

induced NF- κ B activation. The MEK1/2 inhibitor U0126 strongly inhibited NF- κ B activation through blockade of I κ B α phosphorylation, while p38 inhibitor SB203580 did not much influence TPA-induced NF- κ B activation in mouse skin. Taken together, suppression of TPA-induced COX-2 expression by blocking activation of ERK and NF- κ B may account for molecular mechanisms by which curcumin exerts anti-tumor promoting effects on mouse skin tumorigenesis.

[PC1-31] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Proteome Analysis of Apicidin-Treated Human Cervix Cancer Cells

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Apicidin [cyclo(N-O-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-oxodecano y)], a histone deacetylase inhibitor, has been shown to cause growth arrest and morphological change of cancer cells, resulting from the alternation of protein expression, such as p21WAF1/Cip1 and gelsolin. However, proteome of altered by apicidin are poorly studied. In this study, we used a functional proteomics approach to identify the proteome altered by apicidin in HeLa cells at 24hr post-treatment. To identify the proteome altered by apicidin, we used two-dimensional electrophoresis and MALDI-TOF mass spectrometry: We were able to resolve more than 1000 protein spots each in both treated and untreated HeLa cells. We found here that cyclophilin I was decreased by apicidin treatment. Cyclophilin I have been shown to process peptidyl-prolyl cis-trans isomerase activity, which is thought to contribute to the proposed role of cyclophilin I as mediator of protein folding and as chaperones. Also Hsp27 has shown to be modified by apicidin treatment, e.g. phosphorylation or acetylation. This modification might be attributable to the morphological change by apicidin, because Hsp27 phosphorylation has been considered to be closely involved in actin-cytoskeleton rearrangement. These results suggest that apicidin may affect the function of molecular chaperones, and the elucidation of possible role of these proteins is our current subject.

[PC1-32] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Differential involvement of JNK in apicidin-induced apoptosis.

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We previously reported that apicidin induces apoptosis through selective induction of Fas/Fas ligand, resulting in the release of cytochrome C from mitochondria to the cytosol and subsequent activation of caspase-9 and ?. However, we observed that apicidin did not induce the apoptosis in a specific cell line, such as HeLa, which was characterized by nuclear DNA fragmentation. On the basis of these facts, we tested whether JNK activation is involved in cell death induced by apicidin. JNK signaling pathway might be required for the apicidin induction of apoptosis in Jurkat cells but not HeLa cells, because specific inhibition of JNK with SD600125 and dominant negative JNK significantly inhibited PARP cleavage in Jurkat cell, but not HeLa cell. Furthermore, we observed the difference in constantly expressed level of Hsp70, which acts as an anti-apoptotic chaperone through the inhibition of JNK activity via physical interaction, suggesting that this difference might be contributable to the decision between apoptosis and survival in response to apicidin. Therefore, we will attempt to elucidate the possible role of Hsp70 using a specific down regulation with antisense oligonucleotide against Hsp70 in HeLa cell and overexpression of Hsp70 in Jurkat cell, respectively

[PC1-33] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Apicidin Induction of cyclin E might be mediated by Sp1 Transcription Factor

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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 5 α -reductase type II in 293 cells: a new tool for protein purification & inhibitor screening

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Human 5 α -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 stably 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-morpholinonydnonimine 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 PGE₂ were increased. Selective COX-2 inhibition by celecoxib blocked the SNP-induced cell death. While PGE₂ enhanced the SIN-1-mediated cell death, the PPAR-