

Transforming growth factor- β induces growth inhibition and IGF-binding protein-3 production in prostatic stromal cells: abnormalities in cells cultured from benign prostatic hyperplasia tissues

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Abstract

The IGF axis has been implicated in the pathogenesis of benign prostatic hyperplasia (BPH) via the paracrine action of IGFs and IGF-binding proteins (IGFBPs). In this study, we examined the regulation of cell growth and IGFBP-3 secretion by transforming growth factor- β (TGF- β) in prostatic stromal cell (PC-S) cultures from histologically normal tissues and tissues from BPH. PC-S cultures were treated with varying doses of TGF- β 1. Forty-eight hour conditioned media (CM) from these cultures were subjected to Western immunoblotting and ligand blotting for detection and quantification of IGFBPs. IGFBPs-2, -3 and -4 were detected in the CM from normal PC-S cultures. In CM from BPH PC-S, IGFBP-3 levels were 2-fold lower at baseline than in the normal PC-S CM, in addition to the differences in IGFBPs-2 and -5 which we have previously reported. In response to TGF- β 1, a 15-fold increase in the levels of IGFBP-3 was observed in normal PC-S CM, while a mere 2-fold increase was observed in BPH PC-S CM ($P < 0.001$). These findings were confirmed by specific

immunoblotting and immunocytochemistry. IGFBP-3 mRNA levels detected by Northern blotting of total RNA extracted from similar cultures showed the induction of IGFBP-3 expression by TGF- β 1 in normal PC-S and its lack of induction in BPH PC-S. Cell growth inhibition in response to TGF- β 1 correlated with the IGFBP-3 concentrations found in CM. Normal PC-S showed a 60% decrease in cell number after 10 days in media with 1 ng/ml TGF- β 1, compared with the untreated control. The decrease in proliferation observed in comparably treated BPH cells was only 20% ($P < 0.001$). In conclusion, BPH PC-S had a reduced IGFBP-3 response to TGF- β 1 and demonstrated decreased TGF- β 1-induced growth inhibition relative to normal PC-S. We hypothesize that in normal PC-S, TGF- β exerts its anti-proliferative effects by stimulating the production of IGFBP-3, which acts as an inhibitory factor, either by inhibiting IGFs or directly by interacting with cells, and that this process is altered in BPH PC-S.

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Introduction

The insulin-like growth factor (IGF) axis consists of IGF-I and IGF-II, the type I and type II IGF receptors, and six known IGF-binding proteins (IGFBPs-1 to -6). Locally produced IGFs and IGFBPs regulate tissue growth and differentiation (Solberg & Cohen 1996). The IGFBPs are thought to modulate the action of IGFs in several ways, including an inhibitory model in which IGFBPs sequester IGFs from their receptors (Angervo *et al.* 1991, Cohen *et al.* 1993), an enhancing model in which IGFBPs transport IGFs to their site of action (Elgin *et al.* 1987, Conover 1992, Neuenschwander *et al.* 1996), or an IGF-independent model that may involve direct inter-

action of IGFBPs with putative IGFBP receptors (Oh *et al.* 1993a,b, 1995, Valentinis *et al.* 1995, Angelloz-Nicoud *et al.* 1996, Gucev *et al.* 1996, Lalou *et al.* 1996, Gill *et al.* 1997, Rajah *et al.* 1997, Rechler 1997, Zadeh & Binoux 1997), which mediate the growth inhibitory effects of the IGFBPs. IGFBPs are regulated by various endocrine factors and are expressed in specific ontogenic patterns (Solberg & Cohen 1996). In the prostate gland, IGFs and IGFBPs appear to play an important role in the proliferative processes that lead to benign prostatic hyperplasia (BPH) and prostate cancer (Cohen *et al.* 1994b). We have previously demonstrated that prostatic stromal cell (PC-S) cultures from tissues with BPH harbor numerous changes in the IGF axis (Cohen *et al.* 1994a,b, Dong *et al.* 1997).

These changes include a decrease in the levels of the tumor suppressor transcription factor WT-1 leading to increased expression of IGF-1 receptor and IGF-II (Guo *et al.* 1995) as well as a loss of IGFBP-2 and a gain in IGFBP-5 secretion from PC-S (Cohen *et al.* 1994b).

