Novel α-L-Arabinopyranosidase have been purified from *Bifidobacterium berve* K-110, which was isolated from human intestinal microflora with ginsenoside Rb2-hydrolyzing enzyme. α-L-Arabinopyranosidase acted to the greatest extent on p-nitrophenyl-α-L-Arabinopyranoside and ginsenoside Rb2, but did not hydrolyze p-nitrophenyl-α-L-Arabinofuranoside and ginsenoside Rc.α-L-Arabinopyranosidase was purified to apparent homogeneity by a combination of DEAE-Cellulose, Butyl-toyopearl, Hydroxyapatite and Sephacryl S-300 column chromtography with the final specific activity of 8.8μmol/min/mg. Molecular weight of α-L-Arabinopyranosidase is 280 kDa by gel filtration, which is consisted of four identical subunits. (M.W. 72 kDa by SDS-PAGE) Its optimal pH was at pH 5.5-6.0. α-L-Arabinopyranosidase was potently inhibited by Cu2+ and PCMS.

Poster Presentations - Field C3. Cell Biology

[PC3-1] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Caspase-mediated cleavage of CDC6 and its functional implication in apoptotic process.

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Growing evidences suggest that proliferating cells are undergone to the arrest stage of the cell cycle prior to proceeding into apoptotic progression. CDC6, an DNA replication initiation factor has been shown to play an essential role in the regulation of re-initiation of DNA replication during the cell cycle. In the present study, we investigated whether CDC6 is involved in the stage of the cell cycle arrest in apoptotic progression. Here, we provide evidence that CDC6 undergoes caspase 3-mediated cleavage in the early phase of apoptotic progression. Proteolytic cleavage of CDC6 during apoptotic progression appears to occur in general since CDC6 similarly undergoes proteolytic cleavage in apoptotic HeLa cells induced by vaious apototic inducers such as Paclitaxel, Etoposide, and Ginsenoside Rh2. Immunoblot analysis demonstrated that the 62Kda-CDC6 is typically cleaved into a peptide fragment, a 44KDa. Interestingly, the proteolytic cleavage of CDC6 in the apoptotic cells was effectively blocked by treatment of the cells with Z-DEVD-fmk, the caspase 3 inhibitor in HeLa cells. In vitro experiments using [35S]-Methionine labeled CDC6 and recombinant caspase 3 showed that CDC6 is a good substrate for caspase 3. To identify the cleavage site of CDC6 by caspase3, we constructed several point mutants and obtained the CDC6 mutant proteins using the in vitro translation system. The results from the cleavage mapping study showed that the cleavage site is located in the C-terminal that has been reported to contain the Nuclear Export Sequences (NES). From these results, we propose that proteolytic cleavage of CDC6 is functionally relevant event that can results in blocking the translocalization to subcellular location, which in turn, leads the cells into the cell cycle arrest and consequently into apoptosis.

[PC3-2] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Inhibition of PDGF-BB-Induced MAP Kinase(ERK1/2) Activation in Rat Aortic Vascular Smooth Muscle Cells by NQ12

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Several 1,4-naphthoquinone derivatives have been reported to possess many pharmacological effects such as anti-viral, anti-fungal, anti-cancer and anti-platelet activities. We have reported that 2-chloro-3-[4-(ethylcarboxy)-phenyl]-amino-1,4-naphthoquinone(NQ12) had potent inhibitory effect on the platelet aggregation in vitro and thrombosis in vivo. However, little has been known about functional role in vascular smooth muscle cells(VSMCs).

In this study, we examined a possible antiproliferative effect of NQ12 on rat aortic vascular smooth muscle cells(VSMCs). NQ12(1-5 μM) significantly inhibited the PDGF-BB-induced proliferation in a dose-dependent manner on rat aortic VSMCs. We also examined the intracellular signaling effect of NQ12 on the PDGF-BB-induced activation of mitogen-activated protein kinase(ERK1/2) by western blotting in cultured rat VSMCs. Pretreatment of rat VSMCs with NQ12 resulted in a significant inhibition of the PDGF-BB-induced ERK1/2.
These results suggest that the antiproliferative effects of NQ12 may be exerted by the inhibition of the PDGF-BB-induced ERK1/2, which can contribute to prevent atherosclerosis by inhibiting VSMCs proliferation.

[PC3-3] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Overexpression and overactivation of Akt play a critical role in cisplatin resistance

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Chemoresistance is a major obstacle for successful cancer chemotherapy. Although multiple mechanisms have been implicated to be involved in cisplatin resistance, recent evidence has suggested that anti-apoptosis may be a key determinant in chemoresistance. Akt is a serine-threonine kinase known to exert anti-apoptosis effects through several down stream targets. We studied the roles of the Akt in both cisplatin-resistant and 뗌ensitive NIH OVCAR-3 human ovarian cancer cell lines. Treatment of both resistant and sensitive cells with cisplatin stimulated the overexpression of Akt and enhanced phospho-Akt levels in cisplatin resistant cells. Also, we investigated enhanced activation of Akt occurs in cisplatin resistant cells. Taken together, these data demonstrate that apoptotic stimuli activate of Akt and such activation may play a role in the cisplatin resistance.

[PC3-4] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Molecular Cloning and Expression of Recombinant Rat Angiopoietin-1 for the Regulation of Tight Junction Protein Occludin at the Blood-Brain Barrier

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The formation of tight junctions (TJs) is facilitated by the increased expression and phosphorylation of occludin, an integral membrane protein localizing at TJs in endothelial cells, but the physiological regulator of occludin expression is not known.
Angiopoietin-1 (Ang-1) is a recently identified ligand of the endothelium-specific tyrosine kinase receptor Tie-2. Ang-1 knockout mice have hemorrhage from blood capillaries and Ang-1 transgenic mice have leakage-resistant blood vessels. These reports suggest that Ang-1 may control blood-brain barrier (BBB) permeability in vivo. However, the regulation mechanism of BBB permeability by Ang-1 is unclear. Rat is useful animal model to study BBB and TJs, but several investigators have used mouse or human recombinant protein to study Ang-1. In addition, there was no recombinant Ang-1 system to get large amount of protein.
In this study, we isolated a cDNA encoding a 498-amino acid protein from rat placenta using reverse transcription-polymerase chain reaction (RT-PCR). The amplified DNA was cloned into the pGEM-T Easy vector and sequenced. At the level of amino acid sequence, the rat Ang-1 exhibited 97% and 96% identity to its mouse and human homolog, respectively.
The rat Ang-1 was expressed in sf plus insect cells using the Bac-to-Bac baculovirus expression system. The rat Ang-1 gene was cloned into a pFASTBAC HTb donor plasmid, and the recombinant plasmid was transformed into DH10BAC competent cells containing the bacmid. High molecular weight mini-prep DNA was prepared from selected E. coli clones containing the recombinant bacmid. This DNA was used to transfect insect cells. Recombinant baculovirus from the transfection was infected into insect cells for three times. A major band of 65 kDa was detected mainly in the culture supernatant by western blot analysis. The observed molecular mass of the major band was larger than the calculated that of recombinant rat Ang-1 (50 kDa) since Ang-1 contained several potential glycosylation sites.
Moveover, we examined the effect of Ang-1 on TJs function through its effect on the expression of