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

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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

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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

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Viral oncoprotien E6 and E7 are selectively retained and expressed in carcinoma cells infected with human papilloma virus type 16 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, HaCaT, normal keratinocyte cell line, was prepared to establish stable cell line expressing E7 (HaCaT/E7). We have produced and purified recombinant His tagged E7 oncoprotein and the E7-Ni2+- NTA-affinity column was prepared to obtain E7-interacting proteins. The E7-interacting proteins were resolved in 2D-gel and analysed by matrix-assisted laser desorption/ionization (MALDI/TOF). Twenty two spots were identified by MALDI/TOF. Among 20 spots identified in 2D patterns of the cell lysates of both E7-transfectant and mock bound to his tagged E7 recombinant protein, there are 3 spots not yet identified. CGI95 protein, protein similar to MG11, Livin inhibitor-of-apotosis, MLL protein, protein serine kinase c17, CD2 binding protein 1, G1/S-specific cyclin E1, TATA box binding protein-associated factor and uridine-cytidine kinase 2 were up-regulated by E7 and also bound to E7. It is presumed that E7 can evade immune surveillance by modulating the immune-regulatory factors, factors related to cell signaling and apoptosis, and cell cycle regulator (This work was supported from the Molecular Medicine Program, MOST).

[PC1-35] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Comparison of Protien Profiles in Brain Tissue Lysates of Wild Type and α1B Knockout Mice by Protein Chip Arrays and Mass Spectrometry

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To investigate the role of N-type calcium channels (α1B) in the pain perception, the diverse intracellular protein profiles were compared for the lysates isolated from whole brain between wild type and α1B-deficient mice by protein chip arrays using surface enhanced laser desorption ionization (SELDI)/mass spectrometric detection. The lysates of brain tissue were prepared by homogenation and extraction steps, and a range of molecular size of proteins was checked by 10 % SDS-PAGE analysis using molecular weight markers (25-250 KDa). Five protien chips with selective affinity to subset of proteins were utilized in the functional clustering of proteins in the range of 5-100 KDa. Those proteins bound on the chip were ionized by excitation with a laser source and the ions were detected using surface-enhanced laser desorption ionization mass spectrometric analyzer.
Eight protein peaks were identified to be different in relative intensities between wild and mutant: peaks for 7,785 and 8,434 Da in normal phase chip, 22,911 Da in the Cu-affinity capture chip, 8,417 Da(pH 4) and 12,327 Da (pH 6) in the weak cation exchange chip, 14,241 and 50,892 Da (pH 7), and 10,897 Da (pH 9) in strong anion exchange chip. Identification of these peaks will give insight on the establishment of biomarkers for related CNS diseases.

[PC1-36] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Purification and characterization of human pattern recognition proteins(Hu-PRPs) by using PG affinity column

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The innate immune system is composed of constitutive components of immunity-such as phagocytes, complement and defensins-that recognize foreigh or dangerous entities,
mount an initial protective response to kill and clear the deleterious invader or toxin and
often direct the subsequent adaptive response to the invader.
To find new PRPs from human serum against PG-component of gram positive bacteria cell wall, we prepared PG as ligand, and then it was bound to CNBr-sepharose 4B resin. Mannose-binding lectin(MBL) was purified from PG affinity column. As yet, it was known that mannose-binding lectin(MBL) is specific for mannose or N-acetylglucosamine(GlcNAc).