[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.

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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.

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Proteomic Analysis of Gastric Cancer Patient Sera

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