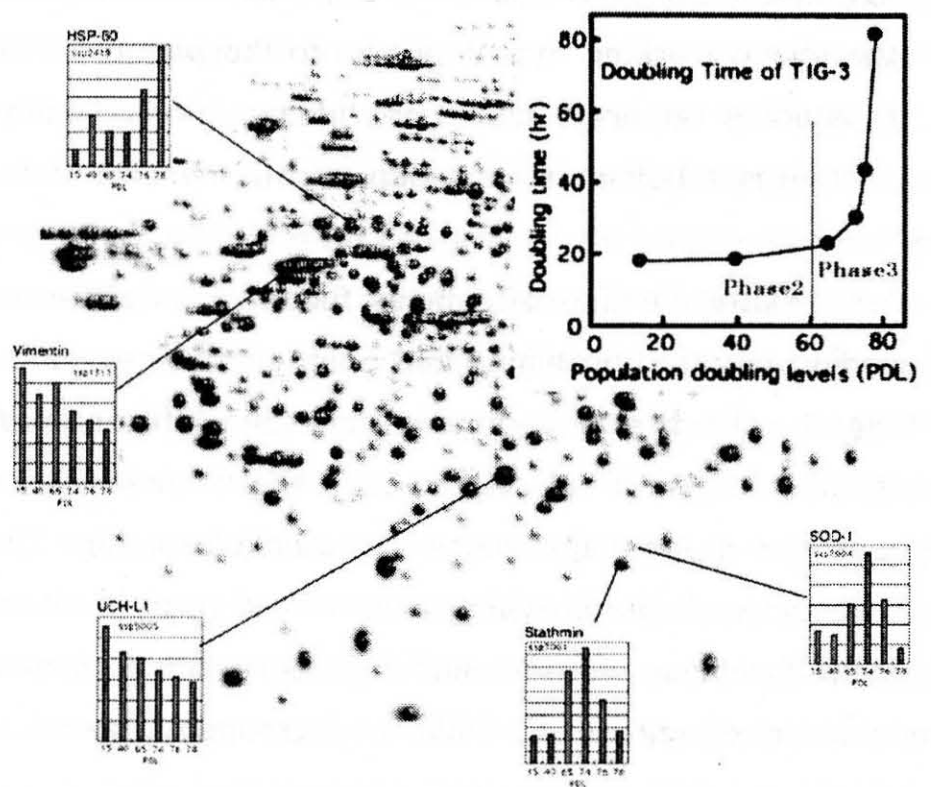


Fig.1 . Typical protein spots, of which relative abundance varied with aging in TIG-3 significantly.

Proteomic analysis of post-mitotic cell aging in mouse brain

2-D gel protein profiling of mouse hippocampus at various ages were carried out for detecting age-related protein alterations in tissues of post-mitotic cells, because

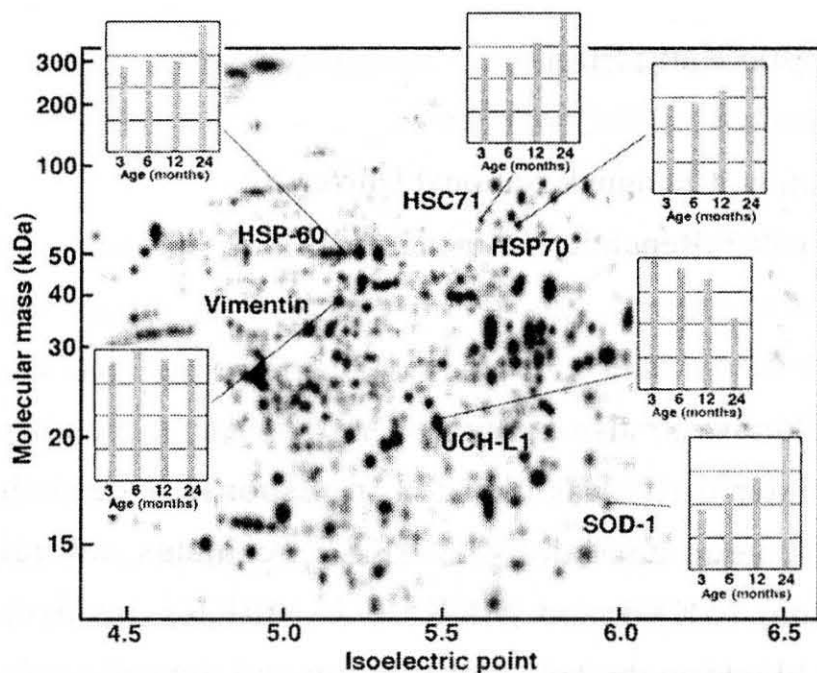


Fig. 2. Typical protein spots, of which relative abundance varied with aging in mouse hippocampus significantly.

In such conditions, decrease of UCH-L1 might cause critical deposition of polyubiquitinated polypeptides, which must be digested in young brain.

Discussions and conclusion

Age-relate increase of superoxide radical scavenger and molecular chaperons were observed commonly in cultured fibroblasts and mouse hippocampus, suggesting that the elevation of oxidative stress is universal phenotype of aged cells. On the other hand the level of UCH-L1 decreased in both cell types, suggesting that the clearance of polyubiquitinated polypeptides might be impaired in aged cells universally. The above alterations may increase the risk of abnormal protein deposit in all types of aged cells.

Thus the comparative analysis of proteomic alteration in aging of various different cell types may give us a lot of information about the universal mechanisms of cellular aging in vitro and in vivo.