level, which was completely abolished by pretreatment with catalase. However, this activation of ERK does not appear to be attributed to nuclear translocation of Smads, because nuclear translocation of Smads in response to TGF-β was not affected by inhibiting ERK signaling pathway, and also treatment with H_2O_2 alone did not cause the nuclear translocation of Smads. On the other hand, ERK inhibition caused the disruption of interaction between Smad3 and Sp1 induced by TGF-β, suggesting that ERK signaling pathway might be necessary for their interaction and essential for the TGF-β induction of $p21^{WAF1/Cip1}$. Taken together, these results suggest that H_2O_2-mediated ERK signaling pathway might be required for $p21^{WAF1/Cip1}$ expression by TGF-β and play as a key determinant for interaction between Smads and Sp1 transcription factor.

[PC1-30] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

15-DEoxy-$d^{12,14}$ Prostaglandin J_2 Rescues Pc12 Cells From Hydrogen Peroxide-induced Apoptosis Through Upregulation Of Heme Oxygenase-1

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Oxidative stress induced by reactive oxygen intermediates (ROIs) has been implicated in a variety of human diseases including cancer, diabetes, rheumatoid arthritis and neurodegenerative disorders. Hydrogen peroxide (H_2O_2), a representative ROI which is produced during the cellular redox process, can cause cell death via apoptosis and/or necrosis depending on its concentrations. 15-Deoxy-$D^{12,14}$ prostaglandin J_2 (15d-PGJ_2), a dehydration product of prostaglandin D_2, has been reported to possess a number of biological activities such as anti-inflammatory, anticarcinogenic, and antioxidative properties. In this study, we have investigated the protective effect of 15d-PGJ_2 on H_2O_2-induced oxidative stress in rat pheochromocytoma (PC12) cells.H_2O_2 treatment caused oxidative PC12 cell death in a concentration dependent manner. PC12 cells treated with H_2O_2 exhibited apoptotic cell death as determined by morphological features, internucleosomal DNA fragmentation, cleavage of poly (ADP-ribose)polymerase, an increased Bax/Bcl-X_L ratio and decreased mitochondrial membrane potential, all of which were inhibited or restored by relatively low concentration of 15d-PGJ_2 pretreatment. In another experiment, PC12 cells treated with 15d-PGJ_2 exhibited transient activation of Akt/protein kinase B as well as extracellular signal-regulated kinase 1/2 and induction of heme oxygenase-1 (HO-1) expression and nuclear translocation of Nrf-2 as an adaptive response to oxidative insult. In conclusion, H_2O_2 caused apoptosis in PC12 cells by inducing oxidative stress, which was effectively protected by 15d-PGJ_2 through augmentation of the cellular antioxidant defence involving HO-1 and Nrf-2.

[PC1-31] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Eupatilin, a Pharmacologically Active Flavone Derived from Artemisia Plants, Induces Cell Cycle Arrest in Ras-Transformed Human Mammary Epithelial Cells

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Extracts of Artemisia asiatica Nakai (Asteraceae) possess anti-inflammatory and anti-oxidative activities. Eupatilin (5,7-dihydroxy-3,4,6-tri-methoxy-flavone), one of the pharmacologically active ingredients derived from Artemisia asiatica, was shown to induce apoptosis in human promyelocytic leukemia (HL-60) cells (H.-J. Seo and Y.-J. Surh, Mutat. Res., 496, 191-198, 2001). In the present study, we examined the cytostatic effects of eupatilin in H-ras-transformed human breast epithelial (MCF10A-ras) cells. Eupatilin inhibited the growth of MCF10A-ras cells in a concentration-dependent and time-related manner as determined by MTT reduction and [^{3}H]thymidine incorporation assays. To determine whether the antiproliferative effects of eupatilin are mediated through dysruption of the cell cycle in MCF 10A-ras, DNA contents were analyzed by the flow cytometry. The area of the peak corresponding to a hypodiploid or apoptotic DNA content didn't change by eupatilin treatment. However, eupatilin (100 μM) blocked the cell cycle progression in both G1/S and G2/M phase. Moreover, eupatilin inhibited the expression of Cdk2, Cdc2, cyclin B1 and cyclin D1, which are responsible for mediating

