[PA4-16] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Identification of a 68 kDa cytosolic, neutral and Mg2+-independent Sphingomyelinase by MALDI-TOF Analysis

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A cytosolic, neutral and magnesium-independent Sphingomyelinase (N-cSMase) is known to play a role in vitamin D3-induced differentiation and neurodegeneration such as Alzheimer's disease and stroke through the production of ceramide, a lipid-derived tumor suppressive mediator. However, little is known about its identity and characteristics. Although we have purified and characterized it as the novel enzyme from mammalian brain, it was not purified to homogeneity yet probably because of its highly hydrophobic properties and the coexistence of several proteins of very similar properties. A number of attempts for the purification implied that among the several proteins from active fractions of the final column, 68 kDa protein paralleled the enzymatic activity, suggesting that it may be the real N-cSMase. When the final enzyme preparations were subjected to a two-dimensional electrophoretic analysis, the 68 kDa protein was separated into four spots. To examine further whether the 68 kDa protein is the enzyme, a specific antibody against them should be prepared. Therefore, first, to identify these spots, protein peptide fingerprinting analysis was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometric analysis. Together, in the present study, the possibility that the 68 kDa protein could be N-cSMase as a novel SMase enzyme will be proposed.

[PA4-17] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Differential Protein and Gene Expression after Adenovirus-Mediated p16 Gene Transfer in Human Non-Small Cell Lung Cancer Cells

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For the safety evaluation of adenovirus-mediated gene therapy, we have investigated gene and protein expression after transduction of adenoviral vector (Ad5CMV-p16) which contains tumor suppressor gene, p16INK4α in human non-small cell lung cancer (A549) cells. We compared the differential gene expression level in the A549 cells treated with Ad5CMV (null type) and Ad5CMV-p16 virus, respectively, by using cDNA membrane chip and oligonucleotide chip. These chips have genes related with signal transduction pathways, cell cycle regulations, oncogenes and tumor suppressor genes. We found that Ad5CMV-p16 virus down regulated 5 genes (cdc2, cdk2, cyclin D3, cyclin B, cyclin E) among 26 genes on cDNA membrane chip (Superarray), 20% or more, but expressions of the other genes did not significantly affected by Ad5CMV-p16 virus. We also found that Ad5CMV-p16 virus compare to Ad5CMV up- or down-regulated 27 genes among 1200 genes on oligonucleotide chip (Mergen), two fold or more. N-ras related gene which was known to be involved in carcinogenesis was found to be up-regulated by treatment of Ad5CMV-p16 virus. We are currently confirming whether N-ras related gene is overexpressed after treatment of Ad5CMV-p16 virus by using RT-PCR. We have conducted two demensional gel electrophoresis to detect any unexpected protein expression by transduction of AdCMV-16 in A549 cells. We found that the expression of several proteins were changed to 3 fold or more by using PDQuest program. These results suggest that we have to consider the potential effects of the other gene and protein expressions except therapeutic gene on the host cells as a safety concerns.

[PA4-18] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Proteomic Analysis of Gastric Cancer Patient Sera

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Cancer is multifaceted disease that presents many challenges to clinicians and cancer researchers searching for more effective ways to combat its often devastating effects. Among the central challenges of this disease, are the identification of markers for improved diagnosis and classification of tumors, and the definition of targets for more effective therapeutic measures. The objective of this study is to identify potential biomarkers for the early detection of gastric cancer in serum. Sera from normal volunteers and patients with gastric cancer were examined by two-dimensional electrophoresis. In this display proteomics technique the serum proteins are first separated by isoelectric point followed by polyacrylamide gel electrophoresis. Of the several consistent changes observed in the cancer sera, the most striking was a large increase in a protein level of a molecular mass of ~45 kDa. To identify this protein, database accessible via the internet, such as the SWISS-2DPAGE database and HSC-2DPAGE was utilized. The protein was identified as haptoglobin, which was further confirmed by matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) analysis of the spot and Western blotting analysis with the anti-haptoglobin antibody. Of interest, haptoglobin has been previously suggested to be elevated in ovarian cancer patients. More detailed studies are underway to examine its relevance to the cancer and to validate its practical application as a biomarker for early detection of gastric cancer.

[PA4-19] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

COX-inhibitors down-regulate TCDD-induced cyp1a1 activity in C57BL/6 mouse and Hepa- I cells.

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In order to understand the mechanism of action of TCDD, we have examined the effect of COX-inhibitors on cyp1a1 activity. We observed the effect of COX-inhibitor on EROD activity in C57BL/6 mouse in vovo. And we also evaluated the effect of COX-inhibitors on cyp1a1 mRNA, mouse cyp1a1 promoter activity and EROD activity in Hepa cell.
When Aspirin were pretreated with 3MC in vivo, the EROD activity that was stimulated by 3MC was inhibited. And Pretreatment of Aspirin, Celecoxib, Nimesulide and other several Cox-inhibitors in vitro, inhibited the TCDD stimulated EROD activity and Luciferase acitivity. In case of cyp1a1 mRNA level, Nimesulide and SB100 were able to decrease cyp1a1 mRNA that was stimulated by TCDD, but other tested COX-inhibitors were not decrease. We don't know this different result exactly.
For the action of Cox-inhibitors on the Cyp1a1, it seems to be important to do pretreatment of these chemicals as apposed to TCDD. In this study, thus, we have suggested that COX-inhibitors such as aspirin, celecoxib, Nimesulide and other several Cox-inhibitors decrease the TCDD induced Cyp1a1.

[PA4-20] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Effects of pyrethroid compounds on alkaline phosphatase activity in estrogen receptor positive human breast cancer cells

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Pyrethroids are one of the most commonly used insecticides in worldwide, but it remains unclear whether pyrethroid compounds possess endocrine disrupting activity or not. T47D cells, an estrogen receptor positive human breast cancer cell line, is known to induce alkaline phosphatase (AlkP) only in response to progestins. Because the action of estrogen may be changed by the action of progestins (Kraus et al, 1995), it is important to examine the potential to produce progestin-mediated effects for determining endocrine disrupting activity of chemicals (DiLorenzo et al, 1991). In this study we investigated the progestagenic/ antiprogestagenic effects of pyrethroid compounds using AlkP activity assay and expression of progesterone receptor in T47D cells. After a 48 hr exposure period, progesterone significantly increased AlkP activity in a dose-dependent manner, and maximum activity was observed at the level of 10^{-8}M. However pyrethroid compounds (bioallethrin, cypermethrin, deltamethrin, fenvalerate, permethrin, sumithrin, and tetramethrin) showed no increase in AlkP activity at any concentration. Among seven pyrethroid compounds fenvalerate and permethrin significantly decreased the progesterone-induced AlkP activity, but only at a relatively high concentration (10^{-5}M). The present study suggests that some pyrethroid compounds (fenvalerate and permethrin) have weak endocrine disrupting effects