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)

Kim Mi-Sung°, Ahn Seong-Min, Moon Aree

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

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

Kim eunMi°, Koo TaeHyeon, Lee JungJoon, Myung PyungKeun, Lee JeongHyung

Korea Research Institute of Bioscience and Biotechnology and College of Pharmacy Chungnam National University

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

Eun-Mi Kim1, 2, TaeHyeon Koo1, Jung Joon Lee1, Pyung-Keun Myung2 and Jeong-Hyung Lee1
1)Korea Research Institute of Bioscience and Biotechnology and 2)College of Pharmacy ChungNam National University.

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

have already existed at the time of surgery. Therefore, understanding how metastasis is regulated at the molecular level is required to devise new modalities of gastric cancer therapy. In present study, we measured the invasive potentials of various Korean-derived gastric cancer cell lines and subsequently performed subtractive suppression hybridization to identify metastasis-related genes by comparing differential gene expression profiles between highly invasive SNU-638 and poorly invasive SNU-484 gastric cancer cell lines. Twenty-two cDNAs were identified as overexpressed genes in SNU-638 cells confirmed by Northern blot analysis. Among them, a splicing variant of aspartyl beta hydroxylase (Humbug) was also identified as a gene overexpressed in metastatic SNU-638 cells. Humbug encodes a protein identical to aspartyl-beta hydroxylase through the NH2-terminal half of the protein, but completely lacks the catalytic domain of aspartyl-beta hydroxylase. Therefore, we further investigated the possible involvement of this gene in metastatic progression of cancer cells. Expression analysis showed that the level of Humbug mRNA was well correlated with invasive and metastatic potential in various gastric cancer cell lines. Moreover, gastric tumor tissue exhibited much higher Humbug mRNA expression than the normal counterparts. Transfection of Humbug cDNA into poorly invasive Az-521 cells resulted in the increase of its migratory and invasive potentials. These results imply that Humbug could be an overexpressed gene during metastatic progression of human gastric cancer cells, and promote tumor cell invasion and metastasis.

[PC1-40] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Modulation of Redox-sensitive Transcription Factor, AP-1 by Aging and Calorie Restriction

Kim HyonJeen°, Jung KyungJin, Kim JiYoung, Chung HaeYoung

College of Pharmacy, Pusan National University, Pusan 609-735, Korea

Oxidative stress is claimed to be the major cause of aging and many age-related diseases. Recent data strongly suggested that the life-prolonging calorie restriction (CR) might retard aging by its anti-oxidative action on the regulation of the intracellular redox status. Currently, there is little information concerning the influences of age and CR on the redox-sensitive transcriptions factor, activator protein-1 (AP-1). In this present study, we investigated whether age affects the regulation of AP-1, and how the *age effect is modulated* by CR. The kidney isolated from Fischer 344 rats at 6, 12, 18, and 24 months of age fed ad libitum (AL) and CR rats were used. Results showed that AP-1 binding activity markedly increased with age in parallel with increased ROS generation, and CR suppressed the activation at the level of 6 months old. Recently, accumulating evidence indicate that mitogen-activated protein kinase (MAPK) cascade can contribute to AP-1-dependent transcription. Results showed that the aging *process strongly enhanced* all three MAPKs activities, while CR markedly suppressed the age-related activation of MAPK. It is known that thioredoxin (Trx), which is mainly in the cytoplasm, quickly translocates into the nucleus and activates AP-1 transcriptional activity by direct association with an intranuclear redox factor, Ref-1. We present evidence that the increased AP-1 activity during aging is correlates with increased nuclear protein levels of Trx and Ref-1. Based on these data, we concluded that the age-related increases in redox-sensitive AP-1 binding activity are associated with increased ROS, and CR modulates the AP-1 activation by suppressing oxidative stress. This molecular insight provides a better understanding of the regulation of cellular events leading to age-associated pathogenic process and furthermore reveals pertinent clues on possible therapeutic intervention.

[PC1-41] [10/19/2001 (Fri) 09:00 - 12:00 / Hall D]

Homology modeling of human TCTP using three different computer programs

Chai JinSun°, Choi InHee, Kim ChoonMi

College of Pharmacy, Ewha Womans University, Seoul, Korea

The translationally controlled tumor-associated proteins (TCTPs) are a highly conserved and abundantly expressed family of eukaryotic proteins that are implicated in both cell growth and the human acute