breast epithelial cells (MCF10A), while both H-ras and N-ras induce transformed phenotype. It has been recently shown that phosphatidylinositol 3-kinase (PI3K) plays an important role on cell migration. In the present study, we wished to investigate the functional role of PI3K in H-ras-induced invasive phenotype in MCF10A cells. The activation of PI3K was examined by detecting phosphorylation of Akt, a downstream molecule of PI3K, by Western blot analysis. We show that phosphorylated Akt level was upregulated both in H-ras MCF10A cells and N-ras MCF10A cells comparing to the parental MCF10A cells while the amount of Akt was equal in the parental, H-ras- and N-ras MCF10A cells. The results suggest that activation of PI3K is not sufficient for invasiveness and motility since PI3K is also activated in the N-ras MCF10A cells which have been shown to be non-invasive and non-motile. We then further investigated the functional significance of PI3K activation in invasion and motility by using the known PI3K inhibitors, LY294002 and wortmannin. Treatment of LY294002 and wortmannin significantly reduced invasive phenotype and motility of H-ras MCF10A cells, suggesting that activation of PI3K is not sufficient, but may be required for H-ras-induced invasion and motility.

[PC1-36] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A Kinetic Assay for the Detection of Prekallikrein

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An improved kinetic assay for prekallikrein activator (PKA), a potential vasodilating agent, has been developed as an indicator for the quality control of human albumin preparation during its production. It consists of two-stage reactions. In the first stage, PKA and prekallikrein were incubated at 37℃ for 45 min to produce kallikrein. The kallikrein, a serine esterase, fromed catalyses the splitting of p-nitroaniline (pNA) from the substrate H-D-Pro-Phe-Arg-pNA (S-2302). The rate at which pNA is released is measured photometrically at 405 nm. Prekallikrein, a substrate of PKA was purified with DEAE ion-exchange chromatography and the major potential variations in the assay were optimized. As a result, the pH 8.0 and ion strength of 150mM sodium chloride were chosen for optimization. Reaction times in the range of 10 and 360 min provided linear dose-response curves. The prekallikrein concentration was adjusted to be in the range of 1:1 and 1:3 dilution to generate a linear standard curve. With optimized variations in the protocol, the reproducibility was tested. In the precision test, coefficeint variation (CV) was below 4% and the dose-response curve showed linearity (R2=0.999). An accuracy test with international standard of PKA afforded the mean of recovery as 97.5%.

[PC1-37] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Overexpression of TIMP-2 by Retroviral Vector Efficiently Inhibits Cell Invaion in H-ras MCF10A Cells: A Gene Therapy Approach

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The matrix metalloproteases (MMPs) play important roles in metastasis and invasion in various cell types. An endogenous inhibitor of MMP, tissue inhibitor of metalloprotease-2 (TIMP-2), has high specificity for MMP-2. An imbalance between MMP-2 and TIMP-2 causes the degradation of the extracellular matrix associated with pathological events including invasion and metastasis. Since TIMPs are secreted molecules, they have the potential to be used for gene therapy of certain tumors. In the 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. Recombinant retrovirus containing TIMP-2 gene was used to infect PG13 cells. When the H-ras MCF10A cells were treated with the conditioned media of PG13, a dose-dependent inhibition of MMP-2 secretion was observed by gelatin zymography. TIMP-2 overexpression mediated by retrovirus significantly reduced the invasiveness of H-

ras MCF10A cells in a dose-dependent manner. Our data confirm the role of TIMP-2 in the downregulation of MMP-2 and invasion in H-ras MCF10A cells and show that retrovirus-mediated delivery of TIMP-2 efficiently inhibits MMP-2 secretion and invasion, suggesting possible application for gene therapy for prevention and treatment of the cancer.

[PC1-38] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A Sensitive Bioassay Method For Measuring Antoproliferative Activity of Transforming Growth Factor β(TGF-β)

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Transforming growth factor-β(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 wished to establish an in vitro bioassay system to seek the most sensitive method that can measure TGF-β activity. We have examined anti-proliferative activity of human TGF-β interim standard (89/514) obtained from National Institute for Biological Standards and Control (NIBSC, UK) in three different cell lines: MCF10A human breast epithelial cells, H-ras transformed MCF10A human breast epithelial cells and CCL-64 mink lung epithelial cells. Among the cell lines tested, CCL-64 cell proliferation were the most sensitively inhibited by treatment of TGF-β in a dose-dependent manner. We then compared two commonly used assays for cytotoxicity: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) assays. XTT assay, when the soluble product was detected at 490 nm, was more sensitive to the treatment of TGF-β dose-dependently. To seek the appropriate cell number for the TGF-β bioassay, 1×10^4, 1×10^5 and 1×10^6 cells were plated in a 96-well plate. Cell number of 10^5 gave the most desirable pattern for anti-proliferative activity of TGF-β. When the incubation time for TGF-β treatment was tested, 24 hr incubation at 37℃, 5 % CO_2 was suitable. Taken together, we have found the experimental protocol which gives the most sensitive quantitation of biological activity of TGF-β: 1×10^5 CCL-64 cells were plated on a 96-well plate and the media was changed to serum free media (phenol red-free) containing various concentrations of TGF-β in pg/ml. Following 24 hr incubation, XTT was treated for 4 hr at 37℃, 5 % CO_2, then absorbance at 490 nm was determined.

[PC1-39] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A Splicing Variant of Aspartyl-beta hydroxylase is associated with Metastatic Progression of Gastric Cancer

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A Splicing Variant of Aspartyl-beta hydroxylase is associated with Metastatic Progression of Gastric Cancer

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Gastric cancer is the most prevalent malignant cancer and the leading cause of cancer death in Korea. Although early detection and improvement of surgical technique have improved the prognosis of gastric cancer, patients with advanced gastric cancer display poor prognosis because systemic metastasis may