[PC3-5] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Effect of MC fraction of Spatholobus suberectus is on induction of apoptosis.

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Spatholobus suberectus is a herb used for treatment of blood stasis. From fractionation screening on cytotoxicity on cancer cells, MC(methylene chloride) fraction of Spatholobus suberectus was most effective. IC50 of Spatholobus suberectus was 20 ug/ml against U937. It also induced DNA fragmentaion clearly from the concentration of 40 ug/ml. We found apoptotic portion in U937 cells stained by Annexin V by FACS analysis and observed apototic bodies by TUNEL method. Furthermore, the treatment of U937 cells with MC(methylene chloride) fraction of Spatholobus suberectus caused activation of caspase-3 protease and subsequent proteolytic cleavage of poly(ADP-ribose) polymerase. These results suggest MC(methylene chloride) fraction of Spatholobus suberectus has apoptototic activity.

[PC3-6] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Cloning of Human Intestinal Taurine Transporter and Detection of Taurine Transporter by RT-PCR in Murine Organs

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Taurine (2-ethaneaminosulfonic acid) is one of the major intracellular β-amino acids in mammals and is required for a number of biological processes, including osmoregulation, antioxidation, and detoxification. The taurine transporter(TAUT), which contains 12 hydrophobic membrane-spanning domains, has been cloned recently from several species and tissues. One step RT-PCR was performed to amplify a cDNA encoding a TAUT in the human intestinal epithelial cells, HT-29. To define the tissue distribution patterns of the TAUT, one step RT-PCR was used to detect cDNA sequence representing mRNA seven different mouse tissues. The coding region of a PCR product was 732 bp long and two oligomers derived from amino acids 31-38 and 317-324 of TAUT. These primers were designed to encode highly conserved amino acid sequences near the transmembrane domains III (IPYFIFLF) and VI (KYKYNSYR). The resulting sequence of human intestinal TAUT cDNA (Accession number of NCBI Genebank is AF346763) was identical to those TAUTs recently determined in the human placenta and retina except 3 base pairs from that of the reported thyroid. The murine TAUT was detected in all of the mouse tissues analyzed such as heart, lung, thymus, kidney, liver, spleen and brain. A large amount of transcript were found in mRNA isolated from the heart, liver, spleen, kidney, and brain. But lung contained a very small amount of transcript.

[PC3-7] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

p38 MAP kinase is involved in ceramide-induced apoptosis in HL-60 cells.

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The lipid mediator ceramide is emerging as a regulator involved in apoptosis, but the role of ceramide in

apoptosis is still unclear. In the present study, we determined if p38 MAP kinase was involved in ceramide-induced cell death. Treatment with SB203580, a p38 MAP kinase inhibitor, protected HL-60 cells from ceramide-induced cell death, which is in accordance with phosphorylation of p38 MAP kinase induced by ceramide. Ceramide elevated caspase-3 and -9 activities and induced translocation of Bax from cytosol to mitochondria. Treatment with SB203580 diminished caspase-3 and -9 induction and blocked Bax translocation induced by ceramide. These results demonstrate that p38 MAP kinase may play an important role in ceramide-mediated cell death in HL-60 cells.

[PC3-8] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Activation of hypoxia-inducible factor-1a in hepatocarcinogenesis by Hepatitis B virus X protein

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Hepatitis B viruse x (HBx) protein has been directly involved in development of hepatocellular carcinoma (HCC), highly vascularized solid tumors. However, the molecular function of HBx remains controversial. We previously reported that HBx increases the expression of vascular endothelial growth factor (VEGF) mRNA in HBx-expressed cells lead to stimulate angiogenesis in vivo mouse Matrigel plug assay. It is well known that hypoxia-inducible factor-1a (HIF-1a) is major regulator of VEGF expression under hypoxia. Herein, We examined the expression of HIF-1a in the liver of transgenic mice expressing the HBx gene. HBx and HIF-1a proteins are highly expressed in the HBx-transgenic liver, in contrast, these proteins are rare or no expressed in normal liver. Also, VEGF protein is highly expressed in HBx-transgenic liver. HBx induces HIF-1a and VEGF protein level in HEK 293 cells in normoxic condition. We further show that HBx blocks the ubiquitination of HIF-1a in normoxia mediated by down-regulation of p53. Our results suggest that HBx induce expression of HIF-1a in the HBV-infected liver and then stimulates angiogenesis, result in development of HCC.

[PC3-9] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Determination of interaction between a novel biotinylated biocompatible polymer and a hepatoma cell line (HePG2)

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Nanoparticles can be delivered to specific sites in the body by size-dependent passive targeting or by active targeting using receptor-mediated interactions. For developing a potential liver cancer targeting system, we attempted to design a receptor-mediated delivery system using biotinylated pullulan acetate (PA/biotin 500). The interactions between the RITC-labeled nanoparticles [pullulan acetate (PA) and PA/biotin 500] and HepG2 cells were quantified by a microplate fluorescence reader and flowcytometer. PA/biotin 500 showed stronger adsorption to HepG2 cells than PA. We investigated the changes of fluorescence intensity as a function of concentration and cultivation time of PA and PA/biotin 500. The fluorescence intensity of HepG2 cells linearly increased with the concentration of PA and PA/biotin 500. The cultivation time of HepG2 cells for the time also affected the fluorescence intensity in similar way. To clarify the specific interaction between RITC-labeled nanoparticles and HepG2 cells, we attempted to use confocal laser microscopy. HepG2 cells were strongly luminated by specific interactions with PA/biotin 500 while the luminescence of PA was a little observed. Therefore, It is suggested that the PA/biotin 500 has a specific interaction with HepG2 cells by ligand-receptor recognition. In conclusion, PA/biotin 500 is a potential drug delivery system for the treatment of liver cancer. The carrier may induce the immunological enhancement activity in the body and attach to hepatoma cell (HepG2) by ligand-