Transforming growth factor- β (TGF- β) has been demonstrated in previous studies to be a potent regulator of IGFBP production by various cell types (Martin & Baxter 1991, Martin *et al.* 1992, Yateman *et al.* 1993, Cazals *et al.* 1994, Durham *et al.* 1994, McCusker & Clemmons 1994, Tsukazaki *et al.* 1994, Guo *et al.* 1995, Han *et al.* 1997, Hwa *et al.* 1997, Rajah *et al.* 1997). In most cells, TGF- β has been shown to be a potent stimulator of IGFBP-3 (Martin & Baxter 1991, Martin *et al.* 1992, Yateman *et al.* 1993, Cazals *et al.* 1994, McCusker & Clemmons 1994, Han *et al.* 1997, Hwa *et al.* 1997, Rajah *et al.* 1997). TGF- β is also known to inhibit the growth of certain cells (Yateman *et al.* 1993, Oh *et al.* 1995, Rajah *et al.* 1997). Using a breast cancer model, Oh *et al.* (1995) demonstrated that the stimulation of IGFBP-3 by TGF- β 2 is critical to TGF- β -induced growth inhibition, and Rajah *et al.* (1997) showed that IGFBP-3 expression is necessary for TGF- β 1-induced apoptosis in a prostate cancer model. Several investigators suggest that IGFBP-3 acts directly through binding to specific cell surface receptors on susceptible cells (Conover 1992, Cohen *et al.* 1993, Oh *et al.* 1993a, 1995, Valentinis *et al.* 1995, Angeloz-Nicoud *et al.* 1996, Gucev *et al.* 1996, Lalou *et al.* 1996, Neuenschwander *et al.* 1996, Gill *et al.* 1997, Karas *et al.* 1997, Leal *et al.* 1997, Mohseni-Zadeh & Binoux 1997, Rajah *et al.* 1997, Rechler 1997, Zadeh & Binoux 1997).

In this study, we examined the effects of TGF- β on growth and IGFBP-3 secretion of normal and BPH PC-S cultures with the hypothesis that the TGF-IGFBP-3-growth axis is also aberrant in BPH.

Materials and Methods

Materials

Iodinated IGF-I and -II were purchased from Amersham (Arlington Heights, IL, USA). Human IGFBP-3 antibodies purified on an IGFBP-3 affinity column were purchased from Diagnostic Systems Laboratories (Webster, TX, USA). TGF- β 1 was purchased from Promega Corp. (Madison, WI, USA). SDS-PAGE reagents were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Primary cell cultures

Prostatic tissues were obtained from radical prostatectomy or open prostatectomy specimens and processed for cell culture as previously described (Peehl 1992, Peehl &

Sellers 1997). The remainder of the prostate was fixed and serially sectioned. Normal cell cultures were derived from tissues with no evidence of BPH or cancer in the sections surrounding the wedge used for culture. BPH cell cultures were derived from BPH tissues with no cancer. Selective culture conditions allowed the establishment of pure stromal strains with no epithelial cell contamination. Stromal cells were maintained in MCDB 105 medium (Sigma) with 10% (v/v) fetal bovine serum (FBS) and 100 μ g/ml gentamicin. Five strains each of BPH and normal PC-S were used altogether. Derivation of pure stromal cells was achieved as follows. Tissue samples were minced and digested overnight in collagenase. After rinsing, the digested tissue fragments were inoculated into one 60-mm dish containing MCDB 105 medium supplemented with 10% FBS and 100 μ g/ml gentamicin. Dishes were incubated in a humidified atmosphere at 37 °C with 5% CO₂/95% air. Generally both stromal and epithelial cells attached within 1 week. The cells were fed every 3 or 4 days until dishes contained abundant stromal outgrowth. During this period, degeneration of epithelial cells was common. Remaining epithelial cells were lost upon the first passage, which was achieved by rinsing the cells twice with HEPES-buffered saline and incubating for 2–3 min at 37 °C with 0.2% trypsin/0.02% EDTA. Cells were resuspended with MCDB 105 containing 10% FBS and gentamicin and briefly centrifuged. The cell pellet was resuspended in MCDB 105 with 10% FBS and gentamicin and transferred to two 60-mm dishes. These secondary cultures, free of epithelial cells, were grown to confluency, then frozen. Cells were thawed and passaged as needed for experimental purposes. Each cell strain was routinely characterized by immunocytochemical labeling and was found to be negative for cytokeratin and factor VIII, but positive for vimentin and fibronectin. Alpha-smooth muscle actin was generally present at low levels.

Conditioned media (CM)

CM were obtained from cell cultures grown to confluency in MCDB 105 with 10% FBS. Cells were then washed twice with HEPES-buffered saline and incubated with serum-free media (with or without TGF- β) for 48 h. Media were collected, centrifuged briefly, and stored at –70 °C until analysis.

Western ligand blots (WLB)

Serum-free CM (50 μ l) was electrophoresed through non-reducing 12.5% polyacrylamide-SDS gels overnight at constant voltage, electroblotted onto nitrocellulose, sequentially washed with NP-40, blocked with 1% BSA in Tris-buffered saline, incubated with 10⁶ c.p.m. ¹²⁵I-IGF-I and -II for 12 h, then exposed to film for 5 days, as

previously described (Cohen *et al.* 1991). Each lane of the autoradiographs was scanned and analyzed densitometrically.

Western immunoblots (WIB)

Detection of CM levels of IGFBP-3 was carried out in the same way as in WLB, except that the nitrocellulose was probed with IGFBP-3-specific antibodies (Diagnostic Systems) using a peroxidase-linked chemiluminescence detection system (Amersham) as previously described (Rajah *et al.* 1997).

