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