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).

Here, we reported MBL purified by using PG affinity column, and its biochemical characterization. MBL might be engaged in activation of complement-MBL mediate pathway.

[PC1-37] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Studies on Inhibitory Effect of Melanoma Cell Invasion by Human Placenta Histone H1

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Histone H1 was purified from human placenta and the inhibitory effect on the invasion of melanoma cell was studied. The histone subraction H1.5 was purified from acid-extract of human placenta. It did not affect the melanoma cell proliferation, and slightly increase the cell motility on gelatin coated membrane. It was detected that histone H1 inhibited the invasion of melanoma cell on Matrigel coated membrane in dose-dependent manner. But, the inhibitory effect of histone H1 on the invasion of melanoma cells were not related to the early expression of MMP gene. These results suggest that the inhibitory action of histone H1 on the invasion of melanoma cells and will provide the possibility of development on anti-invasive agents.

[PC1-38] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Purification of Paraoxonase 1 from Human Plasma

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Human paraoxonase 1 (PON1), an enzyme associated with high density lipoprotein, is responsible for the hydrolysis of organophosphates as well as aryl esters. Especially, the enzyme possesses the ability to protect low density lipoprotein against Cu^{+2} catalyzed oxidation. Here, PON1 is purified from human plasma by using Cibacron Blue 3GA chromatography, DEAE sephacell chromatography, Sephacryl HR-200 chromatoghraphy and Concannavalin A chromatography. Finally, FP-HPLC is utilized to remove other contaminating proteins. The purified paraoxonase, showing a specific activity of 585 μmole /min/mg protein (195 fold) was relatively pure on SDS-PAGE analysis. The enzyme, expressing a Km value of 0.87 mmole, was inactivated irreversibly by p-hydroxymercury benzoic acid and acrolein, indicating that cystein residue exist in the active site. In the addition, the susceptibility of PON1 to Cu^{+2} bound 'OH may support the involvement of histidine residue in active site. Based on the results, human plasma is identical to that from human serum in various physicochemical properties.

[PC1-39] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

The novel peptidoglycan detection system by using prophenoloxidase cascade reaction

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The prophenoloxidase(proPO)-activating system(proPO-AS) is an efficient non-self recognition system in invertebrates that can recognize and respond to micrograms of lipopolysaccharides(LPS) or peptidoglycans (PG) from bacteria and B-1,3-glucans from fungi.
To obtain the solution showing specific phenoloxidase(PO) activity against PG, I have prepared PG-specific solution from Galleria mellonella larvae by using the first and the second Sephadex G-100 gel filtration