[PC3-3] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Transforming Growth Factor-β (TGF-β) Induces Invasion and Migration of MCF10A Human Breast Epithelial Cells

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Transforming growth factor (TGF)-β, a hormonally active polypeptide found in normal and transformed tissue, is a potent regulator of cell growth and differentiation. In this study, we examined the effect of TGF-β on invasion and motility of MCF10A human breast epithelial cells. TGF-β induced migration and invasive phenotype of the parental MCF10A cells in a dose-dependent manner. Activity of MMP-2 promoter was increased by TGF-b, suggesting that the TGF-β-induced invasive phenotype may possibly be mediated by MMP-2 rather than MMP-9. TGF-β-stimulated invasive and migratory properties were decreased by inhibition of the p38 MAPK and ERK pathways by SB203580 and PD98059, respectively. We show that TGF-β induces prominent morphological changes of MCF10A cells which was also abolished by SB203580 and PD98059. The present study suggests that TGF-β-induced cell migration and invasion as well as morphological changes involves activation of both p38 MAPK and ERK pathways and is associated more closely with the expression of MMP-2 rather than MMP-9. [Supported by the Korea Food and Drug Administration Grant (KFDA-03092-LIF-000)]

[PC3-4] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Roles of PI3K and Rac pathways in H-ras induced invasion and motility

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Phosphatidylinositol 3-kinase (PI3K) and Rac play important roles that regulate cellular functions including cell survival and migration. In the present study, we investigated the functional roles of PI3K and Rac1 pathways in H-ras-induced invasive phenotype and motility of MCF10A cells. Akt, a downstream molecule of PI3K, was effectively activated not only by H-ras but also by N-ras, suggesting that the activation of PI3K pathway is not sufficient to induce metastatic potential of MCF10A cells. Inhibition of PI3K pathway by treatment of LY294002 and wortmannin, known PI3K inhibitors, significantly reduced invasiveness, motility and secretion of MMP-2/-9 in H-ras MCF10A cells. The data suggest that the activation of PI3K pathway may not be sufficient but is required for H-ras-induced invasion and motility. We then asked the functional role of Rac pathway in H-ras-induced invasion and migration. Prominent activation of Rac1 was shown only in H-ras-activated cells but not in N-ras-activated MCF10A cells. Functional significance of H-ras-activated Rac1 pathway in invasiveness and cell migration was evidenced by studies using a dominant-negative (DN) construct of Rac1. Blocking Rac1 pathway significantly inhibited the H-ras-induced invasiveness and motility. We also show that the activation of downstream effector molecules, p38 MAPK and ERKs, were inhibited in DN Rac1 transfectants. This study reveals the Rac as a key-signaling molecule differently regulated by H-ras and N-ras, leading to H-ras-specific cell invasive and migratory phenotypes. [Supported by a Korea Health 21 R&D, Ministry of Health and Welfare (02-PJ1-PG10-20801-0001) grant]

[PC3-5] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

The two-tiered activation of JNK1 prolongs cell survival prior to induced apoptosis

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The c-Jun N-terminal kinase (JNK) plays essential roles in apoptosis and cell survival. Because apoptosis is promoted by blocking the MEK kinase1-mediated activation of JNK1, we tested whether JNK1 plays dual roles in apoptosis. We show here that JNK1 activity is differentially up-regulated in a two-tiered fashion by specific mechanisms during taxol- or ginsenoside Rh2-induced apoptosis. The early phase of JNK1 activation, but not

apoptosis is prevented by expressing the dominant negative SEK1 mutant. In contrast, the later phase of activation and apoptosis are equally prevented by expressing p21D112N, an uncleavable version of $p21^{WAF1/CIP1}$. 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

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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 PI3Kγ, 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 Rac1and Cdc42, respectively), and PI3Kγ K832R-transfected cell (catalytically inactive mutant of phosphoinositide 3-kinaseγ (PI3Kγ). 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, PI3Kγ, 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

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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