HeLa cell lysates bound to E6AP protein, there is one protein not yet identified. Heat shock cognate 71 kDa protein, CTCL tumor antigen se2-5, HLA-A24, Ku70-binding protein, amphiphysin isoform 2, GR AF-1 coactivator-1, eukaryotic translation initiation factor 4A, and BTB domain protein, were bound to E6AP. These results suggest that E6AP can have several functions by interacting with several proteins related to transcription/translation and cytoskeleton, and heat shock cognate protein in cervical carcinoma cells (This work was supported from the Molecular Medicine Program M1-0106-00-0078, MOST).

[PC1-28] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Study of Metastatic Potential of Gastric Cancer Cell Lines by Comprehensive Proteomic Analysis

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Several methods have been developed for the comprehensive analysis of gene expression in cancer cells. Usually these approaches in the postgenome era begin with either a portion of the cellular transcriptome using cDNA microarray or a portion of the cellular proteome using a 2-D gel and MALDI-TOF analysis. Each approaches has distinct conceptual and methodological advantages and disadvantages. Whilst many detailed biochemical studies have been performed about them there are few clinically relevant studies using genomic and proteomic methods. Gastric cancer is very popular in Korea and metastasis of it is a main obstacle for the treatment of it. Like as many other cancers human gastric carcinoma often metastasizes to lymph nodes, but the mechanisms responsible for lymph node metastasis are not clearly understood. To investigate them, the factors associated with metastasis were identified using proteomic method. We have studied the protein expression profiles of gastric cancer cell line, OCUM-2M LN, with a high rate of lymph node metastasis and its parental cell line, OCUM-2M, which exhibited a low rate of lymph node metastasis by two-dimensional (2D) gel electrophoresis and MALDI-TOF. Protein expression profiles of OCUM-2MLN and OCUM-2M cell lines were generated by two-dimensional electrophoresis (2-DE). Twenty proteins in these cell lines were identified as differentially expressed ones. Mass spectrometric analysis of these spots revealed the metastasis-stimulatory or inhibitory proteins. This finding may explain a marked acceleration in metastatic potential of OCUM-2LN and we will discuss our findings based on known discovery.

[PC1-29] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

The Investigation of joint diseases in Equine with Biochemical Factors: Analysis of synovial fluid and serum

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A modification of a colorimetric assay was used to determine synovial fluid total and individual sulfated-glycosaminoglycan concentration of joint disease in equine. For the identification of enzymatic digestion products of the equine synovial fluid , strong anion exchange-high performance liqid chromatography (SAX-HPLC) was performed. By the action of chondroitin ABC lyase, Three unsaturated disaccharides 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose(△Di-COS), 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose(△Di-C6S) and 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose(△Di-C4S) were produced from the equine synovial fluid.The synovial fluid low concentration of sulfated-GAG in abnormal samples. Total sulfated-GAG concentrations (mean±SD) were decreased in horses with joint disease (0.17 ㎎/㎖ ± 0.12 ㎎/㎖), but synovial fluid total sulfated-GAG concentrations of normal (0.82 ㎎/㎖ ± 0.24 ㎎/㎖). The mean value of HA on diluted normal and joint diseases serum were found to be 77.00 ± 66.14 ㎍/㎖ and 168.50 ± 147.50 ㎍/㎖ by labeled B-HABP. there appears to be some correlation between joint inflammation and circulating HA levels as determined by experimental studies of animals.

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

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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

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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]