TGF-β maintains dormancy of prostatic stem cells in the proximal region of ducts

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We have previously shown that prostatic stem cells are located in the proximal region of mouse prostatic ducts. Here, we show that this region responds differently to transforming growth factor (TGF)-β than the distal ductal region and that under physiological conditions androgens and TGF-β are crucial overall regulators of prostatic tissue homeostasis. This conclusion is supported by the observations showing that high levels of TGF-β signaling are present in the quiescent proximal region of ducts in an androgen-replete animal and that cells in this region overexpress Bcl-2, which protects them from apoptosis. Moreover, androgen ablation reverses the proximal-distal TGF-β signaling gradient, leading to an increase in TGF-β signaling in the unprotected distal region (low Bcl-2 expression). This reversal of TGF-β–mediated signaling accompanies apoptosis of cells in the distal region and gland involution after androgen withdrawal. A physiological TGF-β signaling gradient (high proximally and low distally) and its functional correlates are restored after androgen replenishment. In addition to highlighting the regulatory role of androgens and TGF-β, these findings may have important implications for the deregulation of the stem cell compartment in the etiology of proliferative prostatic diseases.

Introduction

The prostate is an androgen-dependent organ and is the site of two significant diseases, namely prostate carcinoma and benign prostatic hyperplasia (BPH), the etiologies of which are poorly understood. The presence of a population of long-lived stem cells that survives androgen ablation in the prostate is indicated by the fact that the prostate regenerates normally after more than 30 cycles of involution-regeneration (Isaacs, 1985). As tumor cells may originate within the stem cell population (Reya et al., 2001; Pardal et al., 2003; Beachy et al., 2004; Valk-Lingbeek et al., 2004), the identification of prostate stem cells and an understanding of their biology is important for elucidating the mechanisms by which cancer and other proliferative diseases of the prostate arise.

Stem cells are generally quiescent and reside in a specialized cellular location known as a niche. The niche provides a microenvironment that maintains the balance between quiescence and self-renewal of the stem cell population. The mechanism by which the niche maintains the primitive phenotype of cells is poorly understood. Significant evidence indicates that stem cell homeostasis in some systems is regulated by a balance between the inhibitory effects of TGF-β and the stimulatory effects of mitogenic cytokines, indicating that a balance of stimulatory and inhibitory signals is likely to regulate stem cell quiescence and proliferation (Cashman et al., 1992; Gabrilove et al., 1994; Fortunel et al., 2000b). TGF-β has also been shown to inhibit the proliferation of primitive cells of several origins (Puolakkainen et al., 1994; Potten et al., 1995, 1997; Gorska et al., 1998; Fan et al., 2002). Recently, it has been shown that epidermal label-retaining cells in the hair follicle bulge region (stem cell niche) have an increased expression of latent TGF-β binding protein (LTBP-1), which is necessary for TGF-β activation, and phosphorylated SMAD (pSMAD), which is implicated in TGF-β signaling (Fuchs et al., 2004; Tumbar et al., 2004), indicating that TGF-β activation and signaling may be important in this stem cell niche.

The mouse prostate can be divided into ventral, dorsal, and lateral lobes with each lobe consisting of a branched ductal network that opens into the urethra. Each duct consists of a proximal region (adjacent to the urethra), an intermediate region, and a distal region (Fig. 1). We recently demonstrated that the proximal region of murine prostatic ducts contains a population of cells exhibiting features of stem cells, namely slow-

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Abbreviations used in this paper: BMP, bone morphogenetic protein; BPH, benign prostatic hyperplasia; IGF-1, insulin-like growth factor 1; LTBP-1, latent TGF-β binding protein; MFI, mean fluorescence intensity; pSMAD, phosphorylated SMAD; SCF, stem cell factor; TMLC, TGF-β–responsive mink lung cells.

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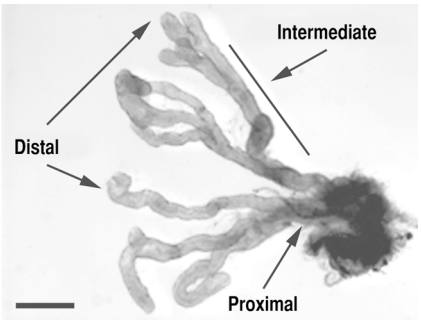


Figure 1. **Microdissected prostatic ducts.** A segment from a microdissected mouse dorsal prostate showing the distal, intermediate, and proximal regions of prostatic ducts. Bar, 1 mm.

cycling cells with high proliferative potential capable of reconstituting branched glandular ductal structures from a single cell (Tsujimura et al., 2002). We propose that the stem cell population in the proximal region of the prostate is kept in a quiescent state in its niche by a balance between TGF-β, which inhibits proliferation of the stem cells, and other mitogenic cytokines that promote proliferation. In this paper, we show that cells in the proximal and distal regions of prostatic ducts respond differentially to TGF-β. In the intact prostate, active TGF-β in the proximal region maintains the stem cells in a quiescent state. During castration-induced involution, TGF-β signaling increases distally. The increased signaling distally results in the apoptosis of cells in this region, leading to involution of the prostate. At the same time, TGF-β signaling decreases proximally, thus "priming" stem cells in this region to respond to mitogenic growth factors. With androgen replacement, the reduced levels of TGF-β signaling in the proximal region, in conjunction with expression of various stimulatory cytokines, facilitates division of the stem cells. Once the prostate has regenerated, TGF-β signaling in the proximal region increases and is again significantly higher than that of the distal region, thereby maintaining quiescence of the stem cells in the proximal region. Our data indicate that Bcl-2 levels are significantly higher in cells in the proximal compared with the remaining regions, thus insuring that these cells are protected from apoptosis after androgen withdrawal and are therefore available to regenerate the gland after subsequent androgen addition.

Results

Proximal cells produce and activate more TGF-β than distal cells

As we propose that stem cells in the proximal region are maintained in a quiescent state by TGF-β, we examined different ductal regions immunohistochemically to determine if regional differences in TGF-β levels were evident. We found that TGF-β is produced by both epithelial and stromal cells in all ductal regions (unpublished data). As TGF-β is secreted as a biologically inactive latent complex that is activated extracellularly (Massague et al., 1991; Abe et al., 1994; Munger et al., 1997) and as commercial antibodies that detect its histological presence do not distinguish between latent and active TGF-β, we

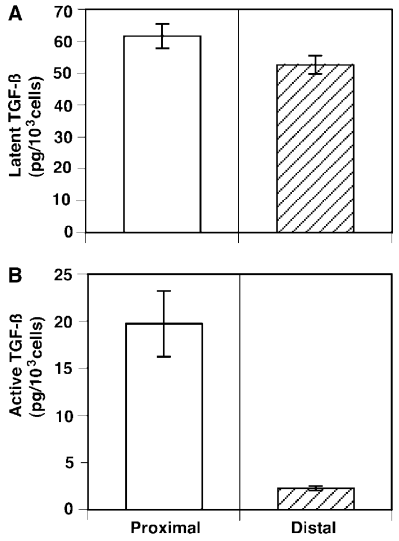


Figure 2. **Proximal cells produce and activate more TGF-β than distal cells.** (A) Heat-activated conditioned medium from proximal and distal cells (4×10^4 cells/24-well dish) was placed on TGF-β–responsive mink lung cells. The amount of total TGF-β was quantified by comparison with a standard curve. (B) Active TGF-β was measured directly by quantifying luciferase activity using a coculture assay in which either proximal or distal cells (4×10^3 cells/well) were combined with TGF-β–responsive mink lung cells (5×10^3 cells/well) in a 96-well plate. The presence of TGF-β in A and B was verified using TGF-β–neutralizing antibodies.

isolated cells from proximal and distal regions and determined the levels of total (latent plus active) and active TGF-β produced by the cells of different ductal regions in vitro. Both proximal and distal cells produced latent TGF-β, with proximal cells producing more of the cytokine than distal cells (61.0 ± 3.1 and 52.8 ± 2.9 pg TGF-β/10^3 cells, respectively; $P < 0.01$; Fig. 2 A). In addition, a significantly larger fraction of the latent TGF-β was activated by proximal cells (19.7 ± 3.5 pg TGF-β /10^3 cells; 32.3% of the latent TGF-β) than by distal cells (2.3 ± 1.2 pg TGF-β /10^3 cells; 4.3% of the latent TGF-β; $P < 0.001$; Fig. 2 B). Thus, proximal cells both produce and activate significantly more TGF-β than do distal cells, suggesting that the quiescence of stem cells in the proximal prostate is maintained by autocrine synthesis and activation of TGF-β.

