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Axin2 Expression Identifies Progenitor Cells in the Murine Prostate

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Abstract

BACKGROUND—We previously reported that prostatic stem/progenitor cells are concentrated in the proximal region of prostatic ducts and express stem cell antigen 1 (Sca-1). As Wnt signaling is important for the maintenance of stem cells, we determined whether Sca-1 expressing cells also express Axin2, as Axin2 expression is highly suggestive of active Wnt signaling.

METHODS—Axin2 promoter reporter mice were used for whole mount and fluorescence activated cell sorting (FACS) analysis to determine its expression in the prostate. Axin2 expressing cells were also examined for the co-expression of Sca-1. We also used a chemical activator of Wnt signaling, BIO, to determine the effects of Wnt signaling on the growth of primary prostate cells in vitro.

RESULTS—We show that Axin2 expression is present in all lobes and is regulated by androgens with the highest Axin2 expression in the lateral and dorsal prostate. Furthermore, a fraction of Axin2 expressing cells co-express Sca-1, suggesting that some progenitor cells have active Wnt signaling. Lastly, we demonstrate that activation of the Wnt pathway may result in increased growth, consistent with a role for Wnt signaling in maintenance and/or expansion of the progenitor cell population.

CONCLUSION—Axin2 expressing cells that co-express Sca-1 are present in all prostate lobes suggesting that progenitor cells reside within the Wnt active population. An understanding of the basic biology of signaling pathways mediating growth in the prostate may lead to rational therapies to treat benign prostatic hyperplasia and prostate cancer.

Keywords

stem cell; Sca-1; androgen; castration; regeneration; Wnt; Axin2

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BACKGROUND

Several intracellular-signaling pathways have been shown to be involved in many functions of stem cells including self-renewal [1–3], maintenance in their niche [4–7], and their transition to a transformed phenotype [8–10]. Activation of the Wnt pathway in adult hematopoietic stem/progenitor cells (HSC) results in an increase in HSC number [11–13], while inhibitors of the Wnt-signaling pathway inhibit HSC growth [14]. Similarly, stabilization of the Wnt activated β-catenin protein results in greater numbers of mammary stem cells in vivo and in vitro [15] eventually resulting in hyperplasia, implicating Wnts in the etiology of transformation. Likewise, Pinto and Clevers [16] demonstrate that Wnt-signaling results in renewal of intestinal crypt cells that replenish the intestinal epithelium, further demonstrating a role for Wnt signaling in the maintenance and/or differentiation of progenitor/stem cells.

In an unstimulated cell, the transcriptional coactivator, β-catenin, is phosphorylated and rapidly turned over via proteosome degradation [17,18]. Upon binding of Wnt ligands to their Frizzled receptor, β-catenin is not phosphorylated and is stabilized in the cytoplasm [19–21]. β-Catenin protein levels accumulate and translocate to the nucleus where they act as transcriptional coactivators for Wnt-signaling regulated genes [22–24]. Recent data strongly suggest that Axin2 is a direct transcriptional target upregulated after activation of Wnt signaling [25–27]. Therefore, analysis of Axin2 expression is likely to be a direct measure of active Wnt signaling.

Unlike the human prostate, the mouse prostate has distinct and separate lobes—ventral prostate (VP), lateral prostate (LP), and dorsal prostate (DP). The ductal architecture of each gland is divided into proximal, intermediate, and distal regions with histologically distinct features suggesting different biological functions in specific regions [28,29]. The proximal region of ducts contains a slow cycling population of stem cells that express high levels of Sca-1 [30–33]. As Wnts have roles in progenitor cell function in a number of tissues, they may also be important in regulating prostate stem and/or progenitor cell homeostasis.

To investigate a possible role for Wnt signaling in mouse prostate progenitor cells, we used two mouse models where a reporter molecule (green fluorescent protein (GFP) or lacZ) was inserted downstream of the Axin2 promoter. We demonstrate that Axin2 expression occurs in all lobes of the prostate in an androgen-dependent manner. We also show that Axin2 expressing cells co-express Sca-1, suggesting that Wnt-signaling occurs in progenitor cells of the prostate. Lastly, using a Wnt-signaling agonist we find that active Wnt signaling may result in increased growth of primary prostate cells in vitro. Together this data suggests that Wnt signaling may be involved in the maintenance/growth of progenitor cells in the prostate.

