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TGF-BETA REGULATES CELL GROWTH AND CAUSES THE TRANSCRIPTIONAL DOWN-REGULATION OF THE SMEMB/NONMUSCLE MYOSIN HEAVY CHAIN B (NMMHC-B) MRNA IN HUMAN PROSTATE STROMAL CELLS

Aims of Study

Benign prostate hyperplasia (BPH), the most common nonmalignant proliferative disorder of aging males, is recognized as a hyperplasia of the stromal and epithelial elements of the prostate. Stereologic morphometric analysis demonstrated an increase in the stroma to epithelium ratio from 2:1 to 5:1 in adenomatous tissue and these changes are related to the development of symptomatic BPH. These findings suggest that BPH is primarily a stromal process. The stromal cells of the prostate play a crucial role in the regulation of prostatic growth and function.

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.

TGF-beta has been shown to stimulate or inhibit the cell growth, induce differentiation and apoptosis depending on the cell culture conditions, the presence of other growth regulatory molecules and the dose and frequency with which they are used. Investigators have demonstrated the phenotypic regulatory effects and growth inhibitory effects of TGF-beta in human prostate stromal cell cultures.

In this paper, we concentrated on the SMemb mRNA expression in proliferating stromal cells and demonstrate the transcriptional down-regulation of SMemb/NMMHC-B by TGF-beta1. The inhibitory effects of TGF-beta1 and SMemb/NMMHC-B phosphorothioate oligodeoxynucleotide (PODN) on stromal cell growth are also determined.

Methods

Primary cultures of prostate stromal cells were established by an explant method from 8 normal prostates. The effects of TGF-beta1 and SMemb/NMMHC-B antisense PODN on stromal cell growth were determined by an MTT conversion assay. The SMemb/NMMHC-B mRNA expression was analyzed quantitatively by Real-time PCR.

Results

The composition of the stromal population was determined by immunohistochemistry. Depending on individual specimens, typical stromal cell cultures contained 90-95 % smooth muscle cells. In the absence of TGF-beta1, cell expressed alpha-smooth muscle actin and vimentin. After TGF-beta1 treatment, the expression of alpha-smooth muscle actin increased and cells also expressed desmin.

TGF-beta1 suppresses the stromal cell growth and SMemb/NMMHC-B mRNA expression in a dose-dependent manner. We first titrated TGF-beta1. TGF- beta1 dose lower than 0.1ng/ml did not have any effect on the cell viability. 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. In the medium without TGF-beta1, elongated cells that were spindle shaped and closely packed were commonly observed. This morphology resembled that of fibroblasts. With TGF-beta1, the cells were flatter, broader and less tightly packed. This morphology resembled that of cultured smooth muscle cells. These morphological changes were remarkable at concentrations greater than 1.0ng/ml. 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 89% (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.

Antisense PODN inhibited SMemb/NMMHC-B mRNA expression and retarded cell growth in prostate stromal cells. To confirm that the growth inhibitory effect of TGF-beta1 correlates to the decreased expression of SMemb/NMMHC-B mRNA, SMemb/NMMHC-B antisense PODN was synthesized and applied to

proliferating stromal cell cultures. Although various doses of antisense PODN were tried, only 20 M of antisense PODN had the effect of decreasing the SMemb/NMMHC-B mRNA expression and to retarding cell growth. Antisense PODN at 20 M caused a reduction in the SMemb/NMMHC-B mRNA expression after 72 hours by 37.6% (SD 3.1%) and a retardation of cell growth by 74.2% (SD 6.4%) at 72 hours No morphological changes were observed in the medium with the equal concentration of antisense PODN.

Conclusions

TGF-beta1 caused the down-regulation of the SMemb/NMMHC-B gene in a dose-dependent manner and this effect paralleled growth inhibition in the human prostate stromal cells. The SMemb/NMMHC-B antisense PODN treatment of stromal cells caused a substantial reduction in the SMemb/NMMHC-B mRNA level and retarded cell growth. These results suggested that the inhibitory effects of TGF-beta1 on the stromal cell growth might be executed, at least partially by the down-regulation of the SMemb/NMMHC-B gene.