As the growth of the prostate is regulated by androgens, changes in androgen status are likely to modulate stem cell homeostasis. We next determined whether androgen removal and replacement affect levels of TGF-β signaling in vivo. TGF-β1–mediated activation of TGF-β receptors induces phosphorylation of SMADs 2 and 3, which then form complexes with SMAD 4 (Massague et al., 1991; Munger et al., 1997). This complex translocates to the nucleus where it modulates the expression of TGF-β–regulated target genes (Massague et al., 1991; Munger et al., 1997). Therefore, the presence of nuclear pSMAD 2 and 3 (pSMAD2/3) was used as an indication of TGF-β receptor activation in vivo. Prostates were removed from intact androgen-replete mice and at 1, 3, 8, and 14 d after castration. After 14 d of involution, some mice were given androgen in the form of a subcutaneous androgen pellet and prostates were removed 1, 3, 8, and 14 d later.

Immunohistochemistry was used to detect the presence of pSMAD2/3 in prostate tissue sections. In the intact androgen-replete prostate (Fig. 3, T0), pSMAD2/3 was present mainly in the proximal region of the prostatic ducts, with little expression in the distal region (62.3 ± 6.8% positive nuclei in the proximal region compared with 15.5 ± 1.6% in the distal region; $P < 0.001$; Fig. 3, A, B, and G). However, 1 d after castration, pSMAD2/3 expression increased markedly in the distal region of the ducts (from 15.5 ± 1.6 to 38.9 ± 4.5% positive nuclei; $P < 0.01$), but remained similar in the proximal region (from 62.3 ± 6.8 to 61.3 ± 5.3% positive nuclei; Fig. 3, C, D, and G). By 8 d after castration, pSMAD2/3 was lower proximally than distally (43.9 ± 1.9 vs. 65.4 ± 7.1% positive nuclei, respectively; $P < 0.01$; Fig. 3, E–G). The number of pSMAD-expressing cells did not change significantly between 8 and 14 d after castration (unpublished data). When androgens were replaced by means of a slow-release subcutaneous androgen pellet (Tsujimura et al., 2002), the levels of pSMAD2/3 returned to the levels detected in the intact prostate within 8 d (65.3 ± 2.8% positive nuclei proximally and 17.1 ± 5.5% distally; $P < 0.001$; Fig. 3 G). A similar pattern of results was obtained when the expression of pSMAD was verified by Western blotting using extracts of prostates and antibodies to this protein (unpublished data).

The incidence of pSMAD expression in basal and luminal cells was also determined as basal and luminal cells have both been shown to form prostatic tissue (Kurita et al., 2004) and as luminal cells are more sensitive to androgen withdrawal than basal cells (English et al., 1987; Evans and Chandler, 1987). In the proximal region of the intact androgen-replete prostate (Fig. 3, T0) more TGF-β signaling was noted in luminal (72.3 ± 2.8% more than in basal cells [52.3 ± 1.3%; $P< 0.01$]) whereas similar numbers of luminal (17.1 ± 0.5%) and basal (13.9 ± 0.8%) cells had active TGF-β signaling in the distal region (Fig. 3 H). In contrast, by 8 d after castration, pSMAD expression in distal luminal cells (90.7 ± 1.3%) was significantly greater than in distal basal cells (40.0 ± 2.7%; $P < 0.001$), whereas approximately equal numbers of proximal basal (42.0 ± 2.5%) and luminal (45.8 ± 2.7%) cells had TGF-β signaling at this time. These data show that TGF-β signaling is more pronounced in luminal than basal cells in the distal region, which may account for the greater apoptosis of this compartment after androgen withdrawal.

These data also indicate that proximal cells produce and activate more TGF-β than distal cells in vitro and that TGF-β signaling is modulated by androgens in vivo. In the intact prostate, TGF-β production and pSMAD signaling is greater in the stem cell–containing proximal region than in the distal region. After castration, the amount of pSMAD signaling decreases in the proximal region, but increases dramatically in the distal region. With androgen replacement, the opposite is observed, with pSMAD signaling decreasing distally but increasing again proximally. These results indicate that the high level of TGF-β signaling in the proximal region in vivo in androgen-replete animals is likely to maintain the quiescence of cells in this region.

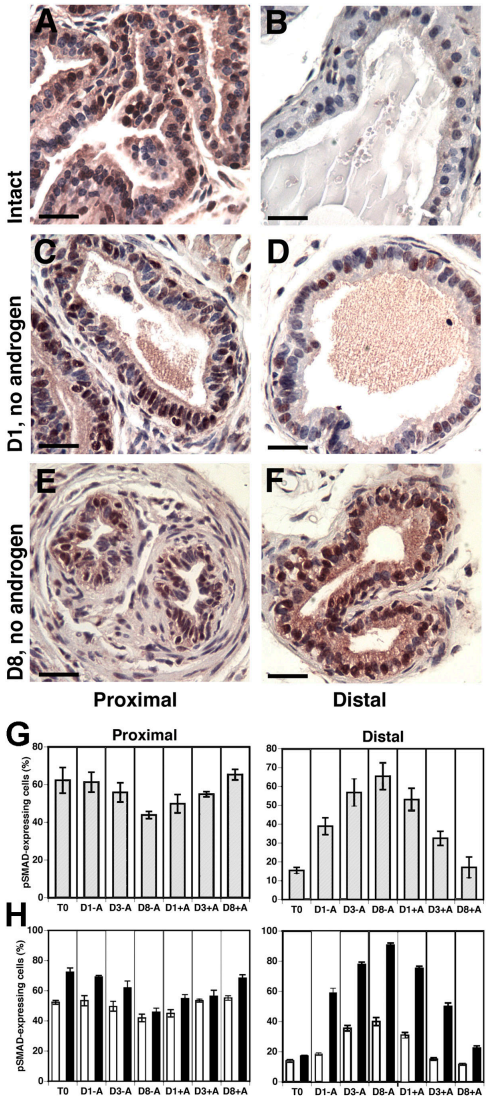


Figure 3. **pSMAD 2 and 3 expression are differentially regulated by androgens in proximal and distal cells.** (A–F) Paraffin sections of the proximal (A, C, and E) and distal (B, D, and F) regions of ducts examined immunohistochemically for pSMAD2/3-expressing cells in tissues removed from androgen-replete animals (A and B), and after androgen deprivation for 1 d (D1, no androgen; C and D) and 8 d (D8, no androgen; E and F). Bars, 100 μm. (G) Quantification of the number of nuclei positive for pSMAD2/3 expression in proximal and distal cells in the intact prostate (T0), the prostate 1 (D1-A), 3 (D3-A), and 8 d (D8-A) after castration and after 1 (D1+A), 3 (D3+A), and 8 d (D8+A) of androgen administration to animals with involuted prostates. (H) Quantification of the number of basal and luminal nuclei positive for pSMAD2/3 expression in proximal and distal cells in the intact prostate (T0), the prostate 1 (D1-A), 3 (D3-A), and 8 d (D8-A) after castration and after 1 (D1+A), 3 (D3+A), and 8 d (D8+A) of androgen administration to animals with involuted prostates. White bars, basal cells; black bars, luminal cells.

TβRI and II expression increases following castration

Because TGF-β signaling along prostatic ducts is differentially regulated by androgens, we determined if the expression of receptors for this cytokine, TβRI and II, were also modulated by androgens. In rats, TGF-β and TβRI and II mRNA and protein increased in the prostate after androgen reduction as a result of castration (Kyprianou and Isaacs, 1989; Kim et al., 1996).

