[PC3-1] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

C2-phytoceramide and Dimethylphytosphingosine induces cell death and apoptosis in human breast cancer cells, MDA-MB-231

Kim Yunwha°, Jeon Soojin, Kim Kisung, Han Youngsoo, Song Jieyoung, Park Changseo, Jung Insung, Hong Sunghee, Yun Yeonsook

Laboratory of Immunology, Korea Institute of Radiological and Medical Sciences, KAERI, 215-4 Gongneung-dong, Nowon-ku, Seoul, 139-706, Korea;Doosan Biotech, Yongin 449-840, Korea;Department of Applied Chemistry, Dongduk Women's University, 23-1

Sphingolipid metabolites have been implicated as an important component of cell signalling, such as cell proliferation, differentiation and apoptosis. But the roles of phytoceramide and its deraivatives are very poorly understood, even though they are abundant in plants, yeasts and animals including humans. We investigated the effects of N-acetyl-C2-phytosphingosine(NAPS) and the analogue of N,N-dimethylsphingosine(DMS), N,N-dimethylphytosphingosine(DMPS), on cell growth inhibition and apoptosis. NAPS and DMPS has the cytotoxic effect on MDA-MB-231 cells in a time- and dose-dependent manner and more toxic than their sphingosine analogues, C2-ceramide and DMS, respectively. Significant apoptosis is induced by treatment of DMPS with 5uM that is detectable at 48 h.
Apoptotic cell death by NAPS and DMPS is mediated by caspase 3. Caspase 3 activity is increased by NAPS(50uM) up to 6-fold at 3 h after treatment , but only 3-fold increased by C2-ceramide. Our results show that the structural difference of hydroxyl group at C-4 of the sphingoid long-chain base is critical for the cytotoxicity and apoptosis.

[PC3-2] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

Estrogen receptor is downregulated by expression of HIF-1a/VP16

Cho JungYoon°, Lee YoungJoo

Department of Bioscience and Biotechnology, Sejong University, Seoul, Korea

Estrogen Receptor is a ligand-activated transcription factor. The concentration of the receptor is a major component that regulates expression of estrogen-responsive genes.We have studied mechanism of estrogen receptor alpha (ERα) downregulation by HIF-1 using HIF-1α/VP16 constructs. ERαis known to be downregulated under hypoxic condition. Transcriptional response under hypoxia is mediated through Hypoxia-inducible factor-1 (HIF-1), a transcription factor that is usullaly degraded but stabilized under hypoxia. We have constructed HIF-1α/VP16 known to be active under normoxia. The HIF-1α/VP16 hybrid protein consisting of DNA-binding and dimerization domains from the HIF-1αsubunit transcriptional activation domain from herpes simplex virus VP16 protein was constructed to create a strong, constitutive transcriptional activator. We have found that ERα was downregulated at the levels of ligand-binding and protein. Expression of HIF-1α/VP16 decreased basal and 17β-estradial-induced estrogen response element-dependent reporter gene activity in COS-ER probably due to decreased level of

activated proteins. Currently, we are investigating the mechanism of downregulation at the transciption level.

[PC3-3] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

The Antiproliferative Effects of Bile Acids and Their Derivatives on HT-29 Human Colon Cancer Cells

Park Sangeun° Yee Su-Bog Choi Hye Joung Chung Sangwoon Park Hwa Sun Yoo Young Hyun1, Kim Nam Deuk

Dept. of Pharmacy, Pusan National University, Pusan 609-735 1Dept. of Anatomy and Cell Biology, Dong-A University College of Medicine and Institute of Medical Science, Pusan 602-714

The antiproliferative effects of bile acids and their derivatives on HT-29 human colon cancer cells were investigated. Ursodeoxycholic acid (UDCA) and its synthetic derivatives, HS-1030 and HS-1183, and chenodeoxycholic acid (CDCA) and its synthetic derivatives, HS-1199 and HS-1200 were employed for this study. General evaluations focusing on cell cycle were conducted in HT-29 human colon adenocarcinoma cell line (p53 mutant type). Although UDCA and CDCA exhibited no significant effect on the cell viability and growth, their synthetic derivatives highly decreased their viability in a concentration- and time-dependent manner as assessed by MTT assay and cell growth study. Flow cytometric analysis demonstrated that the synthetic bile acid derivatives increased G1/S population. Western blotting showed that the expressions of cyclins, cyclin dependent kinase were down-regulated. The cyclin dependent kinase inhibitor, p21, was up-regulated in a p53-independent manner. These findings suggest that these cytotoxic effects of novel bile acid derivatives on human colon adenocarcinoma cells were mediated via apoptosis through a p53-independent pathway.

[PC3-4] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

4-Hydroxy nonenal (HNE) Induces Endothelial cells Apoptosis via iNOS mediated ONOO- generation

Chung Sang Woon°, Yee Su Bog, Choi Hye Joung, Park Sangeun, Jung Kyung Jin, Kim Dae Hyun, Chung Hae Young, Kim Nam Deuk

Dept. of Pharmacy, Research Institute of Genetic Engineering, Pusan National University, Pusan 609-735, Korea

Among the aldehydes derived from lipid peroxidation, 4-hydroxynonenal (HNE) that can be produced from arachidonic acids, linoleic acids, or their hydroperoxides in relatively large amounts in response to oxidative insult. Therefore, HNE might be an important mediator of oxidative stress-induced apoptosis. To study the hypothesis that HNE may induce apoptosis, we estimated cytotoxicity of HNE on YPEN-1 rat prostatic endothelial cells. Anti-proliferative effects were examined by morphological changes and MTT assay after exposure to different concentration (5 ~ 15 μM) of HNE. As results, we observed apoptotic bodies with propidium iodide staining and detected induction of apoptosis by HNE with flow cytometry assay. We also studied apoptosis related events with Western blotting. Cells exposed to HNE for 24 hr resulted in increased poly(ADP-ribose) polymerase cleavage and up-regulation of Bax. In addition, HNE induced intracellular ROS generation and NF-κB expression. Cells exposed to 15 μM HNE for 0.5 ~ 4 hr resulted in increased NF-κB expression. Also, HNE induced COX-2 and iNOS