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ESTABLISHMENT OF *IN VITRO* BIOASSAY FOR TRANSFORMING GROWTH FACTOR (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- 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, 1104, 1105 and 1106 cells were plated in a 96-well plate. Cell number of 1105 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: 1105 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.