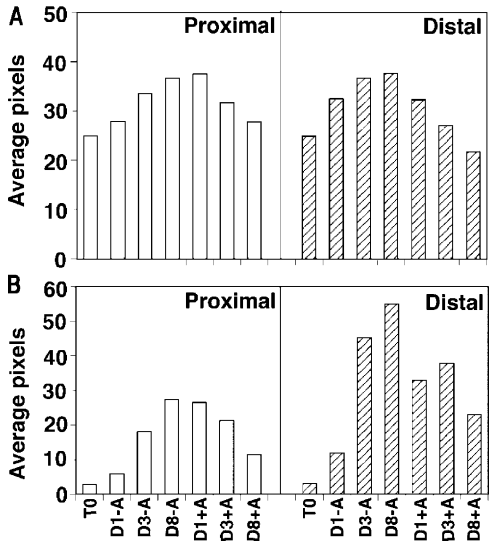


Figure 4. **TβRI expression is regulated by androgens in proximal and distal cells.** TβRI was detected using RT-PCR (A) and Western blot (B) in the intact prostate (T0), the prostate 1 (D1-A), 3 (D3-A), and 8 d (D8-A) after castration and after 1 (D1+A), 3 (D3+A), and 8 d (D8+A) of androgen administration to animals with involuted prostates.

However, no data exist that indicate whether TGF-β receptor expression differs between proximal and distal regions.

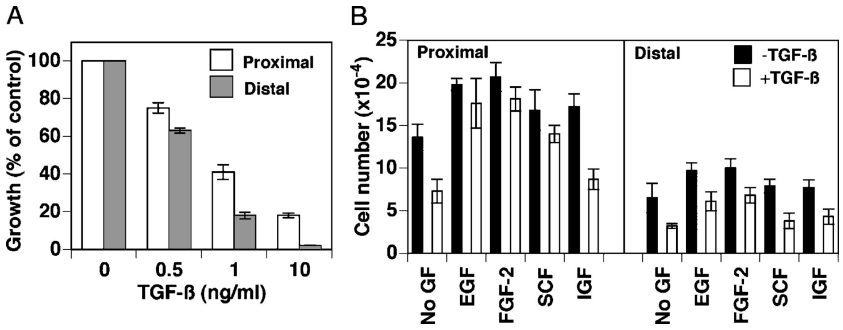
Prostates were removed from mice at time 0 (intact prostate) and 1, 3, 8, and 14 d after castration. After 14 d of involution, some mice were given androgen and prostates were removed 1, 3, 8, and 14 d later. The proximal and distal regions were examined by RT-PCR (Fig. 4 A) and Western blot (Fig. 4 B) analysis to determine whether androgen withdrawal affected the expression of TβRI and II. TβRI mRNA and protein levels increased both proximally and distally after castration and continued to increase until day 8, and then leveled off. After 8 d of involution, receptor mRNA and protein levels in the proximal region were 1.5-fold ($P < 0.01$) and 10-fold ($P < 0.01$) higher, respectively, than those of the androgen-replete prostate. Similar increases in mRNA (1.5-fold; $P < 0.01$) and protein (15-fold; $P < 0.001$) levels were noted in the distal region at this time (Fig. 4, A and B). After the administration of androgens, the levels of receptor mRNA and protein decreased both proximally and distally, although after 8 d of androgen supplementation, protein levels of TβRI remained four- (proximal) or sevenfold (distal) higher than the levels observed in the androgen-replete prostate (Fig. 4, A and B). Similar results were observed with TβRII (unpublished data). Thus, TGF-β receptor expression is diminished by androgens both proximally and distally. The increase in receptor expression in the distal region after castration may result in an increase in sensitivity to TGF-β, leading to apoptosis and the involution of this region of the gland.

TGF-β inhibits proximal cell proliferation, whereas SCF, EGF, and FGF-2 abrogate this effect

The in vitro proliferation of stem cells from a variety of organs is enhanced by mitogenic cytokines, such as stem cell factor (SCF), EGF, insulin-like growth factor 1 (IGF-1), and FGF-2 (Isfort et al., 1997; Mitsunari et al., 1999; Santa-Olalla and Covarrubias, 1999; Fortunel et al., 2000a; Deasy et al., 2002). We therefore examined the effect of these cytokines on the growth of cells in vitro that were isolated from the proximal and distal regions of the ducts.

TGF-β inhibited the proliferation of both proximal and distal cells. Low levels of TGF-β (0.5 ng/ml) inhibited the growth of proximal prostate cells by 24.4% and distal cells by 34.5% ($P < 0.01$). At a concentration of 1.0 ng/ml, TGF-β inhibited proximal and distal growth by 57.5 and 85.6% ($P < 0.001$), whereas 10.0 ng/ml TGF-β inhibited proximal and distal growth by 85.6 and 99.3% ($P < 0.001$), respectively (Fig. 5 A). Thus, distal cells were more sensitive to TGF-β inhibition than were proximal cells ($P < 0.01$ at all concentrations of TGF-β). We determined whether the inhibitory effect of TGF-β on proximal prostate cells could be negated by SCF, EGF, FGF-2, FGF-7, or IGF-1, all of which induce proliferation in several stem cell types (Isfort et al., 1997; Mitsunari et al., 1999; Santa-Olalla and Covarrubias, 1999; Deasy et al., 2002). TGF-β–mediated inhibition of proximal cells was abrogated by the simultaneous addition of EGF, FGF-2, or SCF. Growth of proximal cells in wells receiving EGF (10 ng/ml), FGF-2 (10 ng/ml), or SCF (100 ng/ml) along with TGF-β (0.5 ng/ml) was not significantly different from growth in wells that received the same mitogens but no TGF-β ($P < 0.1$; Fig. 5 B). In contrast, EGF, FGF-2, or SCF only partially reversed the inhibition of growth caused by administration of TGF-β to distal cells ($P < 0.01$; Fig. 5 B). FGF-7 and IGF-1 did not counteract the inhibitory effect of TGF-β on either proximal or distal cells.

Figure 5. **The inhibitory effect of TGF-β on cell growth is opposed by EGF, FGF-2, and SCF.** Cells from the proximal or distal region were seeded (5×10^3 cells/well) on collagen and overlaid with medium containing TGF-β (0.5, 1, or 10 ng/ml; A) and either TGF-β alone (0.5 ng/ml) or TGF-β together with either EGF (10 ng/ml), FGF-2 (10 ng/ml), SCF (100 ng/ml), or IGF-1 (10 ng/ml) (B). Control wells received EGF, FGF-2, SCF, IGF-1, or none of these factors in the absence of TGF-β. Wells were cultured for 10 d, after which cells were enumerated. The y axis in A is normalized to cells that were not treated with TGF-β. The histograms of the proximal and distal cells in B cannot be directly compared with each other because of their differential responses to TGF-β. The comparisons should be made between the control (No GF) and experimental samples within each of the two histograms.



The data also indicate that both proximal and distal cells are sensitive to the inhibitory effects of TGF-β with distal cells being more sensitive than proximal cells. However, in the proximal cells, the inhibitory effect can be counteracted by the addition of mitogenic cytokines, such as EGF, FGF-2, or SCF, suggesting that homeostasis in the proximal region may be maintained by a balance between inhibitory and stimulatory cytokines. The distal region, which does not contain the stem cell compartment, is less sensitive to cytokine-induced reversal of TGF-β inhibition than the proximal region.

We next determined if TGF-β had effects on the growth of clonal cultures of proximal cells. Proximal cells were seeded at limiting dilution in collagen gels. The growth of cells in wells containing a single cell was determined in the absence and presence of neutralizing antibodies to TGF-β. There was significantly higher duct-forming efficiency by single cells in wells containing antibodies to TGF-β with 72.5 ± 5.9% of these wells containing ducts compared with 14.5 ± 3.2% of control wells ($P < 0.001$). In addition, the size of the ducts in wells treated with antibodies to TGF-β was significantly larger (159.2 ± 12.10^3 μm^2) than ducts in control wells (54.3 ± 5.10^3 μm^2; $P < 0.01$). This shows a direct action of TGF-β on single proximal cells and also demonstrates that the production of endogenous TGF-β by proximal cells inhibits their proliferation. We have previously shown that single cells from the proximal region form ducts that contain both basal and luminal cells (Tsujimura et al., 2002).

