

as 2.7Kb vector fragment. Finally, 3.3Kb HindIII DNA fragment could be obtained by deletion analysis.

[PC3-1] [ 10/20/2000 (Fri) 15:30 - 16:30 / [Hall B] ]

### **Sphingosine Accumulation by FTY720 induces Apoptosis in LLC-PK1 cells**

Lee Woo Jin<sup>o</sup>, Yoo Hwan Soo, Lee Yong Moon

College of Pharmacy, Chungbuk National University

FTY720, a synthetic sphingoid base analog, was investigated as a new potent sphingosine kinase inhibitor and increases sphingosine which induces apoptosis in LLC-PK1 cells. FTY720 showed high level of fragmented DNA, induction of caspase-3 like activity and TUNEL staining cells. We have as well found that sphingosine and sphinganine were accumulated endogenously in time- and dose-dependent manner within 12 hr by FTY720 treatment. The activity of sphingosine kinase was also reduced by FTY720 like as other sphingosine kinase inhibitors, N,N-dimethylsphingosine, dl-threo sphinganine. Fragmented DNA content by 20  $\mu$ M FTY720 and by 5  $\mu$ M of exogenously added BSA-sphingosine complex represents typical apoptosis. In the same above conditions, accumulated sphingosine concentration in total cells is almost identical though sphingosine distribution inside cells may be somewhat different. Our results indicate that FTY720 induced apoptosis is associated with inhibition of sphingosine kinase activity and is related to successive accumulation of sphingosine.

[PC3-2] [ 10/20/2000 (Fri) 15:30 - 16:30 / [Hall B] ]

### **Glycolic acid attenuated UVB-induced Activator Protein-1 activation by down regulation of c-fos gene expression in HaCaT cells**

Ahn KS<sup>o</sup>, Hong JT, Jung KM, Kim EJ, Park KS, Lee JK, Kim YK, Park YK\*, Lee SH

Department of Toxicology, National Institute of Toxicological Research, KFDA, \*Graduated School of Biotechnology, Korea University

Glycolic acid is widely used as cosmetic ingredient since it is expected to reduce the wrinkles, roughness, age spots of skin and other signs of sunburn damages. In the previous our in vivo study, we investigated that glycolic acid inhibited UVB-induced mouse papilloma formation in two-stage carcinogenesis model. Modification of UVB-induced Activator Protein-1 (AP-1) activation by glycolic acid was investigated as a possible mechanism in a cultured human keratinocyte cell line, HaCaT. Glycolic acid decreased UVB-induced AP-1 activation. UVB-induced c-fos mRNA and c-Fos protein expression were also attenuated by UVB and glycolic acid co-treatment. Taken together, the ability of glycolic acid for down regulate the expression of AP-1 DNA binding protein may be involved in the attenuation of AP-1 activation. Considering the functional role of AP-1 activation in UVB-induced epidermal carcinogenesis, the attenuation of UVB-induced AP-1 activation by glycolic acid may play in part a role in the inhibitory effect of glycolic acid on UVB-induced skin carcinogenesis.

[PC3-3] [ 10/20/2000 (Fri) 15:30 - 16:30 / [Hall B] ]

### **p27kip1 promotes ceramide-induced apoptosis in HL-60 cells**

Ghil KC, Mun JY, Chun YJ, Kim MY<sup>o</sup>

Both the cyclin-dependent kinase inhibitor p27kip1 and ceramide have been implicated in the regulation of apoptosis. Recently, we demonstrated that ceramide induced apoptotic cell death associated with increase in the level of p27kip1 in HL-60 cells. In the present study, we have overexpressed p27kip1 in HL-60 cells to clarify the role of p27kip1 in ceramide-induced cell death. HL-60/p27kip1 cells treated with ceramide have shown marked increase in apoptotic cell death compared to HL-60 cells. However, overexpression of p27kip1 by itself did not induce apoptosis indicating that p27kip1 alone might not be sufficient to induce apoptosis but promotes ceramide-induced apoptosis in HL-60 cells. Overexpression of p27kip1 did not modify the expression of Bcl-2 protein, but increased Bax protein level without ceramide treatment. Furthermore, overexpression of p27kip1 accelerated ceramide-induced cytochrome c release and poly(ADP-ribose) polymerase (PARP) cleavage in HL-60 cells. Ceramide induced PARP cleavage in HL-60/p27kip1 cells at the time which was not seen in HL-60 cells. These findings indicate that p27kip1 promotes ceramide-induced apoptosis through the elevation of Bax expression and activation of caspase with cleavage of the endogenous substrate PARP.

[PC3-4] [ 10/20/2000 (Fri) 15:30 - 16:30 / [Hall B] ]

### **BCL-2 OR BCL-XL ATTENUATES HYDROGEN PEROXIDE - AND BETA-AMYLOID-INDUCED OXIDATIVE PC12 CELL DEATH**

Jang Jung-Hee<sup>o</sup> and Surh Young-Joon

College of Pharmacy, Seoul National University, Seoul 151-742

Recent studies have revealed that moderate amounts of intracellular reactive oxygen intermediates (ROIs) can cause cell death via apoptosis while their excessive cellular accumulation leads to necrotic cell death. Cell death is regulated by plenty of functional genes and their protein products. Bcl-2 which is an integral intermitochondrial membrane protein blocks cell death induced by wide variety of toxicants. In the present work, we have investigated a possible protective role of bcl-2 in oxidative death induced by hydrogen peroxide and beta-amyloid in cultured PC12 cells. When PC12 cells were treated with hydrogen peroxide or beta-amyloid, they underwent apoptotic death as determined by morphological features, internucleosomal DNA fragmentation and positive in situ terminal end-labeling (TUNEL staining). Hydrogen peroxide or beta-amyloid caused activation of NF-kappa B, which appears to be mediated via transient induction of mitogen-activated protein kinases (MAPKs). Transfection of PC12 cells with bcl-2 or bcl-XL gene rescued these cells from oxidative death caused by either hydrogen peroxide or beta-amyloid. PC12 cells overexpressing the above anti-apoptotic genes exhibited relatively high constitutive NF-kappa B activation, compared with the vector-transfected control cells. Furthermore, NF-kappa B inhibitors, such as pyrrolidine dithiocarbamate or L-1-tosylamido-2-pentylchloromethyl ketone, sensitized PC12 cells to hydrogen peroxide or beta-amyloid. Western blot analyses revealed that bcl-2 transfected PC12 cells exhibited the higher level of p65, the functionally active subunit of NF-kappa B, in nucleus than did the vector-transfected controls. In contrast, relatively small amounts of cytoplasmic inhibitor Ikappa B alpha were present in the cells overexpressing bcl-2. These results suggested that the ubiquitous eukaryotic transcriptional factor NF-kappa B plays a role in cell survival against oxidative stress.

[PC3-5] [ 10/20/2000 (Fri) 15:30 - 16:30 / [Hall B] ]

### **Bax is required for ceramide-regulation of cell death**

Kim HJ<sup>o</sup>, Chun YJ, Kim MY

College of Pharmacy, Chung-Ang University