Northern blots

Total RNA (20 μ g/lane) was electrophoresed through a 1% agarose-formaldehyde gel at constant voltage and capillary blotted onto Zeta-probe membrane (BioRad, Hercules, CA, USA). Membranes were probed overnight with 32 P-labeled IGFBP-3 cDNA fragment (amplified by PCR using primers for a 400 bp fragment of the N-terminal region of human IGFBP-3), washed, and exposed to film as previously described (Cohen *et al.* 1991). RNA loading was controlled for by normalization to actin expression on the same blots. Blots were quantified with the aid of a phosphoimager (Molecular Dynamics, Atlanta, GA, USA).

Immunocytochemistry

Cells were inoculated at 5×10^3 cells per chamber in eight-chamber slides containing MCDB 105 with 10% FBS. Three days later, medium was changed to MCDB 105 without serum and with or without 1 ng/ml TGF- β 1. Four days later, cells were fixed with 2% paraformaldehyde and permeabilized with 95% ethanol. Non-specific binding was blocked with 10% horse serum, then the cells were incubated with a polyclonal rabbit antiserum against IGFBP-3 (1:400) (Diagnostic Systems). Binding of the primary antibody was detected with biotinylated anti-rabbit IgG, the avidin-biotin complex reagent, and the substrate diaminobenzidine as previously described (Peehl & Sellers 1997).

Growth assays

Cells were inoculated at 200 cells/60 mm dish containing MCDB 105 with 10% FBS and experimental factors. After 10 days of incubation, the cells were fixed and stained with crystal violet and relative growth was measured with an image analyzer as previously described (Cohen *et al.* 1991).

Statistical analysis

Experiments were performed in triplicates and repeated three times. Densitometric data were analyzed using

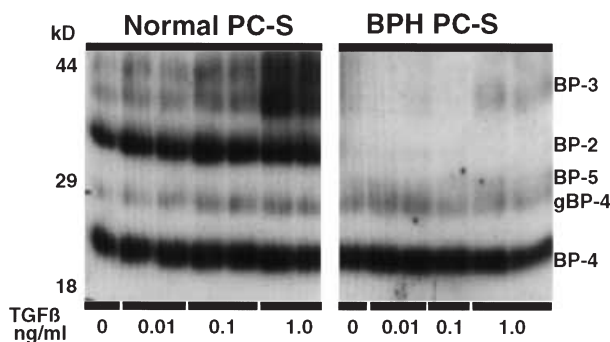


Figure 1 Demonstration of IGFBPs secreted from normal and BPH PC-S. WLB analyses of 48 h CM from normal and BPH stromal cell strains are shown. (gBP-4=glycosylated IGFBP-4.) CM (50 μ l) was electrophoresed on 12.5% SDS-PAGE and visualized by autoradiography of 125 I-IGF-I and -II bindings.

standard statistical methods, including Student's *t*-test and ANOVA. Results are reported as mean \pm S.E.M.

Results

Detection of IGFBPs in PC-S CM

We examined the levels of IGFBPs in PC-S CM obtained from confluent cultures maintained in serum-free medium for 48 h. Figure 1 is an autoradiograph which demonstrates the various IGFBPs which are detectable by 125 I-IGF-labeled WLB. IGFBPs-2 to -5 were identifiable by their respective sizes and were confirmed by specific immunoblots (data not shown). In the control (untreated) lane of normal PC-S CM, bands representing IGFBPs-4, gly-4, -2 and -3 are visible, while in the control lane of BPH PC-S CM, IGFBP-2 (31 kDa) is dramatically reduced in intensity while IGFBP-5 (24 kDa) becomes apparent, as we have previously published (Cohen *et al.* 1994a). IGFBP-4 (24 kDa) levels appear to be constant between the normal and BPH strains. Also noted is IGFBP-3 (40–44 kDa), which is 2-fold lower in BPH vs normal. Studies of three additional normal and BPH strains showed similar findings (shown in Fig. 2).

Effect of TGF- β 1 on IGFBP-3 levels as detected by WLB

CM was collected from confluent cultures maintained for 48 h in serum-free medium with or without TGF- β 1 (0.01–1.0 ng/ml). In Fig. 1, it is observed that TGF- β 1 had a significant, dose-dependent stimulatory affect on the production of IGFBP-3 by normal PC-S. A similar effect is apparent in the BPH PC-S CM, but to a much lesser degree. The other IGFBPs in PC-S CM were not affected by TGF- β treatment. Figure 2 shows a graphical representation of densitometric analysis of mean control and TGF- β 1-stimulated IGFBP-3 levels in the normal ($n=4$