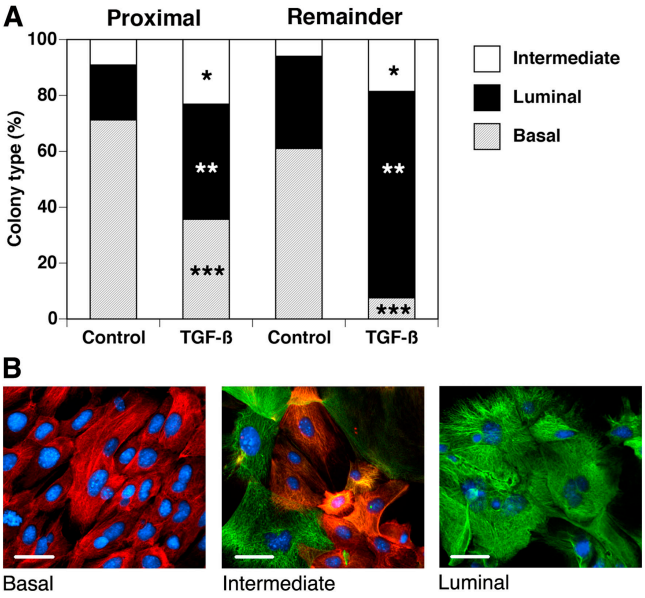


Figure 6. **Proximal cells are more resistant to the differentiating-inducing effects of TGF-β than cells from the remaining regions of ducts.** (A) Cells from the proximal and remaining ductal regions (2,000 cells/collagen-coated 8-well chamber slide) were cultured for 5–7 d and TGF-β (0.1 ng/ml) was added for 48 h after which colonies of >100 cells were counted and examined for evidence of basal and luminal cytokeratins. The data are plotted as the colony type (basal, luminal, or intermediate) as a percentage of the total number of colonies and are the average of four pooled experiments. *, 1.2-fold, $P > 0.05$ NS; **, 1.8-fold, $P < 0.0001$; ***, 4.7-fold, $P < 0.001$. (B) Colonies were examined immunohistochemically using antibodies to basal or luminal cytokeratins and appropriate Alexa Fluor 594 or 488 secondary fluorescent antibodies to determine those colonies that were comprised of basal (red), intermediate (both basal and luminal), or luminal (green) cells. Bars, 50 μm.

Proximal cells are more resistant to the differentiation-inducing effects of TGF-β than cells from the remaining regions of ducts

TGF-β has been shown to induce the differentiation of a rat basal cell line to luminal cells (Danielpour, 1999). Therefore, we determined the effects of TGF-β on the colony composition of cells isolated from the proximal and remaining ductal regions. As TGF-β inhibits cell growth, cells were initially cultured in the absence of TGF-β and, once clonal growth was established, TGF-β (0.1 ng/ml) was added for the final 48 h of culture and its effect on the composition of colonies (basal, luminal, and intermediate [cells containing both basal and luminal cytokeratins]) was determined. The addition of TGF-β to colonies arising from proximal cells resulted in a twofold decrease ($P < 0.001$) in the incidence of basal colonies and 2.1- and 2.5-fold increases in luminal and intermediate colonies, respectively ($P < 0.002$ and $P < 0.003$; Fig. 6 and Tables I and II). Colonies arising from cells isolated from the remaining ductal regions were more sensitive to TGF-β as an eightfold decrease in basal colonies was noted ($P < 0.0001$) concomitant with a 2.2- and a threefold increase in luminal and intermediate colonies, respectively ($P < 0.001$ and $P < 0.02$; Fig. 6 and Tables I and II). Significantly more of the colonies arising from proximal cells had a basal phenotype (35.9 ± 18.9%) after TGF-β addition than those colonies that grew from cells isolated from the remaining ductal regions (7.6 ± 7.9%; $P < 0.001$). Similar significant differences were noted in the incidence of

Table I. **Proximal cells are more resistant to the differentiation-inducing effects of TGF-β than cells from the remaining regions of ducts**

Region	Colony type	Control mean ± SD[a]	+ TGF-β mean ± SD[a]	Fold increase	Fold decrease	p-value
Proximal	Basal	71.2 ± 12.6	35.9 ± 18.9		2.0	<0.001
	Luminal	19.7 ± 10.5	41.2 ± 15.6	2.1		<0.002
	Intermediate[a]	9.3 ± 7.8	23.1 ± 11.9	2.5		<0.003
Remaining	Basal	61.0 ± 26.0	7.6 ± 7.9		8.0	<0.0001
	Luminal	33.0 ± 25.9	73.9 ± 14.8	2.2		<0.001
	Intermediate[a]	6.1 ± 8.3	18.6 ± 10.1	3.0		<0.02

This table and Table II represent a different statistical analysis of the same data set. The data compare the types of colonies in each region in the presence and absence of TGF-β.
[a]Colony type as a percentage of total colonies.

Table II. **Proximal cells form more basal colonies and fewer luminal colonies than cells from the remaining regions of ducts after TGF-β addition**

Colony type	Prox + TGF-β mean ± SD[a]	Rem + TGF-β mean ± SD[a]	Fold increase	Fold decrease	p-value
Basal	35.9 ± 18.9	7.6 ± 7.9		4.7	<0.001
Luminal	41.2 ± 15.6	73.9 ± 14.8	1.8		<0.0001
Intermediate[b]	23.1 ± 11.9	18.6 ± 10.1		1.2	NS

This table and Table I represent a different statistical analysis of the same data set. The data compare the incidence of basal, luminal, and intermediate colonies from each region in the presence of TGF-β.
[a]Colony type as a percentage of total colonies.
[b]Intermediate colonies contained cells that expressed both basal and luminal cytokeratins.

luminal colonies after TGF-β treatment with cells isolated from the remaining ductal regions having 1.8-fold more luminal colonies than proximal cells ($P < 0.0001$; Fig. 6 and Tables I and II). These data show that TGF-β induces differentiation of basal cells to luminal cells and that cells isolated from the proximal region are more resistant to the differentiation-inducing effects of TGF-β than cells from the remaining regions of ducts.

Proximal cells express high levels of Bcl-2

The proximal region of an intact prostate has high levels of TGF-β signaling, and proximal cells produce and activate significantly more TGF-β than distal cells. As high levels of TGF-β have been shown to result in apoptosis after androgen ablation (Kyprianou and Isaacs, 1989), we examined the mechanism whereby proximal stem cells could maintain quiescence in the presence of high levels of TGF-β signaling, whereas distal cells underwent apoptosis under similar conditions. As Bcl-2 prevents apoptosis of hematopoietic stem cells (Domen and Weissman, 2000) and as the overexpression of Bcl-2 has been shown to increase the number of these stem cells (Domen et al., 2000), we examined the expression of Bcl-2 in cells from different regions of prostatic ducts in androgen-replete animals by FACS analysis.

More Bcl-2–expressing cells were found in the proximal region (42.1 ± 6.9%) than in the remaining regions of the prostate (27.5 ± 8.2%; $P < 0.0001$; Fig. 7 A). Proximal cells also expressed 2.2-fold more Bcl-2 per cell than cells from the remaining regions ($P < 0.0001$) as evidenced by an increase in the mean fluorescence intensity (MFI) of these cells (Fig. 7 B). A representative histogram (Fig. 7 C) shows the increase in both intensity of Bcl-2 expression (MFI) and the numbers of cells expressing this antigen in the proximal region (MFI = 599; Bcl-2–expressing cells =36%) compared with the cells in the remaining regions (MFI = 157, Bcl-2–expressing cells = 18%). Furthermore, examination of cells expressing high levels of Bcl-2 (cells with a MFI >800) indicates that the cells from the proximal region contain 7.1-fold more Bcl-2^{high} cells than those in the remaining regions (7.2 ± 2.7 and 1.0 ± 1.0%, respectively; $P < 0.00001$; Fig. 7 D). Thus, cells with the highest Bcl-2 levels are almost exclusively located in the proximal region. The high levels of Bcl-2 in this region may protect proximal cells from the apoptotic events initiated during involution of the gland after androgen deprivation.

