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