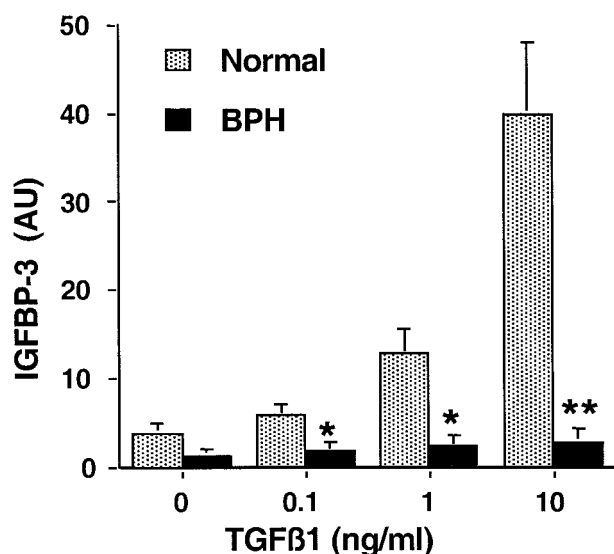


Figure 2 Induction of IGFBP-3 protein secretion by TGF- β 1 in prostate stromal cells. Densitometrically analyzed values of IGFBP-3 glycoprotein levels in the CM of normal ($n=3$) and BPH ($n=3$) PC-S are shown as mean \pm S.E.M. * $P<0.01$, ** $P<0.001$ compared with normals.

strains) vs the BPH ($n=4$ strains) PC-S CM. At baseline, the normal PC-S expressed 2-fold more IGFBP-3 than BPH PC-S (P =not significant) and showed a dose-dependent response to TGF- β 1. Remarkably, when maintained in the presence of 1.0 ng/ml TGF- β 1, the normal cells expressed 15-fold more IGFBP-3 than at baseline while the BPH PC-S grown in the same condition showed only a 2-fold increase in IGFBP-3 levels. The normal PC-S, thus, appear to be six times more responsive to TGF- β 1-stimulation of IGFBP-3 than the BPH PC-S. Identical findings were observed by WIBs (data not shown).

Immunocytochemistry

Immunocytochemical analysis of IGFBP-3 was performed on control and TGF- β 1-treated cultures of normal and BPH PC-S. In Fig. 3, the control normal PC-S showed slight staining for IGFBP-3, whereas the control BPH PC-S had little IGFBP-3 staining. When treated with 1.0 ng/ml TGF- β 1 for 3 days, the normal cells showed marked increase in staining for IGFBP-3. The BPH PC-S showed only a slight increase in staining for IGFBP-3, following treatment with TGF- β 1.

Effects of TGF- β 1 on IGFBP-3 mRNA levels

In order to determine the nature of TGF- β 1 regulation of IGFBP-3, Northern blot studies were conducted on total RNA from normal and BPH PC-S, both with and

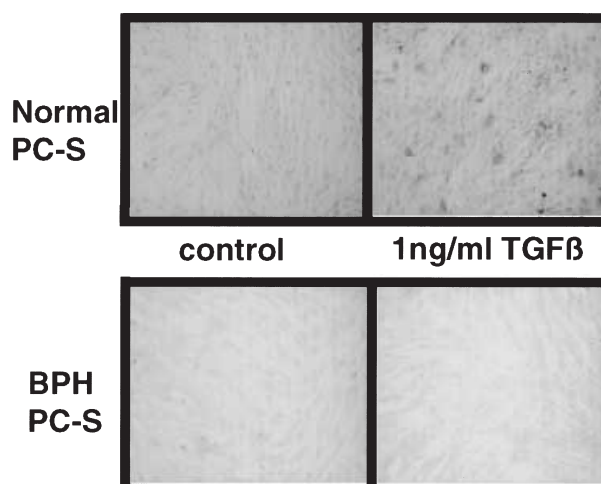


Figure 3 *In situ* induction of IGFBP-3 protein by TGF- β 1 in normal and BPH PC-S. Immunocytochemical staining with IGFBP-3 antibodies in normal and BPH cells are shown. Cultures grown to near confluency were treated for 48 h with 1 ng/ml TGF- β 1.

without TGF- β 1 treatment. Figure 4A depicts total RNA probed for IGFBP-3 message in the same normal and BPH PC-S cultures described earlier. Baseline IGFBP-3 mRNA levels appeared similar in normal and BPH cells. However, consistent with WLB and WIB findings, the normal PC-S were significantly more responsive to TGF- β 1 than BPH PC-S. As shown in Fig. 4B, phosphoimager analysis of three experiments on three normal and three BPH strains demonstrated that at 1.0 ng/ml TGF- β 1, normal PC-S IGFBP-3 mRNA levels were 5-fold higher than those of BPH PC-S ($P<0.001$). This suggests that the increased levels of IGFBP-3 glycoprotein seen in the CM of normal TGF-stimulated PC-S cells are a result of increases in IGFBP-3 mRNA levels, but additional post-transcriptional mechanisms such as modulation of proteolysis or cellular uptake of IGFBP-3 may also contribute to the rise in the CM IGFBP-3 levels.

