Unusual Induction of Cyclooxygenase-2 in H-Ras-Transformed Human Breast Epithelial Cells Undergoing Apoptotic Death

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Cyclooxygenase-2 (COX-2) is an inducible enzyme expressed in response to a variety of proinflammatory. The presence of oncogenic *ras* has been associated with sustained induction of COX-2, which confers resistance to apoptosis. Contrary to the above notion, we found that MCF10A-*ras* cells treated with an anti-tumor agent, ET-18-O-CH3, underwent apoptosis as revealed by proteolytic cleavage of poly(ADP-ribose) polymerase, pro-caspase 3 activity, and TUNEL staining, while the same treatment caused an increased expression of COX-2 as well as the elevated production of prostaglandin E_2(PGE_2). The apoptotic effect of ET-18-O-CH3 involved intracellular accumulation of reactive oxygen species. Treatment of MCF10A-*ras* cells with the selective COX-2 inhibitor celecoxib (50 μM) attenuated ET-18-O-CH3-induced apoptosis as well as COX-2 expression and production of PGE_2, suggesting that unusual expression of COX-2 by ET-18-O-CH3 is causatively implicated in the induction of apoptosis. ET-18-O-CH3 inhibited activation of both Akt/protein kinase B and transcription factor NF-κB that are involved in cell survival pathways. ET-18-O-CH3 also inhibited activation of ERK1/2 and p38. ET-18-O-CH3-induced inactivation of these protein kinases and NF-κB was attenuated by ce ecoxib. Taken together, the above findings suggest that COX-2 up-regulation does not necessarily confer the resistance to apoptosis in *ras*-transformed cells, but may rather sensitize these cells to apoptotic death. This work was supported by the grant (01-PJ1-PG1-01CH05-0001) from the Ministry of Health and Welfare, Republic of Korea.

[OB-1] [04/19/2002 (Fri) 14:30 - 14:40 / Hall A]

Cyclin-Dependent Protein Kinases Play an Essential Role in Apoptotic Progression

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In our earlier report, we have shown that activation of cyclin A-cdk2 activity is an essential event in apoptotic progression of SK-HEP1 cells induced by treatment with ginsenoside-Rh2 (G-Rh2). In the present study, we provide evidence that abnormal activation of cyclin-dependent protein kinases activities are commonly occurring in different cell types induced by various apoptosis inducing systems, including Etoposide, Paclitaxel, and TRAIL. Both Cdk2 and Cdc2 kinase activities were dramatically up-regulated in apoptotic cells induced by treatment with Paclitaxel or TRAIL. By contrast, Cdk2 but not Cdc2 kinase activity was remarkably up-regulated in Etoposide-induced apoptosis. Forced down-regulation of cdk2 activity by ectopic overexpression of p21WAF1/CIP1, a potent inhibitory protein of Cdk2, or that of the dominant negative version of Cdk2 (Cdk2-dn) efficiently and equally blocked the apoptosis progression of the cells induced by three different apoptosis inducers. Overexpression of cyclin A in the cells resulted in a dramatic up-regulation of cyclin A-Cdk2 activity and accordingly, enhanced apoptosis in same system. Ectopic overexpression of dominant negative version of Cdc2 also successfully suppressed the TRAIL- or pacletaxel-induced apoptosis. From these data, we propose that activation of cdk2 and/or cdc2 is a general prerequisite event in apoptotic progression that undergoes mediating through either mitochondria and death-receptor pathways.

