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CELECOXIB ATTENUATES ET-18-O-CH3-INDUCED APOPTOSIS IN H-ras TRANSFORMED HUMAN BREAST EPITHELIAL CELLS

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Cyclooxygenase-2 (COX-2) is an inducible enzyme expressed in response to a variety of proinflammatory agents and cytokines. COX-2 expression has been shown to be elevated in several different types of human cancer. The presence of oncogenic ras has been associated with constitutive induction of COX-2 in certain H-*ras* transformed cells, and COX-2 overexpression confers resistance to apoptosis. Contrary to the above notion, we have found that apoptotic death occurs while the COX-2 expression is induced in the H-ras transformed human breast epithelial cell line (MCF-10A-*ras*) treated with ET-18-O-CH_3 (1-*O*-octadecyl-2-*O*-methyl-glycero-3-phosphocholine), a specific inhibitor of phosphoinositide phospholipase C. Thus, while MCF-10A-*ras* cells were found to undergo apoptosis by treatment with ET-18-O-CH_3 as reveled by proteolytic cleavage of poly(ADP-ribose) polymerase (PARP) and positive terminal deoxynucleotidyl transferase-mediated dUTP nick-labeling (TUNEL), the cells exhibited an increased expression of COX-2 as well as the elevated production of prostaglandins (PGE_2). To determine whether the expression of COX-2 by ET-18-O-CH_3 is associated with the induction of apoptosis, the effects of the selective COX-2 inhibitor celecoxib (SC-58635) on ET-18-O-CH3-induced apoptotic death were examined. Treatment of MCF-10A-*ras* cells with celecoxib (50 μM) attenuated ET-18-O-CH_3-induced apoptosis as well as COX-2 expression and production of PGE_2 in a concentration dependent manner. To delineate the upstream signaling pathways responsible for the apoptosis-inducing activity of ET-18-O-CH_3, we examined the effect of this drug on activation of the anti-apoptotic protein Akt/protein kinase B and also mitogen-activated protein (MAP) kinases such as c-Jun NH_2-terminal kinases (JNK), extracellular signal-regulated kinases (ERK) and p38 MAP kinase that play a role in regulation of *cox*-2 gene expression. ET-18-O-CH_3 inhibited the activation of ERK1/2 and p38 MAPK but not JNK, and also blocked the phosphorylation

of Akt/protein kinase B. Inhibitory effects of ET-18-O-CH_3 on activation of p38 MAPK, ERK1/2, and Akt were attenuated by co-treatment with celecoxib. 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 the transformed cells to apoptotic death.