

Viral oncoprotein 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-apoptosis, 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 Protein Profiles in Brain Tissue Lysates of Wild Type and α 1B Knockout Mice by Protein Chip Arrays and Mass Spectrometry

Lee Jung Ran^o, Kim Daesoo, Choi Myung Ja

Bioanalysis and Biotransformation Research Center,
Korea Institute of Science and Technology, Seoul, Korea

To investigate the role of N-type calcium channels (α 1B) in the pain perception, the diverse intracellular protein profiles were compared for the lysates isolated from whole brain between wild type and α 1B-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 protein 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

Ma YoungGerl^o, Jhun ByungHak, Lee BokLuel

College of Pharmacy, Pusan National University

The innate immune system is composed of constitutive components of immunity-such as phagocytes, complement and defensins-that recognize foreign 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).