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