Discussion

The regulation of stem cell production within an organ is necessary to maintain homeostasis. The mechanism by which this occurs in most organs and the alterations that result in pathological conditions are currently unknown. We have previously shown that prostatic stem cells are concentrated in the proximal region of prostatic ducts (Tsujimura et al., 2002). We now show for the first time that there are regional differences in TGF-β activation and signaling consistent with the proximal location of quiescent prostatic stem cells. Cells from the proximal region of the ducts produce and activate significantly more TGF-β than cells from the distal region but are less sensitive to its inhibitory effects. We also show that TGF-β signaling in various ductal regions is differentially regulated by androgens in a manner that indicates that TGF-β signaling regulates prostatic homeostasis. In androgen-replete animals, TGF-β signaling and Bcl-2 expression are significantly higher in the proxi-

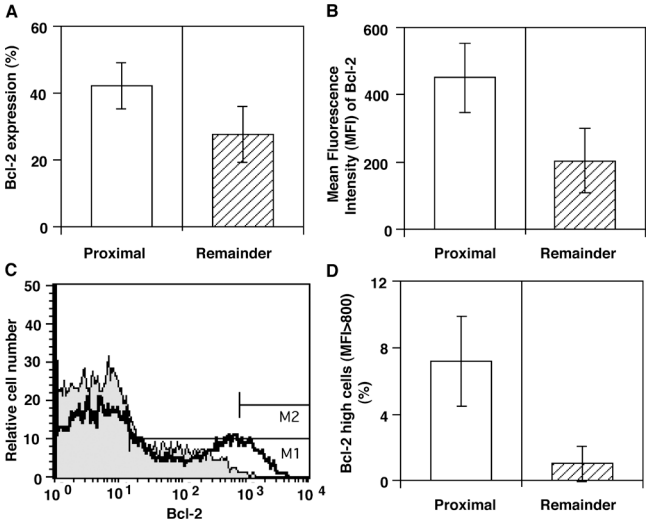


Figure 7. **Bcl-2 is highly expressed by cells from the proximal region of prostatic ducts.** (A) More cells in the proximal region of prostatic ducts express Bcl-2 than those from the remaining regions of ducts ($P < 0.00001$). (B) Cells in the proximal region express higher levels of Bcl-2 per cell, as is indicated by a higher mean fluorescence intensity (MFI) ($P < 0.00001$). (C) A representative histogram of Bcl-2 expression by cells from the proximal region (thick solid line; MFI = 599) and the remaining ductal regions (gray filled area; MFI = 157). The marker M1 is set such that <1% of control cells are included within this marker. The marker M2 delineates Bcl-2^{high} cells with MFI > 800. (D) Cells expressing high levels of Bcl-2 (MFI > 800) are 7.1-fold enriched in the proximal region of ducts compared with those in the remaining ductal regions ($P < 0.00001$).

mal compared with the distal region. The combination of high TGF-β signaling and high Bcl-2 expression promotes quiescence of cells in the proximal region while protecting them from TGF-β–mediated apoptosis. These findings indicate that TGF-β maintains the dormancy of stem cells in the proximal region of prostatic ducts. During castration-induced involution, TGF-β signaling increases distally and decreases proximally. In addition, we have found that several growth factors counteract the inhibitory effects of TGF-β on proximal cells. TGF-β also promotes the differentiation of basal to luminal cells, and proximal basal cells are more resistant to the differentiation-inducing effects of TGF-β than distal basal cells. Stem cell homeostasis in the proximal region may be maintained by an androgen-controlled balance between levels of the inhibitory cytokine, TGF-β, and mitogenic cytokines, such as EGF, FGF-2, and SCF.

Our data indicating that stem cells in the proximal region of androgen-replete animals are maintained in a quiescent state by high levels of TGF-β are strongly supported by the finding that loss of sensitivity to TGF-β in the proximal region of the ventral prostate, due to expression of a dominant-negative TGF-β receptor, results in the accumulation of multiple layers of epithelial cells in this region (Kundu et al., 2000). The proximal region of the prostate is enveloped with a thick band of smooth muscle (Nemeth and Lee, 1996) that produces high levels of TGF-β (Nemeth et al., 1997). The abnormal growth in this region after TGF-β abrogation indicates that TGF-β plays an important role in regulating the growth of the proximal region of prostatic ducts.

Previous work has indicated that TGF-β inhibits the proliferation of prostatic epithelial cells (Kyprianou and Isaacs, 1989). Androgen reduction as a result of castration increases the expression of this cytokine and its receptors, and activates its signaling pathway leading to apoptosis and involution of the gland (Kyprianou and Isaacs, 1988, 1989; Wikstrom et al., 1999). After androgen ablation we show that the increases in TGF-β signaling in the distal region correspond with similar increases in the expression of TβRI and II. Increased expression of TβRs is likely to sensitize the cells to TGF-β, contributing to the enhanced TGF-β–mediated pSMAD signals that result in the castration-induced apoptosis of the distal region. With the replacement of androgens, we show that TβRI and II expression diminishes, thereby facilitating cell growth in the distal region in response to positive proliferative signals, allowing the prostate to regenerate. Proliferating cells have been shown to be preferentially located in the distal region of prostatic ducts (Sugimura et al., 1986a,b; Cunha and Donjacour, 1987; Tsujimura et al., 2002). The TβRI and II levels decrease to a level higher than that of intact animals, an unexplained phenomenon reported previously in rats (Kim et al., 1996). In the proximal region, whereas TGF-β signaling decreases in the involuting prostate, TβRI and II expression increases. In the regenerating prostate, TGF-β signaling increases but TβRI and II expression decreases. This suggests that there is a negative feedback between signaling and receptor expression. We propose that the increase in TβR expression in the involuting proximal prostate sensitizes the cells in this region to TGF-β.

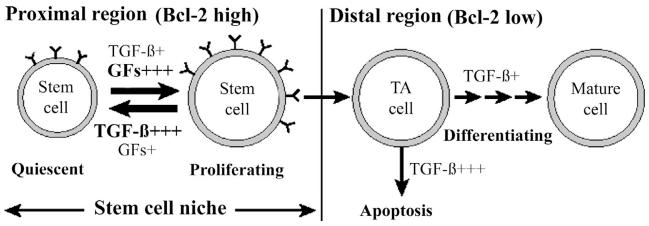


Figure 8. **A model for the regional regulation of prostatic homeostasis.** A schematic diagram showing that in an androgen-replete prostate high levels of active TGF-β and TGF-β–mediated signaling in the proximal stem cell niche maintain the quiescence of stem cells in this region. In the distal region, low levels of active TGF-β and TGF-β–mediated signaling permit the division of transit-amplifying (TA) cells in this region. After castration, the decrease in androgen levels with concurrent increased levels of TGF-β and TGF-β–mediated signaling distally, lead to apoptosis of cells in this region, with the resulting involution of the prostate gland. Simultaneously, the decrease in TGF-β–mediated signaling in the proximal region sensitizes the cells in this region to mitogenic signals. When androgens are readministered these cells respond to androgen-induced mitogenic cytokines (GFs), thus contributing to prostatic regeneration. Simultaneously, as the distal TGF-β signaling activity declines, the transit-amplifying cells in this region also divide, resulting in regeneration of the gland.

The increased sensitivity of this region to TGF-β may therefore restore quiescence once androgens are administered.

Increased levels of TGF-β after castration result in apoptosis and involution of the gland (Kyprianou and Isaacs, 1989). It is therefore of significance that we find that the expression of Bcl-2, an antiapoptotic protein (Adams and Cory, 1998), is considerably higher in proximal cells than in cells from other regions of the prostate. These results suggest that high local levels of Bcl-2 protect the cells in the proximal region from the apoptotic effects of high levels of TGF-β. This region is least affected by castration and remains mostly intact after involution (Sugimura et al., 1986a; Rouleau et al., 1990). Of relevance to our findings is the observation that the apoptotic effects of TGF-β are antagonized by Bcl-2 in prostate cancer cells (Bruckheimer and Kyprianou, 2002), indicating that prostate stem and cancer cells both express high levels of Bcl-2. In addition, Bcl-2 both prevents apoptosis of hematopoietic stem cells and increases their numbers in vivo (Domen et al., 2000; Domen and Weissman, 2000), and it is also highly expressed in keratinocyte populations that are enriched in stem cells (Tiberio et al., 2002). Thus, the expression of high levels of Bcl-2 by prostatic stem cells protects them from the apoptotic events that regulate the growth, differentiation, and involution of the post–stem cell compartment.

