

apoptosis is prevented by expressing the dominant negative SEK1 mutant. In contrast, the later phase of activation and apoptosis are equally prevented by expressing p21<sup>WAF1/CIP1</sup>. Thus, the two-tiered activation of JNK1 is conducted by different mechanisms in a stage-specific manner during apoptosis. We also show that the stable expression of JNK1 suppresses apoptosis, while the dominant negative JNK1 mutant (DN-JNK1) promotes it. In contrast, the transient expression of DN-JNK1 or JBD, a JNK inhibitor suppresses apoptosis. Thus, the early phase of JNK1 activation prolongs cell survival during apoptosis, while the later phase of activation is required for the induction of apoptosis.

[PC3-6] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Activation Of p21-Activated Kinase1 Is Required For Autotaxin-Induced Focal Adhesion Kinase Phosphorylation and Cell Motility in A2058 cells**

**Jung In Duk**<sup>o</sup>, Lee Jangsoon, Yun Seong Young, Park Jun Hong, Park Chang Gyo, Lee Hoi Young  
College of Medicine, Konyang University

Autotaxin (ATX) is a 125-kDa glycoprotein and a strong motogen that can increase invasiveness and angiogenesis, originally isolated from the conditioned medium of human melanoma A2058 cells. And it is a strong. Recently, we suggested that ATX promotes motility via G protein-coupled PI3K $\gamma$ , and Cdc42/Rac1 are essential for ATX-induced tumor cell motility in A2058 melanoma cells. In the present study, we found that activation of p21-activated kinase1 (PAK1) was required for ATX-induced cell motility. ATX activated PAK1 that was blocked by PTX, LY294002, and Genistein, but not by U73122, PD98059, and Y27632. ATX could not activate PAK1 in N17Rac1- or N17Cdc42-transfected cells (dominant negative mutants of Rac1 and Cdc42, respectively), and PI3K $\gamma$  K832R-transfected cell (catalytically inactive mutant of phosphoinositide 3-kinasey (PI3K $\gamma$ ). Transfection of PAK1 mutant (PAK1 K299R) inhibited the phosphorylation of focal adhesion kinase (FAK) and ATX-induced cell motility. These findings strongly indicate that PAK1 is located downstream of Gi, PI3K $\gamma$ , Rac1, Cdc42, and plays a critical role in ATX-induced A2058 cell motility

[PC3-7] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Allicin-induced apoptosis of gastric epithelial cells is associated with changes of caspase-independent effector and involvement of PKA**

**Baeg HyeKyoung**<sup>o</sup>, Rhee Dong-Kwon, Pho Suhkneung  
Sungkunkwan University, College of Pharmacy

Garlic (*Allium sativum*) has been used as a general food and a remedy in Oriental for a long time. Since garlic compounds have been also shown to inhibit growth of tumors and to modulate the activity of carcinogenesis, the effects of allicin on growth and survival in human gastric epithelial cells were evaluated by cell viability, cell cycle analysis and DNA fragmentation. Protein levels of cytochrome C, Bcl-xL, Bax and AIF were detected by Western blotting. Effects of recombinant VacA on caspase proteases activity were also determined. Allicin inhibited cell growth and induced apoptosis in gastric epithelial cells. Treatment resulted in DNA fragmentation and cell cycle analysis revealed subdiploid cells. Allicin also mediated a prolongation of the cell cycle progression in G2 phase. Allicin increased the expression of Bcl-xL, Bax and cytochrome C in gastric epithelial cells. However, cell death was observed with pancaspase inhibitor (Z-VAD-FMK) and the absence of immunoreactivity for caspase-cleaved poly-ADP-ribose polymerase (PARP) was not shown. In addition, the level of AIF, caspase-independent effector, was increased. Apoptosis of gastric epithelial cells by allicin was partially suppressed by a specific protein kinase A (PKA) inhibitor. Taken together, the data suggest that allicin induces caspase-independent apoptosis and apoptotic effects of allicin is mediated through the activation of PKA.

