College of Pharmacy, Pusan National University, Pusan, Korea, Bioinformatics cooperative course, Pusan National University, Pusan, Korea, Department of Pathology, Nagasaki University, School of Medicine, Japan

Mini rat is a transgenic rat strain carrying antisense gene for rat growth hormone (GH), resulting in retarded growth. Mini rats have been used by several investigators as a GH deficiency model. In the present study, we assessed the redox status of mini rat liver to elucidate the effect of aging and calorie restriction (CR). Liver homogenates from mini rats at 31 and 100 weeks of age which were fed ad libitum (AL) and CR were used in the study. Total reactive oxygen species (ROS) generation was determined by dichlorofluorescin diacetate (DCFH-DA) method. We also investigated malonaldehyde (MDA) amounts using thiobarbituric acid (TBA) positive material assay. In addition, the mitochondrial membrane fluidity was measured by fluorescence polarization method. Results showed that total ROS generation of liver increased with age and was reduced by CR. Increased MDA levels might be due to increased ROS generation, which could cause a decrease of mitochondrial membrane fluidity. Moreover, other redox markers such as GSH/GSSG and total SH levels decreased during aging and was maintained by CR. The present findings are in agreement with our previous report showing age-dependent loss of the antioxidant potential and its modulation by CR.

[PC1-34] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A Role of p38 MAPK on H-ras-Induced Invasion and Motility in MCF10A Human Breast Epithelial Cells

Lee Eun-Jung°, Kim Mi-Sung, Moon Aree

College of Pharmacy, Duksung Womens University, Seoul 132-714

One of the most frequent defects in human cancer is the uncontrolled activation of the ras-signaling pathways. We have previously shown that H-ras, but not N-ras, induces an invasiveness and motility in human breast epithelial cells (MCF10A), while both H-ras and N-ras induce transformed phenotype. Since migration plays a crucial role in invasive, we examined motility of MCF10A cells transformed with H-ras or N-ras. We show that cell motility was increased by H-ras, but N-ras suggesting that H-ras-induced invasive phenotype may be mainly due to enhanced cell motility. It has been recently shown that p38, a member of the mitogen activated protein (MAP) kinase family, is important for cell migration. We wished to investigate the functional role of p38 MAP kinase in H-ras-induced invasive phenotype. We show that p38 is prominently activated in H-ras MCF10A cells comparing to the parental MCF10A cells or N-ras MCF10A cells, while no significant difference was found in the activation of stress-activated protein kinase-1/c-Jun N-terminal protein kinase (SAPK-1/JNK). Extracellular signal-regulated protein kinase (ERK)-1,2 were activated in both H-ras and N-ras MCF10A cells. To assess the functional significance of H-ras-activated p38 in invasion and migration, we examined the effect of SB203580 and dominant-negative p38(DN p38). Treatment of SB203580, an inhibitor of p38, reduced invasive activity and motility of H-ras MCF10A cells. H-ras MCF10A cells were transfection with dominant-negative p38 but not dominant-negative JNK-1 inhibited cell migration. These results suggest a possible involvement of p38 in H-ras-induced invasiveness/motility.

[PC1-35] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

A Role of Phosphatidylinositol 3-kinase (PI3K) on H-ras-Induced Invasive phenotype

Shin Il-Chung°, Moon Aree

College of Pharmacy, Duksung Womens University, Seoul 132-714

We have previously shown that H-ras, but N-ras, induces an invasiveness and cell motility in human

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

Shin InSoo°, Sim YunBo, Kim SunNam, Lee KiHong, Yoo SiHyung, Kang HyeNa, Choi SeungEun, Hur Sook Jin, Kang JuSeop, Lee SeokHo, Hong SeungHwa

Biologics Evaluation Department, Korea Food & Drug Administration

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

Ahn Seong-Min°, Sohn Yeowon, Kim Yun-Soo, Moon Aree

College of Pharmacy, Duksung Women's University, Seoul 132-714, KFDA, KRIBB

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-