E101 Characterization of cleaved Tau-induced apoptosis

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Tau, a microtubule-binding protein, has been known to one of the critical pathogens in Alzheimer's disease and in several tauopathies. We have previously shown that Tau is cleaved by caspase-3 during neuronal cell death and expression of cleavage product (Tau-1) induced cell death. Here, we showed that caspase-8 is an essential down-stream caspase of Tau-1(DMVD)-induced apoptosis. Tau-induced apoptosis in SK-N-BE(2)C and B103 neuroblastoma cells was accompanied by proteolytic activation of caspase-6 and caspase-8, which was effectively suppressed by IETD-fmk, a caspase-8 inhibitor, and VEID-fmk, caspase-6 inhibitor, compare to DEVD-cmk, a caspase-3 inhibitor. While C33A cells deficient in caspase-8 expression were resistant to death triggered by Tau-1(DMVD), reconstitution with caspase-8 sensitized C33A cells to Tau-1(DMVD). Dominant negative FADD was also effective to suppress Tau-1 (DMVD)-induced apoptosis. In addition, transient and stable expression of caspase-9 short form as well as Bcl-2 and Bcl-XL blocked the Tau-1(DMVD)-induced apoptosis of neuronal and non-neuronal cells. As a summary, we proposed that caspase-8 plays an important role as a necessary step leading to DTau-induced cell death, implying a putative role of caspase-8 in Alzheimer's disease.

E102 MAP1B bind to p73 and regulates its activity.

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p73, a putative tumor suppressor, is known to induce apoptosis and cell cycle arrest when overexpressed. However, the regulation mechanism through the protein-protein interaction is still investigated. In this study, using the yeast two-hybrid assay with the C-terminal region of p73, we have identified a cDNA encoding MAP1B. MAP1B, microtuble associated protein 1B, is known to stabilize microtuble polymerization. It processed to be a heavy chain and a light chain. In this study, we showed the light chain of MAP1B was binds to p73 in vivo as well as in vitro. MAP1B was originally localized in cytoplasm, while p73 was expressed in nucleus. However, MAP1B was moved into nucleus when it was co-transfected with p73 in 293 cells. To determine the biological function of p73-MAP1B interaction, luciferase assays were performed with reporter plasmid containing p21 promoter. MAP1B slightly increase the transactivity of p73 as dose-dependent manners. We examined the effect MAP1B on the cell death effect function of p73 in the HeLa human cervix epithelial carcinoma cell line by using FACS analysis. MAP1B did not affected on the cell death, but it synergistically increased p73-induced cell death. From the results, we suggest that MAP1B is one of the p73 binding protein and may regulate the p73 activity inducing apoptosis

E103 Protective Effects of Onion Extraction on tert- butyl hydroperoxide-induced Oxidative Damage in HepG2 Humen Liver Cells

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Onion extraction (OE), used Quercetin

products, was studied for its antioxidant bioactivity. In the preliminary study, This antioxidant bioactivity of OE was investigated further using a model of t-butylhydroperoxide(t-BHP)-induced cytotoxicity and genotoxicity in HepG2 human liver cells. Results presented here demonstrate that OE, at concentration of 0.01-1.00 mg/ml, significantly decresed the leakage of lactate dehydrogenase (LDH), and the formation of malondialdehyde (MDA) induced by 30 min treatment of t-BHP (1.5mM) in HepG2 human liver cells. OE also attenuated high level of DNA repaired synthesis. These results lead to speculation that OE presents inhibitory effects against t-BHP caused cytotoxicity and genotoxicity.

E104 Identification of Gastric Cancer Markers from Plasma

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Gastric cancer is the most common cancer in the Korea. It is assumed that unique gastric cancer marker protein can be found from patient's plasma using proteomics technology. The marker protein can be developed for a counter-top diagnosis tools. If the diagnostic tool is available, an early diagnosis of gastric cancer and a rapid diagnosis of gastric cancer recurrence would be possible. The objective of the study is to identify gastric cancer marker proteins from gastric cancer patient's plasma using proteomics technology. In this study, samples from successfully and unsuccessfully chemotherapeutic treated patients' plasmas were analyzed by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). Results from our own studies have shown a similar pattern of changes in protein expression in different gastric cancer samples. More than twelve proteins showed tumor specific alterations. Twelve different proteins disappeared from the plasma which was obtained from a patient whose chemotherapeutic treatment was successful. But the same spots did not disappear from a patient's plasma of unsuccessfully chemotherapeutic treatment. The proteins were characterized by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS). In the future, we are going to validate that the proteins can be used as diagnostic markers using monoclonal antibodies.

E105 Studies on the Calcium Influx Mediated by Glutamate Receptor in Neuronal Cells

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Calcium ion is an important intracellular messenger for the glutamatergic activation in the regulation process of biological rhythm in the mammalian suprachiasmatic nucleus (SCN). Immortalized neuronal cells (SCN2.2) which are derived from the rat SCN are proved to retain its circadian clock properties. The present study was designed to examine whether glutamate induces changes in intracellular calcium in a single cell level using calcium-imaging analysis. L-glutamate (1mM) induced intracellular calcium level: N-Methyl-D-Aspartate (NMDA, 1mM) was effective, while AMPA (1mM) and KA (1mM) failed to do so. We also employed a new calcium-imaging procedure by fluorescence resonance energy transfer (FRET) technique and compared the real-time measurement of calcium changes in a single cell level.