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.

[OC-2] [10/18/2002 (Fri) 16:10 - 16:20 / Hall B]

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

binding of TATA-binding protein (TBP) and its association with p65. More importantly, TPA-induced activation of ERK1/2 resulted in increased interaction of p65 with TBP. These findings suggest that genistein inhibits COX-2 expression and PGE_2 production in MCF10A cells by indicating the transcriptional initiation complex that involves TBP.

[OC-3] [10/18/2002 (Fri) 16:20 - 16:30 / Hall B]

A new mechanism for unsaturated fatty acid biosynthesis in *Streptococcus pneumoniae*

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The anaerobic pathway for unsaturated fatty acid biosynthesis was established in the 1960s in Escherichia coli. The double bond is introduced into the growing acyl chain by FabA, an enzyme capable of both the dehydration of β-hydroxydecanoyl-[acyl carrier protein] (ACP) to trans-2-decenoyl-ACP, and the isomerization of trans-2 to cis-3-decenoyl-ACP. However, there are a number of anaerobic bacteria whose genomes do not contain a fabA homolog, but these organisms nonetheless produce unsaturated fatty acids. We cloned and biochemically characterized a new enzyme in type II fatty acid synthesis from *Streptococcus pneumoniae* R6 that carries out the isomerization of trans-2-decenoyl-ACP to cis-3-decenoyl-ACP, but is not capable of catalyzing the dehydration of β-hydroxy intermediates. This tetrameric enzyme, designated FabM, has no similarity to FabA, but rather is a member of the hydratase/isomerase superfamily. Thus, the branch point in the biosynthesis of unsaturated fatty acids in S. pneumoniae occurs following the formation of trans-2-decenoyl-ACP, in contrast to *Escherichia coli* where the branch point takes place after the formation of β-hydroxydecanoyl-ACP.

[OD-1] [10/18/2002 (Fri) 11:30 - 11:40 / Hall B]

Novel Asymmetric Synthesis of Unsaturated 1,2-Amino Alcohols

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The synthesis of chiral 1,2-amino alcohols has been an area of intense study in the synthetic and industrial fields, because of their important roles in organic synthesis as fundamental building blocks and their occurrence in a number of natural products, drugs, and chiral auxiliaries or ligands. General methods for the synthesis of these compounds can be divided into two large categories: functional group transformations and the C-C or the C-N bond formations. Of these two methods, the former has been used widely so far, including the reduction of α-amino acids, α-amino ketones or α-hydroxy imines, the nucleophilic substitution of 1,2-diols, epoxides, aziridines, cyclic carbonates or cyclic sulfates, the aminohydroxylation or oxymecuration of olefins and the hydroboration of enamines. The latter involves the addition of an organometallic reagent to the N-protected α-amino aldehydes or to the O-protected α-hydroxy imines and coupling of carbanions with imines. Many of these procedures sometimes have one or more problems, for example, low stereoselectivity, limited applications and the use of heavy metals.
Recently, we have developed the novel synthetic methods for N-protected allylic amines from allyl ethers using chlorosulfonyl isocyante (CSI), we found that the reaction of 1,4-diphenylbut-2-enyl methyl ether with CSI gave only one product, methyl N-(1-benzylcinnamyl)carbamate, due to the steric hindrance of the phenyl ring and the formation of a stable conjugated product.