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**Venue: LT7A**

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**Proteomic analyses of signal transduction pathways using 2D DIGE**

Seisuke Hattori

*Division of Cellular Proteomics (BML), Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan*

Two-dimensional (2D) gel electrophoresis is a general tool for proteomic studies. However, proteins of low abundance such as factors involved in signal transduction have been overlooked hidden behind huge spots of cytoskeletal proteins or metabolic enzymes. To overcome this problem, we pre-fractionate proteins of interest before 2D gel electrophoresis. As a 2D system, we employ a fluorescence difference two-dimensional gel electrophoresis system (2D DIGE, GE Healthcare) to detect subtle differences between the samples.

The first pre-fractionation procedure is phospho-protein purification. Phospho-proteins were purified from cells in which a kinase of interest was either activated or suppressed. Profiles of phospho-proteins in these cells were compared by 2D-DIGE. By this approach we succeeded in the identification of nearly 30 substrates of ERK (extra-cellular signal-regulated kinase), half of which are known ERK substrates, indicating the efficacy of our approach. We also identified novel p38 MAP kinase substrate by a similar approach.

Also, we compared lipid raft proteins from activated or quiescent T-cells, which enabled us to identify factors recruited to lipid raft during T-cell activation. Factors that underwent post-translational modification were also identified. Biochemical and histochemical experiments confirmed the translocation of identified proteins from cytosol to lipid rafts.

These results show that 2D DIGE system combined with appropriate pre-fractionation procedures is a very powerful tool to analyze cell signaling pathways.

Reference:

Ueda, K. Kosako, H. Fukui, Y., and Hattori, S. Proteomic identification of Bcl2-associated athanogen 2 as a novel MAP kinase-activated protein kinase 2 substrate. *J Biol Chem.* 279, 41815-41821.