A model for the regional regulation of prostatic homeostasis

These data are the first to show that TGF-β signaling in vivo is regulated by androgens in a differential manner along the proximal-distal prostatic ductal axis and that the inhibitory effects of TGF-β on proximal cells can be negated by several cytokines. We propose a model (Fig. 8) in which the stem cells, concentrated in the proximal niche, are maintained in a dormant state in the androgen-replete prostate by high levels of active TGF-β that are produced by the cells in this region. Lower levels of active TGF-β distally allow proliferation and differentiation of distal cells, thus accounting for the preferential location

of dividing cells in this region (Sugimura et al., 1986a,b; Cunha and Donjacour, 1987; Tsujimura et al., 2002). Stem cells in the proximal region are protected from apoptosis by high Bcl-2 expression. After castration, the decrease in androgen levels with concurrent increased levels of TGF-β (Kyprianou and Isaacs, 1989) and TGF-β signaling distally lead to apoptosis of cells in this region, with the resulting involution of the prostate gland. Simultaneously, the decrease in TGF-β signaling (less pSMAD expression) in the proximal region may sensitize these cells to mitogenic signals. When androgens are readministered these sensitized cells respond to androgen-induced mitogenic cytokines, thus contributing to prostatic regeneration. As the distal TGF-β signaling activity declines in response to androgens, the transit-amplifying cells in this region, which are still capable of proliferation, also divide, resulting in regeneration of the gland.

Our findings that TGF-β signaling is differentially regulated by androgens along the proximal-distal ductal axis and that cells with high Bcl-2 expression are located proximally explains how the proximal region is preserved largely intact after castration whereas the distal region involutes. It is of interest that Bcl-2 expression is increased in both BPH (Kyprianou et al., 1996) and prostate cancer (Bruckheimer and Kyprianou, 2002). Our results may therefore be important in understanding the etiology of prostatic diseases that result in aberrant proliferation of the stem cell compartment. For example, the age-related reduction in androgen levels (van den Beld et al., 2000) may result in less TGF-β–mediated inhibitory signaling in the stem cell compartment. This in turn may sensitize these cells to stimulatory signals resulting in excessive proliferation of stem cells, thus promoting the development of proliferative prostatic diseases.

Materials and methods

Animals

C57Bl/6 mice were housed in a climate-controlled facility and all animal care and procedures were performed in compliance with the New York University School of Medicine Institutional Review Board requirements.

Cell isolation and growth in vitro

Cells from the proximal and distal regions of the prostatic ducts of the ventral and dorsal lobes were isolated as described previously (Tsujimura et al., 2002) and cultured on 96-well collagen-coated plates. The culture medium was DME (Sigma-Aldrich) with 1% FCS (Atlanta Biologicals), and contained 10 μg/ml transferrin, 0.5 μg/ml hydrocortisone, 10 ng/ml cholera toxin, 10^{-7} M dihydrotestosterone, and 0.1 mM phosphoethanolamine (all from Sigma-Aldrich). Growth factors and antibodies were added to the culture medium 3 h after cells were seeded and included 100 ng/ml SCF (R&D Systems), 10 ng/ml EGF (Genzyme), 10 ng/ml FGF-2 (Scios Nova), 10 ng/ml FGF-7 (Scios Nova), 10 ng/ml IGF-1 (Sigma-Aldrich), or 0, 0.5, 1.0, or 10.0 ng/ml TGF-β (R&D Systems). Cytokines and antibodies were added alone or in combination with each other, as indicated in Results. Control wells received either no factors or all the factors. The medium was removed and replaced with fresh medium and additives every 2 d. Cell growth in each assay was measured and quantified as described previously (Tsujimura et al., 2002). Experiments were performed in triplicate.

Clonal cultures from single proximal cells were obtained by seeding cells at limiting dilution in collagen gels in 96-well dishes. Each well was examined for evidence of single cells and the incidence of colony/duct formation and the sizes of the ducts (NIH image analysis program, http://rsb.info.nih.gov/nih-image) were determined in the absence and presence of a neutralizing antibody to TGF-β (20 μg/ml; antibody 1D11.16; R&D Systems), which neutralizes TGF-β1, 2, and 3. Fresh medium–containing antibodies was added at 48-h intervals and the incidence and sizes of ducts was determined after 14 d.

The effects of TGF-β on the colony composition of cells isolated from the proximal and remaining ductal regions was done by seeding 2,000 cells from each region on collagen-coated 8-well chamber slides (Tsujimura et al., 2002) to determine if colonies obtained from cells isolated from these regions of ducts demonstrated differential sensitivity to TGF-β. As TGF-β inhibits cell growth, cells were cultured in the absence of TGF-β for 5–7 d, and once clonal growth was established, 0.1 ng/ml TGF-β was added for the final 48 h of culture in DME containing 10% FCS, and its effect on the composition of colonies of >100 cells (basal, luminal, and intermediate [cells containing both basal and luminal cytokeratins]) was determined. This experiment was performed four times seeding triplicate wells for each condition in each experiment.

Immunohistochemistry

Tissue sections were deparaffinized in xylene, followed by washing in ethanol and rehydration in PBS. Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide in PBS for 30 min at RT. Sections were incubated for 30 min at 37°C in hyaluronidase (1 mg/ml in 0.1 M sodium acetate, pH 5.5). Slides were rinsed briefly and were incubated for 1 h at RT with 5% normal goat serum in TBS containing 1% BSA. pSMAD2/3 was detected by incubating sections with a polyclonal rabbit anti-pSMAD2/3 antibody (10 μg/ml; Santa Cruz Biotechnology, Inc.) for 24 h at 4°C. Slides were washed, and the secondary antibody (goat anti–rabbit HRP; R&D Systems) was added for 1 h at RT. Slides were washed and the Nova red substrate kit (Vector Laboratories) was used to detect HRP activity. At least 200 nuclei were counted for each time point in duplicate slides from each of two animals. Basal and luminal cells were scored based on their location within ducts. Experiments were performed in triplicate. Basal and luminal cytokeratins in colonies cultured from different regions of ducts were visualized using antibodies to CK 5 (Babco/Covance) and CK 8 (RDI) and appropriate Alexa Flour 594 or 488 secondary fluorescent antibodies (Molecular Probes). Slides were mounted using Fluoromount G (Southern Biotechnology Associates, Inc.). Fluorescent images were captured using a fluorescent microscope (model Axioskop 2; Carl Zeiss MicroImaging, Inc.) using lens 20×/0.50 with a digital camera (model CA742-95; Hamamatsu) using Openlab 2.2.5 software. Light microscopic images were captured using a DMLD light microscope (Leica) using lens 20×/0.40 with a digital camera (model 3.2.0; Diagnostic Instruments) using Spot software. Image processing was done using Adobe Photoshop.

TGF-β production

Production of TGF-β was determined by a TGF-β bioassay, which uses TGF-β–responsive mink lung cells (TMLC; Abe et al., 1994; Salm et al., 2000b) and measures luciferase activity produced by these cells as a consequence of TGF-β activation. Proximal and distal cells were seeded at 4×10^4 cells/ml in 24-well dishes in the culture medium described in Tsujimura et al. (2002), which was replaced by 2 ml of 0.1% BSA in DME after 24 h. Both total and active TGF-β secreted into the 0.1% BSA medium were quantified. Total TGF-β production was measured after converting latent TGF-β in the medium to its active form by heat activation at 80°C. A neutralizing antibody to TGF-β (20 μg/ml; antibody 1D11.16; R&D Systems), which neutralizes TGF-β1, 2, and 3, was added to triplicate samples of conditioned medium to confirm that the luciferase activity measured in the assay was generated by TGF-β. All experiments were performed in triplicate.

