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.