cell cycle progression, while it increased the expression of cyclin-dependent kinase inhibitors such as $p27^{kip1}$ and p53. In addition, expression of $p21^{waf/Cip1}$ was decreased at both protein and mRNA levels. Eupatilin also inhibited the activation of ERK1/2 as well as expression of Raf-1 and Ras in MCF10A-ras cells. The inhibitory effect of eupatilin on cyclin D1 expression is mediated by targeting the Raf/MEK/ERK signaling cascades. Eupatilin didn't change activation of Akt, an important component of pro-survival signaling pathways. In conclusion, the anti-proliferative effect of eupatilin is associated with its inhibition of ERK1/2 activation and subsequent blocking of both G1/S and G2/M phases of cell cycle progression in MCF10A-ras cells.

[PC1-32] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Docking of Retinol into the 3D Structural Model of Human TCTP Constructed by Homology Modeling

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TCTP is presented to have a retinol binding protein (RBP)-like structure by domain search. Human cellular RBP (CRBP) plays a key role in the intercellular transfer of retinol. Modulation of its expression is known to contribute to tumor growth and progression via retinoid-mediated signaling. Changes in the expression of TCTP have also been reported to be associated with carcinogenesis. Therefore, the attempt to establish the interactive relationship between the human TCTP and CRBP with retinol will be helpful in further understanding the cell signaling of TCTP. To this day, the three dimensional (3D) structure of the TCTP has not been known. In this study, the 3D model of the protein was constructed using MODELLER program of homology modeling technique. Docking of retinol into this model was performed with QXP program in which both protein and ligand are simulated flexibly. In order to find a possible binding site of retinol in the TCTP, multiple alignments were carried out with the sequences of the TCTP and those of the RBP and CRBP, respectively. The docking result of retinol into TCTP was compared with the binding modes of retinol with the RBP in both crystal and docked (rmsd=0.84Å) structures. Retinol interacted with the residues of the TCTP that were correlated with those of the active sites of the RBP and CRBP. The docking result of retinol into CRBP corresponded well with the suggested binding modes of the published data. Docking energy showed that retinol has been stably docked into the two proteins. These results suggest that retinol could bind to the TCTP and might contribute to the comprehension of the process of carcinogenesis.

[PC1-33] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Development of efficient detection methods of CDK2 (or 4) activities for mass screening

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Mammalian cell cycles are tightly regulated by cyclins, cyclin dependent kinase (CDK), Retinoblatoma (Rb) protein, and cellular CDK inhibitors (CDKI). Cyclin dependent kinases (CDK) are key enzymes regulating eukaryotic cell cycle. And also it is recognized that the abnormal increase of CDK activities is one of the common events in human cancer and CDK inhibitors have therapeutic values in cancer treatment. Until now it is known that over 10 different CDKs participate in cell cycle regulation.In most studies, CDK activities are measured by radioisotopic methods using $g\text{-}^{32}P$ labeled ATP for the accurate measurements of kinase activities. However it is not appropriate for mass screening of CDK inhibitors because the method is dangerous, uneasy and high cost process.Here we tried to set up new methods to simply measure CDK2 or 4 activities using fluorecence labeled peptide substrates and agarose gel electrophoresis. The substrate is designed to be N-terminally FITC-conjugated peptide consisting of both CDK2 (or 4) binding domain and phosphorylation domain in Rb protein. The substrate was well phosphorylated by immunoprecipitated cyclin D-CDK4 complex. Furthermore it was revealed that partially purified cell extracts could be used as CDK2 (or 4) enzyme sources using competitor test. To test whether this assay system is applicable to mass screening, we screened some compounds showing CDK inhibition and confirmed that they inhibit Rb phosphorylation in cell-based assay. Taken together, efficient new methods for measuring CDK2 (or 4) activities was established.