METHODS

Animals

C57BL/6 mice were obtained from Charles River (Wilmington, MA). Axin2-GFP and $Axin2^{+/lacZ}$ mice were generated as previously described [26,27] and kindly provided by Dr Frank Costantini at Columbia University, New York. All mice were housed in climate controlled animal research facilities at New York University, and all experiments were performed in compliance with institutional review board requirements. In some instances, castrated animals were replenished with androgen by intraperitoneal injection of testosterone propionate (4 μg/g body weight/day).

Tissue Preparation and Analysis

Animals were sacrificed and the urogenital tract was removed en bloc and transferred to Hank's balanced salt solution (HBSS) (Cellgro). The VP, LP, and DP were dissected in HBSS under a Leica S6D dissecting microscope using 25-gauge needles [29]. Images were acquired using an Optronics digital camera (Model 60800) and MagnaFire software (Optronics) attached to a Leica MZFLIII dissecting microscope.

To prepare single cell suspensions, prostates were removed from C57BL/6 and Axin2-GFP mice and individual lobes were separated into two regions: (i) the proximal region, which comprises those ducts nearest the urethra, and (ii) the remaining regions, which include the intermediate and distal regions [30]. Each sample was incubated in 0.5% collagenase (type II clostridiopeptidase A; Sigma–Aldrich) for 1 hr with periodic agitation followed by digestion in 0.25% trypsin (BD Biosciences) for 7 min at 37°C. Cells were suspended in growth medium containing conditioned medium from a prostatic smooth muscle cell line, PSMC1 [34,35], and used for further analysis.

LacZ Staining

Prostates were fixed in glutaraldehyde fixative (5 mM EGTA (Sigma), 2 mM $MgCl_2$ (Sigma), 0.1 M NaPi pH 7.3 (Sigma), 0.1% glutaraldehyde (Fisher), 1.5% formaldehyde (Fisher)) for 30 min at room temperature. Prostates were removed from the fixative and washed three times for 30 min/wash in 0.02% NP-40 (Sigma) in 1 × PBS at room temperature. Prostates were then transferred to the staining solution (5 mM $K_3FE(CN)_6$ (Sigma), 5 mM $K_4Fe(CN)_6$ (Sigma), 2 mM $MgCl_2$ (Sigma), 0.01% deoxycholate (Sigma), 0.02% NP-40 (Sigma), 1 mg/ml X-gal (Roche) in 1 × PBS) and monitored until adequate staining had developed (approximately 1 hr). Once staining was achieved, prostates were washed three times with 1 × PBS, post-fixed overnight in 4% paraformaldehyde (Electron Microscopy Sciences) in 1 × PBS, and photographed as previously described.

Cell Preparation and FACS Analysis

Single cell digests were resuspended in FACS buffer (HBSS containing 0.5% BSA (Sigma–Aldrich) and 2 mM EDTA (Sigma–Aldrich)). Fc receptors were blocked with mouse anti-mouse CD16/32 antibodies (Caltag) and rat IgG (Caltag) for 10 min on ice. The cells were then incubated with phycoerythrin (PE)-conjugated rat anti-mouse Sca-1 (Caltag) antibody (100 ng/ml) or control rat IgG2a-PE (100 ng/ml) (Caltag) for 30 min on ice then washed with FACS buffer. In all analyses, the dye 7-aminoactinomycin D (1 μg/ml) was added 5 min before analysis, to exclude dead cells [31]. Cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson), using CELLQUEST software (Becton Dickinson).

Cell Growth Assays

Each population of cells was seeded at 10^4 cells/well on collagen-coated 96-well plates (Falcon) [36]. Cells were cultured for the indicated number of days in medium containing 1 μM methylBIO or 1 μM BIO [37] compound kindly provided by Dr Ali Brivanlou (Rockefeller University, New York). Following culture, cells were removed from collagen-coated plates by a 20 min incubation with 0.5% collagenase (type II clostridiopeptidase A; Sigma–Aldrich) and disaggregated into a single cell suspension by a 7 min incubation in 0.25% trypsin (BD Biosciences). Trypan blue (Sigma–Aldrich) exclusion was used to determine cell viability.

RESULTS

Axin2 Expression Is Highest in the Lateral and Dorsal Lobes of the Mouse Prostate and Is Androgen Dependent

We have shown that prostatic stem cells reside in the proximal region of ducts and that progenitor cells express Sca-1 [30–32]. As Wnt signaling has a role in several aspects of stem cell and tumor biology [38,39], we determined whether evidence of Wnt signaling was present in prostate progenitor cells. This was accomplished using Axin2 expression in the prostate as an indication of Wnt-signaling using two Axin2 reporter mouse models. In these models, either a destabilized form of GFP (Axin2-GFP) or lacZ ($Axin2^{+/lacZ}$) is expressed under control of the Axin2 promoter [26,27]. Whole mount analysis of the mouse prostate showed that the LP expressed the most Axin2 with less expression evident in the VP or DP (Figs. 1 and 2). This indicates that Axin2 (Wnt activity) is expressed in all lobes of the mouse prostate with the highest levels occurring in the LP.

