γ antagonist, GW9662, rendered PC12 cells sensitized to SIN-1. The above findings suggest possible involvement of COX-2 induction and PG synthesis in regulating nitrosative PC12 cell death. PGE_2 may mediate apoptosis induced by peroxynitrite in PC12 cells. On the other hand, 15d-PGJ_2 may act as a negative feedback mediator of COX-2 signaling cascades.

[PC1-36] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Autotaxin-induced tumor cell motility requires the activation of Rac/Cdc42, PAK, and FAK

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Cell motility plays important physiological roles in embryogenesis, immune defense, wound healing, and metastasis of tumor cells. Cell motility of normal cells is tightly regulated, while tumor cell motility is aberrantly regulated or autoregulated. Autotaxin (ATX) is a 125-kDa glycoprotein, originally isolated from the conditioned medium of human melanoma A2058 cells. ATX stimulates random (chemokinetic) and directed (chemotactic) motility of human tumor cells at high picomolar to low nanomolar concentrations. Recently, ATX has been shown to augment invasive and metastatic potential of ras-transformed cells. In MatrigelTM invasive assays, NIH3T3 cells with full length ATX cDNA demonstrated greater spontaneous and ATX-stimulated invasion than control. In addition, in vivo study showed that combination of ATX expression with ras transformation amplified tumorigenesis and metastatic potential compared to ras-transformed control, suggesting that ATX augments cellular characteristics necessary for tumor aggressiveness. In the present study, we investigated the intracellular signaling pathway of ATX. Unlike N19Rho expressing cells, the cells expressing N17Cdc42 or N17Rac1 showed reduced motility against ATX. In addition, ATX increased PAK activity and phosphorylated focal adhesion kinase. Since FAK in cells expressing N17Rac1 or N17Cdc42 was not phosphorylated by ATX, FAK appears to be located downstream of Cdc42/Rac1. Collectively, these data indicate that Cdc42, Rac1, and FAK are involved in ATX-induced tumor cell motility in human melanoma cells.

[PC1-37] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Involvement of G1 arrest and caspase-3 activation in apoptosis induced by bovine lactoferricin

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We investigated the effect of bovine lactoferricin (Lfcin-B) on cell cycle regulation and caspase activation in tumor cells. Treatment with Lfcin-B resulted in the production of intracellular reactive oxygen species (ROS) during apoptosis of THP-1 cells. Biochemical analysis revealed that Lfcin-B-induced apoptosis, the cell cycle arrest and caspase activation were completely abrogated by addition of an antioxidant such as N-acetylcysteine (NAC). In cell cycle analysis using the bromodeoxyuridine (BrdU) labeling method, it was shown that Lfcin-B blocked the progression of the cell cycle to S phase (G1 arrest) in THP-1 cells undergoing apoptosis. In coincidence with G1 arrest, the results of western blot analysis showed that treatment with Lfcin-B prominently decreased the expression of Cyclin D2, CDK2, CDK4 and Cyclin E molecules responsible for progression to S phase. In addition, treatment with Lfcin-B enhanced the intracellular activity of caspase-3 and ?8 in the early period of apoptosis. When we investigated the correlation of ROS production, G1 arrest and caspase-3 activation in apoptosis induced by Lfcin-B, it was revealed that ROS regulated G1 arrest and caspase activation at a point of up-stream of the apoptosis cascade.

[PC1-38] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Retrovirus-mediated Delivery of TIMP-2 Inhibits Migration, Invasion and Angiogenesis

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An imbalance between matrix metalloproteinase (MMP)-2 and its endogenous inhibitor, tissue inhibitor of metalloproteinase (TIMP)-2 causes the degradation of the extracellular matrix associated with pathological events including invasion, metastasis and angiogenesis. Since TIMPs are secreted molecules, they have the potential to be used for gene therapy of certain tumors. In present study, we have studied the retrovirus-mediated delivery of TIMP-2 in H-ras MCF10A cells in which MMP-2 was shown to be responsible for the H-ras-induced invasive phenotype. PG13 cells, packaging cells, were infected with the recombinant retrovirus containing TIMP-2 gene (rRetTIMP-2). Recombinant retrovirus containing LacZ (rRetLacZ) was used as a control. Overexpression of TIMP-2 was detected in H-ras MCF10A cells infected by rRetTIMP-2 after 5 days in culture. Retroviral delivery of TIMP-2 in H-ras MCF10A cells caused a significant downregulation of MMP-2 in a dose-dependent manner as evidenced by Western blot and gelatin zymography. Migration and invasive phenotype H-ras MCF10A cells were markedly inhibited by rRetTIMP-2 infection compared to the cells infected with rRetLacZ. In addition, retroviral delivery of TIMP-2 efficiently inhibited angiogenesis of HUVECs dose-dependently as evidenced by in vitro tube formation assay. Taken together, we show that the downregulation of MMP-2 by TIMP-2 overexpression inhibits migrative and invasive properties of H-ras MCF10A cells and angiogenesis of HUVECs, suggesting a possible application for gene therapy to prevent and treat cancer.

[PC1-39] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Identification and characterization of a novel cancer/testis antigen gene

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We applied serological analysis of cDNA expression library technique to identify cancer-associated genes. We screened cDNA expression libraries of human testis and gastric cancer cell lines with sera of patients with gastric cancers. We identified a gene whose expression is testis-specific among normal tissues. We cloned and characterized this novel gene. It contains D-E-A-D box domain and encodes a putative protein of 630 amino acids with possible helicase activity. It showed wide expression in various cancer tissues and cancer cell lines. The corresponding gene was named cancer-associated gene (CAGE). PCR of human x hamster Radiation Hybrids showed localization of CAGE on the human chromosome Xp22. Transient transfection of CAGE showed predominantly nuclear localization. Both western blot and plaque assay indicated seroreactivity of CAGE protein. . It was shown that CAGE expression was induced in cell cultures treated with 5-aza-2'-deoxycytidine. This suggests that methylation plays a role in regulation of CAGE expression. Direct sequencing of the CAGE gene after sodium bisulfite treatment of genomic DNA revealed that the methylation of CpG sites had occurred in those cancer cell lines without CAGE expression. This confirms that the methylation of CpG sites is associated with silencing of CAGE expression. We are currently carrying out transient transfection experiment to check whether methylation repress transcription of CAGE in cells containing transcription factors required for expression. Because CAGE is expressed in a variety of cancers but not in normal tissues except testis, this gene can be a target of antitumor immunotherapy.

[PC1-40] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

GDNF Enhances Hs683 Human Glioma Cell Migration: Possible Involvement of MAPKs

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Glial cell-derived neurotrophic factor (GDNF) is a potent neurotrophic factor that enhances survival of midbrain doparminergic neuron. GDNF and its receptors are widely distributed in brain and are believed to be involved in the control of neuron survival and differentiation. In this study, we examined the effect of GDNF on proliferation and migration of Hs683 human glioma cells. GDNF markedly enhances proliferation and migration of Hs683 cells in a dose-dependent manner. Since involvement of mitogen-activated protein kinases (MAPKs) in the cellular effect of GDNF has been suggested, we wished to investigate the activation of JNK, ERK-1,2 and p38 by GDNF treatment in Hs683 cells. GDNF (80 ng/ml) prominently increased phosphorylated form of p38 without affecting total p38 level. A kinetic study of GDNF-induced p38 activation showed that p38 was maximally activated within 30 min after GDNF treatment and decreased at 1 hr in the Hs683 cells. Activation of other MAPKs, JNK and ERK-1,2, was also detected upon GDNF treatment, to a lesser degree compared to p38. Our data suggest that the