[OC-1] [04/19/2002 (Fri) 14:40 - 14:50 / Hall A]

Celecoxib Down-regulates Phorbol Ester-induced Expression of Cyclooxygenase-2 through Inhibition of AP-1 and C/EBP Transcripton Factors in Mouse Skin In Vivo

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Cyclooxygenase (COX) catalyzes the rate-limiting step in the formation of prostaglandins from arachidonic

acid. There are two isoforms of COX, designated as COX-1 and COX-2. While COX-1 is constitutively expressed in most tissues, COX-2 can be induced transiently by proinflammatory cytokines, endotoxins, growth factors, oncogenes, UV and mitogens. Elevated levels of COX-2 have been observed in cancers of breast, colon, and lung as compared with the surrounding normal tissues. Based on these findings, it is conceivable that targeted inhibition of inappropriate or abnormal up-regulation of COX-2 is one of the most broadly effective and promising approaches to cancer chemoprevention. Celecoxib, a selective COX-2 inhibitor, has been reported to prevent experimentally induced colon, breast and skin carcinogenesis. Moreover, daily intake of celecoxib resulted in significant reduction of polyps in patients with familial adenomatous polyposis. In the present study, we examined the effect of celecoxib on COX-2 induction in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated mouse skin. Topical application of 0.1, 1.0, or 10 μ mole celecoxib onto shaven backs of female ICR mice (6 to 7 wk of age) 30 min prior to 10 nmole TPA inhibited expression of COX-2 protein and subsequent production of prostaglandin E_2 in a dose-related manner. Celecoxib inhibited activation of AP-1 and C/EBP transcription factors in a dose dependent manner. Furthermore, celecoxib inhibited both catalytic activity and phosphorylation of ERK1/2. These results suggest that celecoxib suppresses TPA-induced COX-2 expression in mouse skin by blocking activation of ERK, which appears to be mediated by transcription factors such as AP-1 and C/EBP.

[OC-2] [04/19/2002 (Fri) 14:50 - 15:00 / Hall A]

BETA-AMYLOID INDUCES OXIDATIVE DNA DAMAGE AND CELL DEATH: POSSIBLE INVOLVEMENT OF INFLAMMATORY CASCADES

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Oxidative stress induced by reactive oxygen and/or nitrogen species has been considered as a major cause of cellular injuries in a variety of neurodegenerative disorders including Alzheimer's disease (AD). Inflammatory as well as oxidative tissue damage has been implicated in pathophysiology of AD, and non-steroidal anti-inflammatory drugs have been reported to have beneficial effects in the treatment or prevention of AD. In this study, we have investigated the molecular mechanisms underlying oxidative and inflammatory cell death induced by beta-amyloid, a neurotoxic peptide associated with senile plaques formed in the brains of patients with AD. Rat pheochromocytoma (PC12) cells treated with beta-amyloid exhibited increased intracellular accumulation of reactive oxygen species and underwent apoptotic death as determined by characteristic morphological features, internucleosomal DNA fragmentation and positive in situ terminal end-labeling (TUNEL staining). beta-Amyloid treatment also led to the cleavage of poly(ADP-ribose) polymerase, the increased Bax/Bcl-XL ratio and the decreased mitochondrial membrane potential in PC12 cells. Furthermore, transfection of PC12 cells with *bcl-2* rescued these cells from apoptotic death induced by beta-amyloid. beta-Amyloid caused activation of NF-κB and AP-1, which appeared to be preceded by activation of mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase 1/2 (ERK1/2), p38 MAPK and c-Jun N-terminal kinase/stress-activated protein kinase. Exposure of PC12 cells to beta-amyloid resulted in time-dependent induction of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase. beta-Amyloid-induced cell death was attenuated by pretreatment with the selective COX-2 inhibitor celecoxib or the peroxynitrite scavenger ergothioneine. These results suggest that prooxidative and proinflammatory mechanisms are involved in the oxidative and/or nitrative cell death in mediating the neurodegeneration associated with AD.

[OD-1] [04/19/2002 (Fri) 15:00 - 15:10 / Hall A]

Cytotoxic Terpenoids from the Sponge Sarcotragus Species

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Twenty-one new and two known terpenoids were isolated from the marine sponge Sarcotragus sp., including twelve furanosesterpene and their derivatives, seven new pyrrolosesterterpenes two trinorsesterterpenes, and two diterpenes were isolated from the same sponge by bioactivity-guided