Cell proliferation studies

We and others have reported the dose-dependent inhibition of prostatic stromal growth by TGF- β (Story *et al.* 1993, Kassen *et al.* 1996, Peehl & Sellers 1997). Proliferation studies were performed to compare the responsiveness to TGF- β 1 of BPH and normal PC-S. Attenuated growth inhibition of BPH PC-S by TGF- β 1 paralleled the diminished induction of IGFBP-3 by TGF- β 1 in these cells. Cells were inoculated into serum-supplemented media with TGF- β 1 ranging from 0.001 to 1 ng/ml. Ten days later, growth was quantified and compared with that in the absence of added TGF- β 1. Figure 5 shows that TGF- β 1 at 1 ng/ml suppressed the growth of normal PC-S strains ($n=5$) by 68%. In contrast, the same amount of TGF- β 1 inhibited the growth of BPH PC-S strains

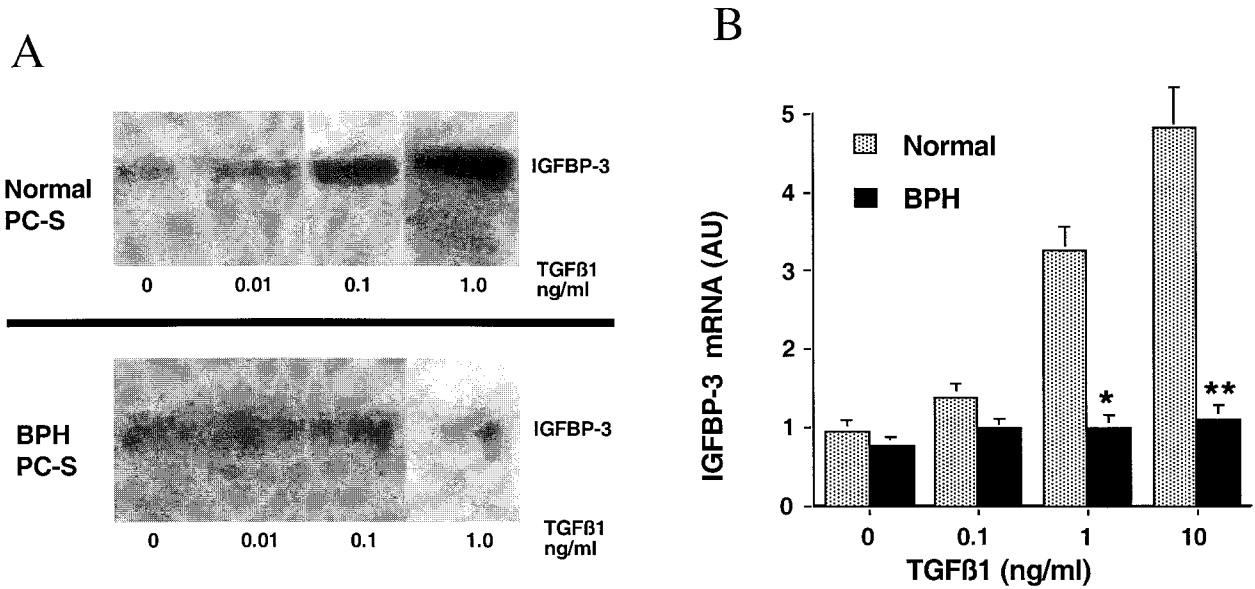


Figure 4 Induction of IGFBP-3 mRNA by TGF- β 1 in normal and BPH PC-S. (A) Northern blot analysis of total RNA extracts from normal and BPH stromal cells treated with varying doses of TGF- β 1 for 48 h; 20 μ g per lane of total RNA were used and the blots were probed with the IGFBP-3 cDNA. (B) Phosphorimager analyzed normalized means \pm S.E.M. of three strains each of normal and BPH PC-S stimulated with TGF- β 1.

($n=5$) by only 26%. Therefore, BPH PC-S were significantly less responsive than normal PC-S to the growth-inhibitory activity of TGF- β , similar to their decreased response to TGF- β induction of IGFBP-3 secretion.

Identification of IGFBP-3 association proteins in PC-S

Since IGFBP-3 has been shown to mediate the effects of TGF- β in other types of cells by an IGF-independent

mechanism, we examined PC-S for the presence of candidate IGFBP-3 receptors/association proteins. Figure 6 shows a reverse ligand blot of normal and BPH PC-S cellular proteins. After separating the proteins by SDS-

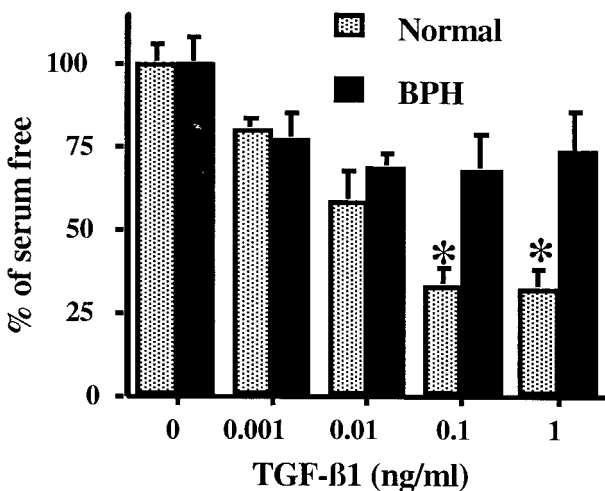


Figure 5 Growth inhibition of normal vs BPH PC-S by TGF- β . Five different strains each of normal PC-S and BPH PC-S were grown in the presence of varying concentrations of TGF- β 1 for 10 days. On the 10th day, growth was quantified. Shown are means \pm S.E.M.

