373

Suzuki K[1], Obara K [1], Kobayashi K[1], Yamana K [1], Itoi T [1], Takahashi K [1]
1. Division of Urology, Graduate School of Medical and Dental Science, Niigata University

TGF BETA1 REGULATES CELL DIFFERENTIATION AND CAUSES THE UP-REGULATION OF CONNECTIVE TISSUE GROWTH FACTOR IN HUMAN PROSTATE STROMAL CELL

Hypothesis / aims of study

Connective tissue growth factor (CTGF) is a fibrogenic cytokine, that involve many biological process such as cell proliferation, survival, migration, differentiation, and angiogenesis. This molecule also play a role in osteogenesis, in chondrogenesis, in the development vasculature, in placentation, and in wound heeling. Recently, overexpression of CTGF is correlated to various pathological condition, such as fibrosis, scleroderma, atheroscleosis, and renal disease, in which it acts as a transforming growth factor beta (TGF beta) downstream mediator.

TGF beta has been identified as potential candidates for regulation of stromal cell function, and as a modulator of connective tissue function in the human prostate. Previous works have shown that TGF beta has a potential role for cell differentiation and collagen production in prostate stromal cells. However, the precise role of CTGF in prostate is unclear. The aim of current study was to investigate the effects of TGF beta on induction of CTGF and exrtacellular matrix synthesis in primary culture of human prostate stromal cells. The effects on stromal cell differentiation has also demonstrated concentrated on the SMemb/NMMHC-B and SMMHC isoform SM2, the marker of phenotypic change of smooth muscle cells, in response to TGF-beta stimulation.

Study design, materials and methods

Primary cultures of human prostate stromal cell were established from 9 normal prostates by explant method. The effect of TGF beta1, at concentrations 0.1, 1, 10ng/ml,on stromal cell growth were determined by MTT conversion assay. The Real-time PCR analysis for SMemb/NMMHC-B and SM2 expression were performed in the ABI PRISM 7700 Sequence Detection System. Immunoblotting were performed to characterize stromal cell culture and to monitor the phenotypic changes after TGF beta1 treatment using monoclonal antibodies against to alpha smooth muscle actin (SM actin), desmin, and vimentin. The expression of CTGF was examined by the RT-PCR and immunoblotting. Fibronectin expression following TGF beta1 stimulation was also examined by immunoblotting.

Results

In the absence of TGF-beta1, cell expressed SM actin and vimentin. After TGF-beta1 treatment, the expression of SM actin increased in dose dependent manner.

The cells were incubated into serum-supplemented media with TGF-beta1 ranging from 0.1 to 10 ng/ml. Ninety-six hours later, the growth was quantified and compared with that in the absence of added TGF-beta1. TGF-beta1 at 1, 5, and10ng/ml suppressed the growth of the stromal cells by 72% (SD 4.8%), 62% (SD 3.2%) and 56% (SD 4.3%) and caused a reduction in the SMemb/NMMHC-B mRNA expression after 96 hours by 71% (SD 5.1%), 52% (SD 4.4%) and 38% (SD 2.6%), respectively. The growth inhibition by TGF-beta1 paralleled the diminished expression of SMemb mRNA. TGF-beta1 at 1, 5, and10ng/ml caused the up-regulation of the SM2 mRNA expression after 96 hours by 2.1 fold (SD 0.4), 3.0 fold (SD 0.2) and 5.3 (SD 0.4), respectively.

CTGF mRNA expression was detected in all nine prostate stromal cells. Immunoblotting demonstrated that both CTGF and fibronectin protein expression were up-regulated in dose dependent manner by TGF beta1 stimulation.

Interpretation of results

TGF beta1 caused the down-reguration of the SMemb/NMMHC-B gene and up-reguration of the SM2 gene in dose-dependent manner and this effect paralleled growth inhibition in the human prostate stromal cell. The expression of both CTGF and fibronectin were induced in dose-dependent manner by TGF beta1 treatment.

Concluding message

These results suggested that CTGF may play a role for TGF beta1 downstream mediator for extracellular matrix production in human prostate stromal cell.