TGF-β activation in a coculture assay

TGF-β activation was measured using a coculture assay that combined either the proximal or the distal cells with TGF-β–responsive mink lung cells (Abe et al., 1994; Salm et al., 2000b). Coculture of the prostate cells with TMLC increases the sensitivity of the TGF-β assay by allowing any active TGF-β to be captured immediately by the indicator cells and thus measures directly the amount of active TGF-β generated by the prostate cells. Proximal or distal cells (4×10^3 cells/well) were coseeded with TMLC (5×10^3 cells/well) in primary culture medium in a 96-well plate. Neutralizing antibodies to TGF-β were added to control wells to confirm that the luciferase activity measured in the assay was generated by TGF-β. Active TGF-β was measured as described previously (Abe et al., 1994; Salm et al., 2000b). Experiments were performed in triplicate.

RT-PCR

Semi-quantitative RT-PCR was used to examine expression of message for TGF-β receptors I and II (TβRI and TβRII). Mice were castrated to initiate prostate involution by androgen withdrawal. Two mice were killed and their prostates removed at each of the following time points: day 0 (intact T0 control) and days 1, 3, 8, and 14 after castration. Castrated mice with involuted prostates were given testosterone subcutaneously (slow-release

subcutaneous androgen pellet; Innovative Research). Two mice were killed and their prostates removed at each of the following time points: days 1, 3, 8, and 14 after androgen supplementation. In all cases, the proximal and distal regions were dissected from both the ventral and the dorsal prostates in collagenase as previously described (Tsujimura et al., 2002). Total RNA was extracted using TriZol (Invitrogen). 1 μg of total RNA was amplified for the required products using the Titan One Tube RT-PCR System kit (Roche). Primers used were as follows: β-actin forward, GAC GTC ATG AAG ACT TGC TG; β-actin reverse, ACA GCA GCA AAG CCT GTT GG; TβRI forward, TTG CTG GAC CAG TGT GCT TCG; TβRI reverse, CCA TCT GTT TGG GAT ATT TGG CC; TβRII forward, AGC AGA AGC TGA GTT CAA CCT GGG; TβRII reverse, GGA GCC ATG TAT CTT GCA GTT CCC. Amplification conditions were as follows: 42°C for 55 min; 94°C for 2 min (×1 cycle); 94°C for 1 min; 55°C for 1 min; 72°C for 1 min (×25 cycles); 72°C for 10 min (×1 cycle). Products were resolved on a 1% agarose gel in Tris acetate buffer and photographed. Images were scanned, and the average pixel intensity for each band was measured using UN-SCAN-IT software (Silk Scientific Software). Experiments were performed in triplicate.

Western blot analysis

The expression of TβRI and II proteins was examined by Western blot analysis. Prostate cells were prepared as described for the RT-PCR experiments. Protein was extracted in lysis buffer (Salm et al., 2000a), quantified using a BCA kit (Pierce Chemical Co.), and equal amounts of protein were loaded on 8 or 10% reducing SDS-PAGE. Gels were electroblotted onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore) with transfer buffer containing 10% (vol/vol) methanol. Blots were incubated for 1 h at RT with appropriate primary antibodies. HRP-labeled secondary antibodies were added for 1 h at RT. Bands were visualized by ECL (Amersham Biosciences). Western blot films were scanned and the average pixel intensity for each band was measured using UN-SCAN-IT software. Experiments were performed in triplicate.

Cell preparation for FACS analysis

Cells were isolated from the proximal region of prostatic ducts (adjacent to the urethra) and from the remainder of the prostate (includes the distal and intermediate regions of ducts; Tsujimura et al., 2002). The cells from these two regions were dissociated by incubation with 0.5% collagenase Type II (Sigma-Aldrich) in HBSS plus 7.5% FCS for 45 min at 37°C, followed by digestion in 0.25% trypsin for 8 min at 37°C. Cells were washed and resuspended in PBS, fixed in PFA, and permeabilized with Tween 20. Cells were resuspended in PBS supplemented with BSA (0.1%), sodium azide (0.01%), and aprotinin (20 μg/ml; FACS buffer). Fc receptors were blocked with mouse CD16/32 antibodies (Caltag Laboratories) for 10 min on ice, and cells were subsequently incubated with 20 μg/ml anti–Bcl-2–PE (Santa Cruz Biotechnology, Inc.) or control IgG1-PE (DakoCytomation) for 30 min on ice and washed twice with FACS buffer. Cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson) and the data were analyzed using CellQuest software (Becton Dickinson).

Statistical analysis

The results are depicted as the means ± SD of each set of data. Comparisons between groups were made using the 2-tailed, paired *t* test, or in the case of different sized samples, the Mann Whitney U test. A p-value of <0.05 is considered statistically significant.

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References

Abe, M., J.G. Harpel, C.N. Metz, I. Nunes, D.J. Loskutoff, and D.B. Rifkin. 1994. An assay for transforming growth factor-beta using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. *Anal. Biochem.* 216:276–284.

Adams, J.M., and S. Cory. 1998. The Bcl-2 protein family: arbiters of cell survival. *Science.* 281:1322–1326.

Beachy, P.A., S.S. Karhadkar, and D.M. Berman. 2004. Tissue repair and stem cell renewal in carcinogenesis. *Nature.* 432:324–331.

Bruckheimer, E.M., and N. Kyprianou. 2002. Bcl-2 antagonizes the combined apoptotic effect of transforming growth factor-beta and dihydrotestosterone in prostate cancer cells. *Prostate.* 53:133–142.

Cashman, J.D., A.C. Eaves, and C.J. Eaves. 1992. Granulocyte-macrophage colony-stimulating factor modulation of the inhibitory effect of transforming growth factor-beta on normal and leukemic human hematopoietic progenitor cells. *Leukemia.* 6:886–892.

Cunha, G.R., and A. Donjacour. 1987. Stromal-epithelial interactions in normal and abnormal prostatic development. *Prog. Clin. Biol. Res.* 239:251–272.

Danielpour, D. 1999. Transdifferentiation of NRP-152 rat prostatic basal epithelial cells toward a luminal phenotype: regulation by glucocorticoid, insulin-like growth factor-I and transforming growth factor-beta. *J. Cell Sci.* 112:169–179.

Deasy, B.M., Z. Qu-Peterson, J.S. Greenberger, and J. Huard. 2002. Mechanisms of muscle stem cell expansion with cytokines. *Stem Cells.* 20:50–60.

Domen, J., and I.L. Weissman. 2000. Hematopoietic stem cells need two signals to prevent apoptosis; BCL-2 can provide one of these, Kitl/c-Kit signaling the other. *J. Exp. Med.* 192:1707–1718.

Domen, J., S.H. Cheshier, and I.L. Weissman. 2000. The role of apoptosis in the regulation of hematopoietic stem cells: overexpression of Bcl-2 increases both their number and repopulation potential. *J. Exp. Med.* 191:253–264.

English, H.F., R.J. Santen, and J.T. Isaacs. 1987. Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. *Prostate.* 11:229–242.

Evans, G.S., and J.A. Chandler. 1987. Cell proliferation studies in the rat prostate: II. The effects of castration and androgen-induced regeneration upon basal and secretory cell proliferation. *Prostate.* 11:339–351.

Fan, X., G. Valdimarsdottir, J. Larsson, A. Brun, M. Magnusson, S.E. Jacobsen, P. ten Dijke, and S. Karlsson. 2002. Transient disruption of autocrine TGF-beta signaling leads to enhanced survival and proliferation potential in single primitive human hemopoietic progenitor cells. *J. Immunol.* 168:755–762.

Fortunel, N., J. Hatzfeld, S. Kisselev, M.N. Monier, K. Ducos, A. Cardoso, P. Batard, and A. Hatzfeld. 2000a. Release from quiescence of primitive human hematopoietic stem/progenitor cells by blocking their cell-surface TGF-beta type II receptor in a short-term in vitro assay. *Stem Cells.* 18:102–111.

Fortunel, N.O., A. Hatzfeld, and J.A. Hatzfeld. 2000b. Transforming growth factor-beta: pleiotropic role in the regulation of hematopoiesis. *Blood.* 96:2022–2036.

