Induction of Apoptosis by N-phenyl-O-phenylthionocarbamate substitutes in SK-MEL-28 human skin cancer cell line

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In this study, anti-cancer effect of N-phenyl-O-phenylthionocarbamate (PPTC) substitutes was investigated, and the apoptotic mechanism by these substrates was examined on SK-MEL-28 human skin cancer cell line. SK-MEL-28 cell was treated at various concentrations for 48hr, and then MTT assay was performed to gain IC_{50} value and examine cytotoxicity of PPTC substitutes, and quantitative structure-activity relationship (QSAR) between cell and these PPTC substitutes was examined. 4-CH_3 (IC_{50}:44.9uM) and 3-CH_3 (IC_{50}:52.6uM) substitute of the PPTC substitutes were more cytotoxic SK-MEL-28 cell than HaCat human keratinocyte cell, and approached at LUMO value (0.8245e.v.). 4-CH_3 and 3-CH_3 substitutes induced apoptosis on SK-MEL-28 cell. TUNEL assay, DNA fragmentation, and EM photograph experiments were performed and identified that DNA cleavage, nuclear condensation, blebbing, and apoptotic body were appeared. In order to examine apoptotic receptor stimulated by these substitutes, FACS was used and identified that Fas (CD95) was apoptosis inducing receptor. Caspase-8 activated by Fas-induced apoptotic death signal, caspase-3 activated by caspase-8, and PARP cleavaged by caspase-3 were investigated by western-blotting and fluorometer experiments. And cell cycle change and apoptosis percent according times and concentrations were examined through flow cytometer. Also, change of proteins in SK-MEL-28 cell during apoptosis process was investigated. 2D gel was performed, and the function, and sequence of proteins were identified through MALDI-TOF mass analysis. From this result, expression or suppression of apoptosis-associated and cell cycle-associated proteins was identified.

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GENISTEIN INHIBITS NF-κB-DEPENDENT COX-2 INDUCTION IN HUMAN BREAST EPITHELIAL CELLS BY MODULATING THE ACTIVATION OF TATA-BINDING PROTEIN

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Genistein has been shown to possess chemopreventive potential, but its underlying molecular mechanisms are largely unclear. In the present study, we have investigated the effects of genistein on induction of cyclooxygenase-2 (COX-2) that plays an important role in the pathophysiology of carcinogenesis as well as in mediating inflammation. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) caused transient increases in COX-2 expression and prostaglandin E_2 (PGE_2) production in MCF10A cells, which was inhibited by genistein pretreatment. Mitogen-activated protein kinases (MAPK), are considered to be upstream signaling enzymes responsible for controlling NF-κB activation and subsequent induction of COX-2. TPA transiently induced activation of ERK1/2 and native p65 of NF-κB. Pharmaceutical inhibition with PD98059 and U0126 or dominant-negative knockout of ERK1/2 not only suppressed phosphorylation of p65, but also down-regulated NF-κB-dependent COX-2 induction by TPA. Genistein treatment attenuated TPA-induced activation of ERK1/2 and phosphorylation of native p65. While, genistein failed to inhibit TPA-induced DNA binding of NF-κB, it blocked its transcriptional activity induced by TPA. The compound significantly reduced the DNA