Tech., Sungkyunkwan University, College of Medicine, Konyang University

Histone deacetylases (HDAC) activity is associated generally with transcriptional repression. We have reported previously that apicidin, a histione 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

Lee ChangHoon°, Park WonSeok, An SuMi, Nam GaeWon, Kim KwangMi, Kim SeungHoon, Lee ByeongGon, Jang IhSeop

Skin Research Team, Skin Research Institute, Pacific R&D Center 314-1 Bora-ri, Kiheung-eup, Yongin-si, Kyoungg-do,

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

Lim SoYoung°, Jang JungHee, Na HyeKyung, Surh YoungJoon

College of Pharmacy, Seoul National University

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-