Fuchs, E., T. Tumbar, and G. Guasch. 2004. Socializing with the neighbors: stem cells and their niche. *Cell.* 116:769–778.

Gabrilove, J.L., K. White, Z. Rahman, and E.L. Wilson. 1994. Stem cell factor and basic fibroblast growth factor are synergistic in augmenting committed myeloid progenitor cell growth. *Blood.* 83:907–910.

Gorska, A., H. Joseph, R. Derynck, H. Moses, and R. Serra. 1998. Dominant-negative interference of the transforming growth factor beta type II receptor in mammary gland epithelium results in alveolar hyperplasia and differentiation in virgin mice. *Cell Growth Differ.* 9:229–238.

Isaacs, J.T. 1985. Control of cell proliferation and cell death in the normal and neoplastic prostate: a stem cell model. Department of Health and Human Services, Washington, DC. 85–94 pp.

Isfort, R., D. Cody, S. Stuard, C. Randall, C. Miller, G. Ridder, C. Doersen, W. Richards, B. Yoder, J. Wilkinson, and R. Woychik. 1997. The combination of epidermal growth factor and transforming growth factor-beta induces novel phenotypic changes in mouse liver stem cell lines. *J. Cell Sci.* 110:3117–3129.

Kim, I.Y., H.J. Ahn, D.J. Zelner, L. Park, J.A. Sensibar, and C. Lee. 1996. Expression and localization of transforming growth factor-beta receptors type I and type II in the rat ventral prostate during regression. *Mol. Endocrinol.* 10:107–115.

Kundu, S.D., I.Y. Kim, T. Yang, L. Doglio, S. Lang, X. Zhang, R. Buttyan, S.J. Kim, J. Chang, X. Cai, et al. 2000. Absence of proximal duct apoptosis in the ventral prostate of transgenic mice carrying the C3(1)-TGF-beta type II dominant negative receptor. *Prostate.* 43:118–124.

Kurita, T., R.T. Medina, A.A. Mills, and G.R. Cunha. 2004. Role of p63 and basal cells in the prostate. *Development.* 131:4955–4964.

Kyprianou, N., and J.T. Isaacs. 1988. Identification of a cellular receptor for transforming growth factor-beta in rat ventral prostate and its negative regulation by androgens. *Endocrinology.* 123:2124–2131.

Kyprianou, N., and J.T. Isaacs. 1989. Expression of transforming growth factor-beta in the rat ventral prostate during castration-induced programmed cell death. *Mol. Endocrinol.* 3:1515–1522.

Kyprianou, N., H. Tu, and S.C. Jacobs. 1996. Apoptotic versus proliferative activities in human benign prostatic hyperplasia. *Hum. Pathol.* 27:668–675.

Massague, J., J. Heino, and M. Laiho. 1991. Mechanisms in TGF-beta action. *Ciba Found. Symp.* 157:51–59.

Mitsunari, M., T. Harada, M. Tanikawa, T. Iwabe, F. Taniguchi, and N. Terakawa. 1999. The potential role of stem cell factor and its receptor c-kit in the mouse blastocyst implantation. *Mol. Hum. Reprod.* 5:874–879.

Munger, J.S., J.G. Harpel, P.E. Gleizes, R. Mazzieri, I. Nunes, and D.B. Rifkin. 1997. Latent transforming growth factor-beta: structural features and mechanisms of activation. *Kidney Int.* 51:1376–1382.

Nemeth, J.A., and C. Lee. 1996. Prostatic ductal system in rats: regional variation in stromal organization. *Prostate.* 28:124–128.

Nemeth, J.A., J.A. Sensibar, R.R. White, D.J. Zelner, I.Y. Kim, and C. Lee. 1997. Prostatic ductal system in rats: tissue-specific expression and regional variation in stromal distribution of transforming growth factor-beta 1. *Prostate.* 33:64–71.

Pardal, R., M.F. Clarke, and S.J. Morrison. 2003. Applying the principles of stem-cell biology to cancer. *Nat. Rev. Cancer.* 3:895–902.

Potten, C., G. Owen, D. Hewitt, C. Chadwick, H. Hendry, B. Lord, and L. Woolford. 1995. Stimulation and inhibition of proliferation in the small intestinal crypts of the mouse after in vivo administration of growth factors. *Gut.* 36:864–873.

Potten, C., J. Wilson, and C. Booth. 1997. Regulation and significance of apoptosis in the stem cells of the gastrointestinal epithelium. *Stem Cells.* 15:82–93.

Puolakkainen, P., J. Ranchalis, W. Gombotz, A. Hoffman, R. Mumper, and D. Twardzik. 1994. Novel delivery system for inducing quiescence in intestinal stem cells in rats by transforming growth factor beta 1. *Gastroenterology.* 107:1319–1326.

Reya, T., S.J. Morrison, M.F. Clarke, and I.L. Weissman. 2001. Stem cells, cancer, and cancer stem cells. *Nature.* 414:105–111.

Rouleau, M., J. Leger, and M. Tenniswood. 1990. Ductal heterogeneity of cytokeratins, gene expression, and cell death in the rat ventral prostate. *Mol. Endocrinol.* 4:2003–2013.

Salm, S.N., Y. Koikawa, V. Ogilvie, A. Tsujimura, S. Coetzee, D. Moscatelli, E. Moore, H. Lepor, E. Shapiro, T.T. Sun, and E.L. Wilson. 2000a. Generation of active TGF-beta by prostatic cell cocultures using novel basal and luminal prostatic epithelial cell lines. *J. Cell. Physiol.* 184:70–79.

Salm, S.N., Y. Koikawa, V. Ogilvie, A. Tsujimura, S. Coetzee, D. Moscatelli, E. Moore, H. Lepor, E. Shapiro, T.T. Sun, and E.L. Wilson. 2000b. Transforming growth factor-beta is an autocrine mitogen for a novel androgen-responsive murine prostatic smooth muscle cell line, PSMC1. *J. Cell. Physiol.* 185:416–424.

Santa-Olalla, J., and L. Covarrubias. 1999. Basic fibroblast growth factor promotes epidermal growth factor responsiveness and survival of mesencephalic neural precursor cells. *J. Neurobiol.* 40:14–27.

Sugimura, Y., G.R. Cunha, and A.A. Donjacour. 1986a. Morphological and histological study of castration-induced degeneration and androgen-induced regeneration in the mouse prostate. *Biol. Reprod.* 34:973–983.

Sugimura, Y., G.R. Cunha, A.A. Donjacour, R.M. Bigsby, and J.R. Brody. 1986b. Whole-mount autoradiography study of DNA synthetic activity during postnatal development and androgen-induced regeneration in the mouse prostate. *Biol. Reprod.* 34:985–995.

Tiberio, R., A. Marconi, C. Fila, C. Fumelli, M. Pignatti, S. Krajewski, A. Giannetti, J.C. Reed, and C. Pincelli. 2002. Keratinocytes enriched for stem cells are protected from anoikis via an integrin signaling pathway in a Bcl-2 dependent manner. *FEBS Lett.* 524:139–144.

Tsujimura, A., Y. Koikawa, S. Salm, T. Takao, S. Coetzee, D. Moscatelli, E. Shapiro, H. Lepor, T. Sun, and E. Wilson. 2002. Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. *J. Cell Biol.* 157:1257–1265.

Tumbar, T., G. Guasch, V. Greco, C. Blanpain, W.E. Lowry, M. Rendl, and E. Fuchs. 2004. Defining the epithelial stem cell niche in skin. *Science.* 303:359–363.

Valk-Lingbeek, M.E., S.W. Bruggeman, and M. van Lohuizen. 2004. Stem cells and cancer; the polycomb connection. *Cell.* 118:409–418.

van den Beld, A.W., F.H. de Jong, D.E. Grobbee, H.A. Pols, and S.W. Lamberts. 2000. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. *J. Clin. Endocrinol. Metab.* 85:3276–3282.

Wikstrom, P., P. Westin, P. Stattin, J.E. Damber, and A. Bergh. 1999. Early castration-induced upregulation of transforming growth factor beta1 and its receptors is associated with tumor cell apoptosis and a major decline in serum prostate-specific antigen in prostate cancer patients. *Prostate.* 38:268–277.