Irrespective of the explanation, it is cleat that measurement of the level of HA in biological fluids may provide a useful marker for monitoring the onset and progression of a number of important diseases and disorders.

[PC1-30] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

MALDI-TOF MS Approach to Proteomics : Identification of the E6AP-interacting factors in SiHa cervical cancer cells

Lee Kyungae°, Kho Changwon, Chung Yeongu, Park SungGoo, Cho YongKweon, Cho YoungSik, Park SurNie, Yoon Doyoung

Korea Research Institute of Bioscience and Biotechnology, Changwon National University

Human papillomaviruses have been recognized as the primary cause of cervical cancer. Viral oncoproteins are selectively retained and expressed in carcinoma cells infected with human papillomavirus and cooperated in immortalization and transformation of primary keratinocytes. E6 associated protein (E6AP) is a 100kDa cellular protein which mediates the stable association of the high-risk HPV E6 protein with tumor suppressor protein p53, resulting in the degradation of p53. E6AP was known as E3 ubiquitin-protein ligase, which has been proposed to play a role in defining the substrate specificity of the ubiquitin-proteasome degradation. In order to identify the E6AP-interacting molecules, SiHa cervical carcinoma cells having HPV 16 genome, was used. We have produced his tagged E6AP and E6AP-Ni2+-NTA-affinity column was prepared to obtain E6AP-interacting proteins. The E6AP-interacting proteins were resolved in 2D-gel and analysed by matrix-assisted laser desorption/ionization (MALDI/TOF). Among 21 proteins identified in 2D patterns of SiHa cell lysate bound to E6AP protein, there are 2 proteins not yet identified. Desmocollin, NT2RM1000563 protein, GR AF-1 coactivator 3, SMF protein, CD2 binding protein 1, Rab interacting lysosomal protein, pigment epithelium-derived factor, A20-binding inhibitor of NF-κB activation-2, GTP-binding protein, PACSIN3, novel protein similar to mouse thrombospondin type 1 were bound to E6AP. These results suggest that E6AP can have several functions by interacting with the cell adhesion molecules, immune-regulatory factors, cell cycle regulators and cell signaling regulating factors in SiHa cells (This work was supported from the Molecular Medicine Program, MOST).

[PC1-31] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

2-DE/MALDI-TOF MS Analysis of Age-dependent Mitochondrial Proteome in Rat Liver

Kim ChulHong°, Park DaeUi, Hong SeongEui, Chung AnSung, Chung HaeYoung

Pusan Bioinforamtics and Biocomplexity Research Center, Pusan National University, College of Pharmacy, Pusan National Univsersity

Mitochondria is called power plant of the cell because they product biological energy, ATP, using electron transport system and proton pump. But this system generates ROS, which cause mitochondrial damage and cell apoptosis. Furthermore, proteins are damaged by this oxidative stress in aging process. That is one of the most possible factor responsible for the functional destruction in aged tissues. To study the age-dependent proteome profile of mitochondrial proteome, we used 2D-electrophoresis and MALDI-TOF MS technique, in young (6 months of age) and aged (24 months of age) rats. As a result of image analysis and MALDI-TOF/MS of 64 spots from silver-stained 2D gel, we identified 45 spots by peptide mass fingerprint. Among them, 30 sopts showed age-dependent expression patterns (increase or decrease) between young and old rat liver mitochondria proteome. But in calorie-resticed old rat, these patterns were reversed. These results suggest that age-related changes of mitochondrial proteome are responsible for functional loss of organ in aging process and further study of the age-dependent interaction mechanism of these proteins can give us the key to elucidate aging process.

[PC1-32] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

MALDI-TOF MS Approach to Proteomics : Identification of the E7-interacting factors in C33A cervical cancer cells

Kho ChangWon°, Lee KyungAe, Park SungGoo, Myung PyungKeun, Park SuenNie, Choe YongKyung, Yoon DoYoung, Park ByoungChul

Korea Research Institute of Bioscience and Biotechnology, *School of Pharmacy, Chungnam National University

Viral oncoprotiens are selectively retained and expressed in carcinoma cells infected with HPV and cooperated in immortalization and transformation of primary keratinocytes. E7 protein interacts with the retinoblastoma protein, which results in dissociation of the E2F-1 transcription factor and activation of genes related to DNA synthesis and cell proliferation. In order to identify the E7-interacting molecules, HPV-negative C-33A cervical cancer cell line, was prepared to establish stable cell line expressing E7. We have purified his tagged E7 oncoprotein. E7-Ni2+-NTA-affinity column was prepared to obtain E7-interacting proteins and E7-interacting proteins were resolved in 2D-gel electrophoresis and analysed by matrix-assisted laser desorption/ionization (MALDI/TOF). Among 12 proteins identified in 2D patterns of E7-transfectant and mock cell lysate bound to E7 protein by MALDI/TOF, there are 3 proteins not yet identified. ATP synthase, glucocorticoid receptor AF-1 coactivator-1, tumor protein p73 (p53-like transcription factor) and CTCL tumor antigen se2-5 which is a cutaneous T-cell lymphoma-associated antigen, were downregulated whereas kinesin, Ku70-binding protein which may play a role in DNA repair pathway, latent transforming growth factor beta were upregulated by E7 and were bound to E7. It is presumed that E7 can evade immune surveillance by suppressing or inducing the immune-mediated factors, cell cycle regulators and cell signaling regulatory factors (This work was supported from the Molecular Medicine Program M1-0106-00-0078, Ministry of Science and Technology).

[PC1-33] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Probing for Differentially Expressed Genes in Aged Monkey Muscle by cDNA-Representational Difference Analysis

Sung BoKyung°, Jung KyungJin, Je JungHwan, Lee JunSik, Chung HaeYoung

College of Pharmacy, Pusan National University, Pusan 609-735, Korea

In laboratory rodents, there are several age-dependent physiological and biochemical changes in skeletal muscle, including increased steady-state levels of oxidative damage to lipids, DNA, and proteins. We have used representational difference analysis (RDA) to identify up- and down-regulated genes in skeletal muscle from aged rhesus monkey. cDNA-RDA was performed using small amounts of mRNA pool to reverse transcribe the cDNAs from muscles of young and old monkeys, which are 6 and 25 years of age, respectively. The cDNA-RDA led to the isolation of several distinct clones that were specifically up- and down-regulated in the aged monkey muscle. Several genes up- and down-regulated in aging monkey were identified in the present study. Differential expressions of these genes were confirmed by semiquantitative RT-PCR approach. Our results lead to a better understanding of the molecular mechanisms of aging process and possibility of candidates of aging biomarkers in primates.

[PC1-34] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

MALDI-TOF MS Approach to Proteomics: Identification of the E7-interacting factors in HaCaT keratinocyte cells by proteomics

Lee KyungAe°, Kho ChangWon, Park SungGoo, Cho YongKweon, Choe YongKyung, Park SueNie, Yoon DoYoung

Division of Life Science Korea Research Institute of Bioscience and Biotechnology, Department of Biochem and Health Science, Changwon National University, Division of Viral Products, Korea Food and Drug Administration