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Histone deacetylases (HDAC) activity is associated generally with transcriptional repression. We have reported previously that apicidin, a histone deacetylase inhibitor, inhibited the proliferation of tumor cells via induction of p21 WAF/CIP1 . We extended our study to identify the effect of apicidin on the expression of other cell cycle regulatory protein, such as cyclin E, a critical regulator of the transition from G1 into S phase. Treatment of HeLa cells with apicidin result in the activation of cyclin E transcription that led to elevated cyclin E protein levels and to regulated positively mRNA levels of cyclin E. This transcriptional activation appears to be mediated by protein kinase C (PKC), because a PKC inhibitor attenuated the activation of cyclin E promoter and the expression of cyclin E induced by apicidin. In spite of cyclin E induction, p21 WAF/CIP1 induced by apicidin specifically bound with cdk2/cyclin E complexes, leading to decrease of cdk2 activity and subsequent arrest of cell cycle at G1 phase. There is much circumstantial evidence that the control of cyclin E expression is implicated in both E2F transcription factors and the retinoblastoma protein (pRB). However, transcriptional activation of cyclin E by apicidin might be mediated by sp1-binding sites, because mutation of the known E2F-binding sites in the cyclin E promoter did not block the activation by apicidin. Promoter activity and protein expression of cyclin E were significantly decreased by mithramycin, a specific inhibitor of sp1, and dominant-negative sp1 construct. Therefore, we make an attempt at the analysis of cyclin E promoter by the subject currently.

[PC1-34] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Stable expression of N-terminal 3X-FLAG tagged human 5a-reductase type II in 293 cells: a new tool for protein purification & inhibitor screening

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Human 5-reductase type II(5AR2) is an important target for the treatment of benign prostatic hyperplasia. In this study we describe the establishment of cell line which stably expressed 3X FLAG tagged human 5AR2. We used this cell line as a cell based assay tool and source for 5AR2 enzyme. First a plasmid (3XFLAGpCMV10-5AR2) for the expression of 5AR2 was constructed by the use of the vector 3XFLAGpCMV10 and transfected into the HEK 293. By selection with G418 sulfate, ten HEK 293 single cell clones were obtained of which three stably exhibited high 5AR2 activity. One single cell clone (HEK293-5AR2) was selected for further study. By Western blot analysis, it turned out that the selected cell line express stablely 3XFLAG tagged 5AR2 protein, and 3XFLAG tagged 5AR2 protein was purified via immunoprecipitation using anti-FLAG monoclonal antibody attached agarose(anti-FLAG M2 affinity gel). The newly established cell line was also used for testing standard compounds on their inhibitory effect on human 5AR2. Using this whole cell assay, inhibitors with IC50 values in the nanomolar range could be identified. In conclusion, we constructed stable cell line which expresses 3XFLAG tagged 5AR2, this cell line can be used as a tool for cell based screening and a source for human 5AR2.

[PC1-35] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Involvement of Proinflammatory Cascades in Nitrosative Damage in PC12 Cells

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Recent studies suggest that inflammatory events are implicated in a variety of human diseases including cancer and neurodegenerative diseases, and non-steroidal anti-inflammatory drugs have beneficial effects in treatment or prevention of these disorders. It has been reported that expression of cyclooxygenase (COX)-2 and nitric oxide synthase and subsequent production of prostaglandin (PG) and nitric oxide (NO), respectively are elevated in many inflammatory disorders. In the present study, we have investigated a possible involvement of reactive nitrogen species in COX-2 signaling cascades in PC12 cells. Treatment of PC12 cells with sodium nitroprusside (SNP), a NO generator or 3-morpholinosydnonimine hydrochloride (SIN-1), a peroxynitrite donor, induced oxidative cell death. During apoptotic cell death induced by SNP or SIN-1, expression of COX-2 and peroxysome proliferator-activated receptor-γ (PPAR-γ) and production of PGE_2 were increased. Selective COX-2 inhibition by celecoxib blocked the SNP-induced cell death. While PGE_2 enhanced the SIN-1-mediated cell death, the PPAR-

γ antagonist, GW9662, rendered PC12 cells sensitized to SIN-1. The above findings suggest possible involvement of COX-2 induction and PG synthesis in regulating nitrosative PC12 cell death. PGE_2 may mediate apoptosis induced by peroxynitrite in PC12 cells. On the other hand, 15d-PGJ_2 may act as a negative feedback mediator of COX-2 signaling cascades.

[PC1-36] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Autotaxin-induced tumor cell motility requires the activation of Rac/Cdc42, PAK, and FAK

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Cell motility plays important physiological roles in embryogenesis, immune defense, wound healing, and metastasis of tumor cells. Cell motility of normal cells is tightly regulated, while tumor cell motility is aberrantly regulated or autoregulated. Autotaxin (ATX) is a 125-kDa glycoprotein, originally isolated from the conditioned medium of human melanoma A2058 cells. ATX stimulates random (chemokinetic) and directed (chemotactic) motility of human tumor cells at high picomolar to low nanomolar concentrations. Recently, ATX has been shown to augment invasive and metastatic potential of ras-transformed cells. In MatrigelTM invasive assays, NIH3T3 cells with full length ATX cDNA demonstrated greater spontaneous and ATX-stimulated invasion than control. In addition, in vivo study showed that combination of ATX expression with ras transformation amplified tumorigenesis and metastatic potential compared to ras-transformed control, suggesting that ATX augments cellular characteristics necessary for tumor aggressiveness. In the present study, we investigated the intracellular signaling pathway of ATX. Unlike N19Rho expressing cells, the cells expressing N17Cdc42 or N17Rac1 showed reduced motility against ATX. In addition, ATX increased PAK activity and phosphorylated focal adhesion kinase. Since FAK in cells expressing N17Rac1 or N17Cdc42 was not phosphorylated by ATX, FAK appears to be located downstream of Cdc42/Rac1. Collectively, these data indicate that Cdc42, Rac1, and FAK are involved in ATX-induced tumor cell motility in human melanoma cells.

[PC1-37] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Involvement of G1 arrest and caspase-3 activation in apoptosis induced by bovine lactoferricin

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We investigated the effect of bovine lactoferricin (Lfcin-B) on cell cycle regulation and caspase activation in tumor cells. Treatment with Lfcin-B resulted in the production of intracellular reactive oxygen species (ROS) during apoptosis of THP-1 cells. Biochemical analysis revealed that Lfcin-B-induced apoptosis, the cell cycle arrest and caspase activation were completely abrogated by addition of an antioxidant such as N-acetylcysteine (NAC). In cell cycle analysis using the bromodeoxyuridine (BrdU) labeling method, it was shown that Lfcin-B blocked the progression of the cell cycle to S phase (G1 arrest) in THP-1 cells undergoing apoptosis. In coincidence with G1 arrest, the results of western blot analysis showed that treatment with Lfcin-B prominently decreased the expression of Cyclin D2, CDK2, CDK4 and Cyclin E molecules responsible for progression to S phase. In addition, treatment with Lfcin-B enhanced the intracellular activity of caspase-3 and ?8 in the early period of apoptosis. When we investigated the correlation of ROS production, G1 arrest and caspase-3 activation in apoptosis induced by Lfcin-B, it was revealed that ROS regulated G1 arrest and caspase activation at a point of up-stream of the apoptosis cascade.

[PC1-38] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Retrovirus-mediated Delivery of TIMP-2 Inhibits Migration, Invasion and Angiogenesis

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