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ACTIVATION OF CD95 RECEPTOR MODULATES CYP3A4 EXPRESSION THROUGH CERAMIDE AND NITRIC OXIDE PRODUCTION IN HUMAN COLON CARCINOMA HT-29 CELLS

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The CD95 (Fas, APO-1) is known to play a key role as a transmembrane cell surface receptor that mediates apoptosis of many cell types through binding with Fas ligand or cross-linking to agonistic anti-Fas antibody. Activation of CD95 provokes a potent and rapid responses in a variety of cell types by unknown mechanism. The specific aim of this work is to investigate the possible role of the CD95-mediated signaling pathway on the expression of cytochrome P450 3A4 (CYP3A4), one of the major drug-metabolizing enzymes in liver and intestinal tissues. We focused on inducible nitric oxide synthase (iNOS) and ceramide during activation of CD95 in human colon carcinoma HT29 cells. Production of nitric oxide in cells was significantly enhanced by anti-Fas antibody treatment in a concentration-dependent manner. Immunoblot analysis showed the expression of iNOS protein was also stimulated by treatment with anti-Fas antibody while human CYP3A4 expression was strongly down-regulated. CD95 activation also enhanced intracellular concentration of ceramide. Synthetic C_6-ceramide induced iNOS and suppressed P450 3A4 expression. *N*-acetylcysteine, a free radical scavenger, inhibited this expression of iNOS. The addition of iNOS antisense oligonucleotide prevented ceramide-dependent iNOS induction and restored CYP3A4 suppression. The strong suppression of CYP3A4 by CD95 activation may cause severe changes on drug metabolism in human gastrointestinal tissues. These results suggest a new role of CD95-mediated signaling pathway on the metabolisms in human colon carcinoma cells.