[PC3-8] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Inducing effect of helenalin on the differentiation of HL-60 leukemia cells**

**Kim Seung Hyun**<sup>o</sup>, Kim Tae Sung  
*College of Pharmacy, Chonnam National University*

Helienalin, a cell-permeable pseudoguaienolide sesquiterpene lactone, is a potent anti-inflammatory agent that inhibits NF- $\kappa$ B DNA binding activity by selectively alkylating the p65 subunit of NF- $\kappa$ B. Transcription factors such as NF- $\kappa$ B provide powerful target of drugs to use in the treatment of cancer. Human promyelocytic leukemia HL-60 cells are differentiated into monocytic or granulocytic lineage when treated with 1,25-dihydroxyvitamin D<sub>3</sub> [1,25-(OH)<sub>2</sub>D<sub>3</sub>] or all-trans-retinoic acid (ATRA), respectively. In this study, we investigated the effect of helienalin on the differentiation of HL-60 leukemia cells. Helienalin by itself induced HL-60 cell differentiation via inhibition of NF- $\kappa$ B activity in a concentration-dependent manner, and also markedly increased the degree of HL-60 cell differentiation when simultaneously combined with low doses of either 1,25-(OH)<sub>2</sub>D<sub>3</sub> or ATRA. Flow cytometric analysis indicated that helienalin induced HL-60 cell differentiation into granulocytes, and stimulated 1,25-(OH)<sub>2</sub>D<sub>3</sub>- and ATRA-induced differentiation into monocytes/macrophages and granulocytes, respectively. Moreover, PKC and ERK inhibitors inhibited HL-60 cell differentiation enhanced by helienalin, while PI3-K and p38 MAPK inhibitors did not. These results indicated that helienalin induced and enhanced HL-60 cell differentiation via the inhibition of NF- $\kappa$ B activity and activation of PKC and ERK pathways.

[PC3-9] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Up-regulation of Cyclin A-Cdk2 activity is associated with depolarization of mitochondrial membrane potential during apoptosis of human hepatoma SK-HEP1 cells induced by treatment with panaxadiol**

**Park Byoung Duck**<sup>o</sup>, Jin Ying Hua, Yim Hyungshin, Lee Seung Ki  
*College of Pharmacy, Seoul National University*

Here we show that panaxadiol, a ginseng saponin with a dammarane skeleton, induces acute apoptotic cell death in human hepatoma SK-HEP-1 cells as evidenced by analysis of DNA fragmentation, caspase activation, and changes in cell morphology. The kinetic study showed that panaxadiol-induced apoptosis is associated with depolarization of mitochondrial membrane potential and cytochrome c release. Sequential activations of caspases-9, and -3, or -7, but not of caspase 8 coincide well in a time dependent manner with mitochondrial membrane depolarization and cytochrome c release from mitochondria during apoptosis of SK-HEP-1 cells induced by treatment with panaxadiol. To further investigate the molecular mechanisms underlying the panaxadiol-induced apoptosis of the cells, we examined whether activities of Cyclin-dependent protein kinases, Cdk2 and Cdc2 are up-regulated during apoptosis of the cells by immune-complex kinase assay. Cdk2 kinase activity, but not the Cdc2 kinase activity is markedly up-regulated and the time-dependent up-regulation correlates well with the mitochondria membrane depolarization and cytochrome c release. In the presence of olomoucine or roscovitine, specific Cdk inhibitors, the depolarization of mitochondrial membrane potential and apoptotic progression are equally and effectively prevented in panaxadiol-treated SK-HEP-1 cells. These results indicated that the induction of apoptosis in human hepatoma cells treated with panaxadiol requires the up-regulation of Cdk2 kinase activity that is functionally associated with depolarization of mitochondrial membrane potential and accordingly apoptosis progression.

[PC3-10] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Monitoring the Expression Profiles of Doxorubicin-Resistant Acute Myelocytic Leukemia Cells by DNA Microarray Analysis**

**Song Ju Han**<sup>o</sup>, Kim Tae Sung  
*College of Pharmacy, Chonnam National University*

Anticancer drug resistance occasionally occurs in malignant hematologic diseases such as acute myelocytic leukemia (AML) treated with chemotherapy and is a major problem to complete remission. Malignant cells primarily induce intrinsic resistance to treatment of anticancer drug, but gradually obtain acquired resistance to cytotoxic activities of chemotherapy. In this study, we monitored the expression profiles of doxorubicin resistance-