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-