MCF10A Human Breast Epithelial Cells

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The matrix metalloproteases (MMPs) play important roles in invasion, metastasis and angiogenesis in various cell types. Tissue inhibitor of metalloprotease (TIMP)-2, an endogenopus inhibitor of MMP-2, has been shown to inhibit invasion and metastasis. We have previously shown that MMP-2 is responsible for the H-ras-induced invasive and migrative phenotypes in MCF10A human breast epithelial cells. Here, we investigated the effect of TIMP-2 overexpression on invasion and migration in H-ras MCF10A cells. Human TIMP-2 gene was effectively introduced into H-ras MCF10A cells by retrovirus-mediated gene delivery. TIMP-2 overexpression mediated by retrovirus significantly inhibited invasiveness and migration of H-ras MCF10A cells in a dose-dependent manner. We also show the antiangiogenic effect of TIMP-2 gene delivery. Taken together, our study shows that retrovirus-mediated delivery of TIMP-2 efficiently inhibits metastatic progression of ras-trnasformed human breast epithelial cells, suggesting a potential use of the TIMP-2 gene therapy for the treatment of breast cancer.
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[PC3-14] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Novel Cell-based Protease Assay System for Molecular Cell Biology and Drug Discovery

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Recently development of cell-based assay systems which are useful in molecular cell biology and drug discovery attracts significant attention. Here, we introduce a new technologies for monitoring enzyme activity and its inhibition inside living cells. Among various enzymes, proteases are important targets for studying various biological and disease-related processes such as viral infections, apoptosis and Alzheimer's disease. In this study, a sensitive cell-based protease detection system that enables direct fluorescence detection of a target protease and its inhibition inside living cells is introduced. The CellPA^{TM} system provides a fluorescent molecular beacon protein comprising an intracellular translocation signal sequence(s), a protease-specific cleavage sequence(s) and a fluorescent marker sequence(s). The molecular beacon protein is designed to change its intracellular translocation upon cleavage by a target protease, e.g., from cytosol to a subcellular organelle, or from a subcellular organelle to cytosol or another subcellular organelle. Details of the mechanism and level of the protease action can be monitored at a single cell level, and accordingly the cell population in terms of the level of the protease activity can be accurately enumerated. The clear change in the fluorescence image of the cell makes the CellPA^{TM} system as an ideal tool for various life science and drug discovery researches including the HTS&HCS applications. Various formats of the CellPA^{TM} system for monitoring HCV NS3 protease, caspase-3, caspase-8, β-secretase etc. will be presented.

[PC3-15] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Erythropoietin increases neuronal cell differentiation : association of transcriptional factors AP-1 and NF-κB activation

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Erythropietin (EPO), a hematopoietic factor is also required for normal brain development, and its receptor is localized in brain. Therefore, it is possible that EPO could act as a neurotropic factor inducing differentiation of neurons. The present study, we therefore investigated whether EPO can increase differentiation of undifferentiated cortical neuron isolated from postneonatal (Day 1) rat brains and PC12 cell, undifferentiated dopaminagic cell line. EPO dose (1-100 U/ml) dependently increased cell differentiation and expression of differentiation marker gene (neurofilament and tyrosine hydroxylase) in both cells. Since our previous study (Jung et al., 2003, Mol.

Pharmacol 63:607-616) showed that transcription factor AP-1 is important signal factor involved in PC12 cell differentiation, we further determined AP-1 and other transcription factor NF-κB activation during cell differentiation. Concomitant with cell differentiation, AP-1 and NF-κB was activated at lower dose (0.5-5 U/ml) of EPO in a dose dependent manner. In addition, in the presence of anti-EPO antibody, the effect of EPO was partial blocked. These data show that EPO induced neuronal cell differentiation, and transcriptional factor AP-1 and NF-κB may be involved in neuronal cell differentiation.

[PC3-16] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

The Effect of Anticarcinogenic Activity of Rhodiola Sachalinesis Extract

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This study was performed to determine the anticarcinogenic activity of the Rhodiola Sachalinesis Extract (RS) on several microorganisms and human cancer cell lines. Among the various solvent fractions of RS, the ethylether partition layer (RSMEE) showed the strongest antimicrobial activity, ethylacetate partition layer (RSMEA) resulted in good antimicrobial activity. We also determined the effect of RS extract and fractions on cytotoxicity, and chemopreventive effect on human cancer cells. The experiment was conducted to determine cytotoxicity of RS partiton layers on HepG2, HeLa, HT-29 and MCF-7 cells by MTT assay. Among the various partition layers of RS, RSMEE were showed the strongest cytotoxic effects on all cancer cell lines. The Quinone reductase induced activities of HepG2 cell, the ethylether partition layer (RSMEE) was 3.21 times more effective compared to the control value of 1.0. This value was significantly higher than that of previous results using the other materials. Therefore, vased on these studies, RS may be developed into a potentially usful antimicrobial and anticarcinogenic agents.

[PC3-17] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Activation of MKK6 induces invasive and migrative phenotypes in MCF10A human breast epithelial cells

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Ras expression has been suggested as a marker for tumor aggressiveness of breast cancer, including the degrees of invasion and tumor recurrence. We previously showed that p38 MAPK is a key signaling molecule differentially regulated by H-ras and N-ras, leading to H-ras-specific cell invasive and migrative phenotypes in human breast epithelial cells (Cancer Res.: 63, 5454-5461, 2003). In this study, we further investigated the role of p38 MAPK pathway in the induction of metastatic potential in MCF10A cells as a "gain of function" study. We established stable transfectants of MCF10A expressing constitutively activated mutant of MAP kinase kinase (MKK)-6, the direct upstream activator of p38 MAPK. We show the induction of invasion and cell migration with specific upregulation of MMP-2 in these cells, demonstrating the role of p38 MAPK pathway in the metastatic potential in MCF10A cells. [Supported by a grant (R04-2003-000-10063-0) from the Basic Research Program of the Korea Science & Engineering Foundation]

[PC3-18] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Characterization of Erythropoietin Producing Cell Lines after Introduction of Urea Cycle Enzymes, Carbamoly Phosphate Synthetase and Ornithine Transcarbamoylase

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