

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 $\beta$ (TGF- $\beta$ )**

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Transforming growth factor- $\beta$ (TGF- $\beta$ ), 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- $\beta$  activity. We have examined anti-proliferative activity of human TGF- $\beta$  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- $\beta$  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- $\beta$  dose-dependently. To seek the appropriate cell number for the TGF- $\beta$  bioassay,  $1 \times 10^4$ ,  $1 \times 10^5$  and  $1 \times 10^6$  cells were plated in a 96-well plate. Cell number of  $10^5$  gave the most desirable pattern for anti-proliferative activity of TGF- $\beta$ . When the incubation time for TGF- $\beta$  treatment was tested, 24 hr incubation at 37°C, 5% CO<sub>2</sub> was suitable. Taken together, we have found the experimental protocol

which gives the most sensitive quantitation of biological activity of TGF- $\beta$ :  $1 \times 10^5$  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- $\beta$  in pg/ml. Following 24 hr incubation, XTT was treated for 4 hr at 37°C, 5% CO<sub>2</sub>, 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