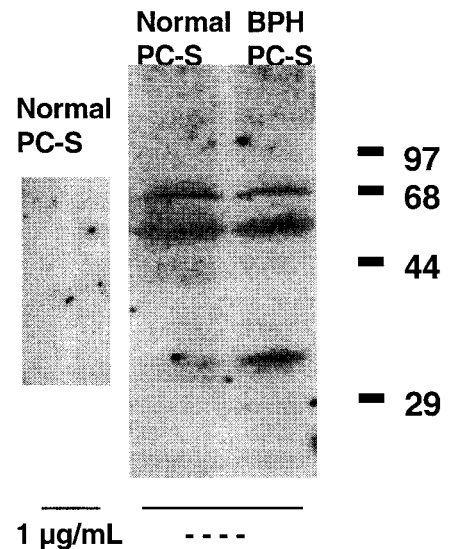


Figure 6 Demonstration of putative IGFBP-3 receptors in PC-S cells. Reverse WLBs were performed on whole cell lysates which were run on 12% SDS-PAGE and blotted onto nitrocellulose membrane. The membrane was then incubated overnight with 125 I-IGFBP-3. Bands at 35, 50 and 55 kDa represent putative IGFBP-3 receptors. These bands are not seen in the presence of excess unlabeled IGFBP-3.

PAGE, the proteins were transferred to a nitrocellulose membrane, which was hybridized with ^{125}I -IGFBP-3. The subsequent autoradiogram revealed three significant bands, present in protein extracts from both normal and BPH PC-S. These bands, all of nearly equal intensity, were approximately 36, 52 and 60 kDa. These putative IGFBP-3 receptors/association proteins may provide an IGF-independent pathway for IGFBP-3-mediated growth inhibition and are similar in size to the putative receptors/association proteins reported for IGFBP-3 by Rajah *et al.* (1997) and Oh *et al.* (1993b). There were no significant differences between the intensity of the bands when five BPH and five normal strains of PC-S were compared (data not shown). To determine the specificity of ^{125}I -IGFBP-3 binding to its association proteins, nitrocellulose on which cell lysates from PC-S were electroblotted was incubated with ^{125}I -IGFBP-3 in the presence of unlabeled excess (1 $\mu\text{g}/\text{ml}$) of glycosylated recombinant human IGFBP-3 resulting in a loss of the bands.

Discussion

In this study, we correlated changes in IGFBP-3 expression and secretion (by multiple methods) with the growth-inhibitory activity of TGF- β in several strains of normal and BPH PC-S. We observed a dramatic induction of the growth-suppressing IGFBP-3 in normal strains, while only a slight induction was observed in BPH strains. Significantly, growth response to TGF- β correlated with IGFBP-3 induction. At the highest tested concentration of 1 ng/ml TGF- β 1, normal PC-S showed a nearly 70% suppression of growth at 10 days relative to control conditions while BPH PC-S cell numbers were only reduced by 20% in response to TGF- β 1. While these two observations are merely correlative at this stage, we propose that the decreased induction of IGFBP-3 in BPH PC-S may lead to the abnormal growth inhibition by TGF- β in this pathological state. We further suggest that the effects of IGFBP-3 in PC-S are mediated both by binding to endogenously produced IGF-II, whose secretion by PC-S we have previously demonstrated (Cohen *et al.* 1994a,b, Dong *et al.* 1997), and by binding directly to IGFBP-3 association proteins which we have now identified in PC-S.

The IGF axis is a critical component in the growth, differentiation and death of many cell types. The ontogeny and tissue-specific expression of the various components of the IGF axis are tightly regulated by hormonal, metabolic and genetic factors (Solberg & Cohen 1996). Changes in these regulatory factors and the levels of IGF axis components in the cellular environment can have profound effects on cellular growth and proliferation. The prostate gland represents a microenvironment wherein IGF axis-mediated autocrine-paracrine growth regulation has been repeatedly demonstrated (Cohen *et al.* 1994a,b,

Angelloz-Nicoud *et al.* 1996, Dong *et al.* 1997, Hwa *et al.* 1997, Rajah *et al.* 1997).

