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ROLE OF TGF-BETA1 IN CELL PHYNOTYPE MODULATION AND EXTRACELLULAR MATRIX PRODUCTION IN CULTURED HUMAN PROSTATE STROMAL CELLS.

Hypothesis / aims of study
The stromal cells of the prostate play a crucial role in the regulation of prostatic growth and function. To clarify the etiology of benign prostatic hyperplasia, we have to concentrate on not only cell growth but also extracellular matrix production.Transforming growth factor-beta1 (TGF- beta 1) has been shown to stimulate or inhibit the cell growth, induce differentiation and apoptosis depending on the cell culture conditions. Embryonic smooth muscle myosin heavy chain (SMemb/NMMHC-B) is most abundantly expressed in proliferating smooth muscle cells and correlates to the phenotypic changes of contractive type to proliferative type. Connective tissue growth factor (CTGF) has been reported as a novel fibrotic mediator produced following TGF- beta1 stimulation. The aim of the current study was to investigate the effects of TGF- beta1 on stromal cell growth and SMemb/NMMHC-B mRNA and SM2 isoform of smooth muscle myosin heavy chain mRNA expression and to clarify the correlation of CTGF expression and fibronectin (FN) synthesis after TGF- beta1 stimulation in human prostate stromal cells.

Study design, materials and methods
Primary cultures of prostate stromal cells were established by an explant method from 9 normal prostates. The effects of TGF- beta1 on stromal cell growth were determined by an MTT conversion assay. The CTGF and FN mRNA expression was detected by RT-PCR. The SMemb/NMMHC-B and SM2 mRNA expression were analyzed quantitatively by real-time PCR. The expressions of CTGF and FN protein following TGF- beta1 treatment were examined by immunoblotting. The inhibitory effects of CTGF blockade on TGF- beta1-induced FN synthesis were also determined using CTGF antisense oligodeoxynucleotide.

Results
TGF- beta1, at concentrations of 1.0, 5.0 and 10 ng/ml suppressed cell growth by 72%, 62%, 56%, respectively. TGF-beta1, at concentrations of 1.0, 5.0 and 10 ng/ml, down-regulated the SMemb/NMMHC-B mRNA expression by 71%, 52%, 38% and up-regulated the SM2 mRNA expression by 2.1, 3.0, 5.3 fold respectively. CTGF expression was detected in all nine prostate stromal cells by RT-PCR and immunohistochemistry. The up-regulation of both CTGF and FN protein by TGF- beta1 treatment was demonstrated in a dose-dependent manner. CTGF antisense oligodeoxynucleotide inhibited TGF- beta1-stimulated FN synthesis.

Interpretation of results
These results demonstrated that TGF- beta1 modulates smooth muscle cell phenotype from proliferative type to contractile type, and the inhibitory effects of TGF- beta1 on stromal cell growth correlates to the down-regulation of the SMemb/NMMHC-B gene. Meanwhile, in human prostate stromal cells , CTGF plays a crucial role in extracellular matrix production as a TGF- beta1 downstream mediator.

Concluding message
These results suggested that CTGF blockade is likely to be a therapeutic target against BPH.

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HUMAN SUBJECTS: This study was approved by the The ethics committee of Faculty of Medicine, Niigata University and followed the Declaration of Helsinki Informed consent was obtained from the patients.