Many agents have been identified as having growth modulating effects correlated with changes in IGFBP levels. Several trophic hormones have been shown to suppress IGFBP production by their target cells, suggesting that such suppression may be part of the stimulatory action of trophic peptides. These include thyroid-stimulating hormone in thyroid cells, follicle-stimulating hormone in Sertoli cells and granulosa cells and luteinizing hormone in Leydig cells (Solberg & Cohen 1996). Conversely, many growth inhibitory agents have been shown to induce IGFBP production by target cells. These include vitamin-D₃ in bone and prostate cells, tumor necrosis factor- α in fibroblasts, and retinoic acid in breast cancer cells (as reviewed in Solberg & Cohen 1996). Most of these examples have been shown to be linked to regulation of the respective mRNA levels of the IGFBPs involved in the actions of the regulatory factors mentioned. The hypothesis commonly proposed regarding this highly regulated IGFBP secretion from cells is that local IGFBP levels govern the proliferation and survival of the cells by regulating the levels of free autocrine IGFs available to interact with the IGF receptor. Alternatively, we and others proposed that the levels of IGFBPs, particularly IGFBP-3, directly regulate cell growth and death via the recently proposed IGFBP-3 receptors/association proteins (Conover 1992, Cohen *et al.* 1993, Oh *et al.* 1993a, 1995, Valentinis *et al.* 1995, Angelloz-Nicoud *et al.* 1996, Gucev *et al.* 1996, Lalou *et al.* 1996, Neuenschwander *et al.* 1996, Gill *et al.* 1997, Karas *et al.* 1997, Leal *et al.* 1997, Mohseni-Zadeh & Binoux 1997, Rajah *et al.* 1997, Rechler 1997, Zadeh & Binoux 1997).

The results of our study provide a possible mechanism for the unregulated prostate cell growth observed in BPH. The potent inhibitory response of normal PC-S to TGF- β concomitant with a strong induction of the growth-inhibitory IGFBP-3 may be important in the regulation of prostate tissue growth and maintenance. The reduced response of BPH stromal cells to TGF- β may represent a critical abnormality which plays a role in the growth dysregulation observed in BPH tissues.

We have previously proposed that reduction in the levels of the expression of the tumor suppressor transcription factor, WT-1, are related to the BPH phenotype (Dong *et al.* 1997). In that study we compared 25 strains of PC-S from normal, hyperplastic and malignant origins. WT-1 mRNA levels were reduced to 10% of normal in BPH PC-S strains, while no change was observed in cancer PC-S. IGF-II mRNA levels inversely correlated with WT-1 levels in that study, being 10-fold elevated, as did IGF-1R mRNA levels, being 3-fold elevated (Cohen *et al.* 1994a, Dong *et al.* 1997). WT-1 is known to regulate these two genes as well as additional target genes and specifically has been shown to regulate TGF- β 1 expression (Dey *et al.* 1994). It is unknown at this time whether

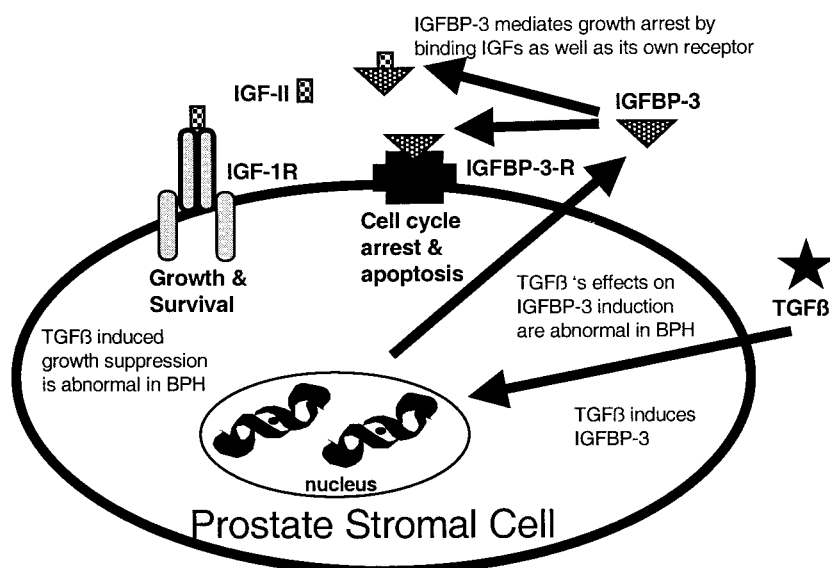


Figure 7 A theoretical model of TGF- β action in PC-S and its abnormalities in BPH. TGF- β inhibits cell growth by stimulating IGFBP-3 secretion. IGFBP-3, in turn, acts to block IGF-dependent proliferation, as well as directly inhibiting growth via its own receptor. In BPH, an impaired TGF- β action on IGFBP-3 and growth inhibition is recognized.

the TGF- β signal transduction cascade is regulated by WT-1. It is also unclear what role WT-1 plays in regulating IGFBP gene expression.

It is attractive to postulate that several IGFBPs including IGFBPs-2, -3 and -5 are WT-1 dependent as all three of these are dysregulated in BPH in the face of altered WT-1 levels (as seen in Fig. 1). Studying 19 different strains of PC-S, we have previously shown that the mRNA expression and protein secretion of IGFBP-2 is dramatically abolished in BPH vs normal and cancer strains (Cohen *et al.* 1994a). IGFBP-5, which is not seen in the secretions of normal PC-S strains, is present in significant amounts in BPH PC-S strains. These two genes are co-localized and tightly linked at chromosomal region 2q33-34 and may therefore be subject to coordinated regulation by WT-1 or other transcriptional regulators. Unlike the IGF-inhibitory nature of IGFBP-2, IGFBP-5 has been demonstrated to be growth enhancing in certain systems (Solberg & Cohen 1996). Thus, multiple changes in the IGF axis are apparent in BPH stroma. Together, these molecular changes may be responsible for the dysregulated growth of the hyperplastic prostate. In our previous study of IGFBPs in BPH and normal PC-S strains (Cohen *et al.* 1994a), we did not observe significant differences in unstimulated IGFBP-3 levels in the CM from these two strain types. IGFBP-3 levels were approximately 35% lower at baseline in BPH strains. Similarly, IGFBP-3 levels were 50% lower (but not statistically different) in BPH vs normal strains in the current study. The significant difference between normal and BPH

IGFBP-3 levels was only apparent after TGF- β 1 stimulation. This observation also correlates with the finding that the altered growth phenotype of BPH only becomes manifest in an environment in which TGF- β is present.

BPH is an extremely common disorder, affecting a large proportion of elderly men. The etiology of this condition remains unknown, but it appears to be related to local factors rather than to systemic hormonal changes. The trophic systems that may influence prostate growth, such as the androgenic and somatotrophic pathways, normally operate at a reduced, rather than increased tone at the age during which BPH develops. Histologically, the changes that occur in the hyperplastic prostate are reminiscent of a regression to a fetal-like state, and include a proliferation of the stroma followed by epithelial growth (McNeal 1990). The loss of IGFBP-3 response to TGF- β in the prostatic stroma as well as the other described differences in the IGF axis are compatible with the above hypothesis. Our findings of abnormal TGF- β response in cultured cells could well be related to increased proliferation *in vivo*.

In separate studies, we have shown the growth inhibition by TGF is accompanied by the induction of a smooth muscle cell phenotype (Peehl & Sellers 1997). Preliminary studies with BPH PC-S indicate that TGF- β induces smooth muscle differentiation equivalently in BPH and normal PC-S (D M Peehl, unpublished observations). This observation implies that the aberrant responses of BPH PC-S to TGF- β in terms of IGFBP-3 secretion and growth inhibition that we have noted in the current study are not the result of loss of TGF- β receptors.

Instead, lack of induction of IGFBP-3 and loss of growth control by TGF- β may be related to aberrations in downstream signaling events in BPH PC-S.

The nature of the TGF- β -induced growth inhibition of PC-S cells may involve the induction of either cell cycle arrest or apoptosis. In other cellular systems, TGF- β is known to mediate both of these processes (Oh *et al.* 1995, Rajah *et al.* 1997). We and others have recently shown that IGFBP-3 directly mediates cell cycle arrest and apoptosis in fibroblasts derived from IGF-1 receptor knock-out mice (Valentinis *et al.* 1995, Rajah *et al.* 1997), in PC-3 prostate cancer cells (Rajah *et al.* 1997) and in breast cancer cells (Gill *et al.* 1997, Nickerson *et al.* 1997). The connection between IGFBP-3 and these processes is further substantiated by the fact that IGFBP-3 is a p53 response gene (Buckbinder *et al.* 1995, Friedlander *et al.* 1996, Ludwig *et al.* 1996). The ability of IGFBP-3 to regulate cell growth and death in environments which have IGF-I or IGF-II action via the IGF receptor is intuitive due to the well-described high-affinity binding of IGFBP-3 to the IGFs, which exceeds the affinity of IGFs to the IGF-1 receptor (Solberg & Cohen 1996). Alternatively, IGF-independent actions of IGFBP-3 may be operative through binding to specific cell surface receptors/association proteins which may mediate (as yet uncharacterized) signal transduction leading to the described effects. These putative receptors, similar in their electrophoretic mobility to the IGFBP-3 association molecules we identified in Fig. 6, bind IGFBP-3 specifically, and with high affinity (Oh *et al.* 1993b). IGFBP-3 may also bind an additional molecules including the type V TGF- β receptor, matrix and cell surface structures, or the IGF-1 receptor (Karas *et al.* 1997, Leal *et al.* 1997, Mohseni-Zadeh & Binoux 1997).

In summary, as illustrated in the cartoon in Fig. 7, we have demonstrated that PC-S strains isolated from patients with BPH harbor a defect in TGF- β action on the induction of IGFBP-3. The reduced IGFBP-3 in BPH PC-S may lead to the observed decrease in the inhibitory effect of TGF- β on PC-S growth seen in BPH strains. The action of IGFBP-3 on cell growth in PC-S may be mediated by binding to IGF-II or by interacting directly with IGFBP-3 receptors.

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