The prostate is an androgen-dependent gland able to undergo over 30 cycles of involution followed by regeneration [40] indicating the presence of a population of stem cells able to survive involution and promote regeneration upon androgen replenishment. Axin2-GFP mice were therefore castrated (androgen deficient) and androgens replenished in an alternate group of mice in order to determine the effect of androgens on Axin2 expression. In whole mounts of prostates of mice that had been castrated for 7 days, GFP is almost undetectable in all lobes of the prostate (see LP in Fig. 3D) demonstrating a dependence on androgens for Axin2 expression. Conversely, in mice that had been castrated for 7 days followed by 7 days of androgen replenishment, GFP expression was partially restored (Fig. 3F) 1 week after androgen supplementation, albeit still below levels seen in intact Axin2-GFP mice (Fig. 3B). The effects of androgen on Axin2 expression were also measured by determining the fraction of cells expressing GFP using the FACS (Fig. 3G). The incidence of Axin2-GFP positive cells was measured in the LP from intact Axin2-GFP mice, castrated Axin2-GFP mice, and Axin2-GFP mice castrated for 7 days followed by daily testosterone injections for 7 days. The LP of castrated mice contained 2.1 ± 0.7% Axin2-GFP expressing cells versus 7.1 ± 1.0% present in animals supplemented with androgen for 7 days (P <0.03) (Fig. 3G). It is possible that levels would have risen further had androgen supplementation been continued for a longer period of time and that they may have approached levels noted in intact animals. However, the levels present after 7 days of androgen supplementation (7.1 ± 1.0%) are not significantly different from those of intact mice (14.2 ± 3.3, P =0.22). This indicates that Axin2 expression is an androgen-dependent event.

To quantitate the number of GFP^{+}/Axin2 expressing cells in the prostate, proximal and remaining regions of individual lobes from the Axin2-GFP reporter mice were analyzed by FACS for GFP expression. As stem cells are concentrated in the proximal region of prostatic ducts, we examined the proximal and remaining regions of individual lobes to determine whether Axin2 expression was concentrated in cells of the proximal region. We found no significant difference in Axin2 expression along the proximal–distal ductal axis of the VP, LP, or DP (Table I), suggesting that Wnt signaling may not preferentially occur in the proximal stem cell enriched region. While no differences in Axin-2 expression among the remaining regions of ducts were noted, we found that the proximal region of the DP had more Axin2 expressing cells (6.8 ± 1.4%) than the proximal region of the VP (4.7 ± 1.0%) (P <0.01) (Table I). Furthermore, the proximal LP contained significantly more Axin2 expressing cells (12.3 ± 3.0%) than the proximal DP (6.8 ± 1.4%) (P <0.01) (Table I). This indicates that the proximal lobes may be phenotypically different from each other in terms of Wnt signaling which might imply different functions for Wnt in this region of the lobes.

Axin2 Expressing Cells Are Sca-1Positive

We examined Axin2 expressing cells for their co-expression of Sca-1 as progenitor cells of the prostate express this antigen [31,33]. When we compared the number of $GFP^+/Sca\text{-}1^+$ (double positive) expressing cells in the proximal region with remaining regions of ducts in individual lobes, we found that only the VP had significantly different numbers of $GFP^+/Sca\text{-}1^+$ cells in the pVP (1.3 ± 0.3%) compared to the rVP (0.2 ± 0.1%) (P <0.02) (Table IIa and Figs. 4 and 5A). This indicates that the proximal region of the VP contains more progenitor cells that may be undergoing Wnt signaling than the distal region. A comparison of either the proximal or remaining regions of the individual lobes with each other indicated that there were significantly more $GFP^+/Sca\text{-}1^+$ cells in the pDP (4.3 ± 1.0%) compared to the pVP (1.3 ± 0.3%) (P <0.03) and significantly more $GFP^+/Sca\text{-}1^+$ cells in the rDP (4.0 ± 1.0%) compared to the rVP (0.2 ± 0.1%) (P <0.02) (Table IIa and Figs. 4 and 5A). This indicates that the DP contains more progenitor ($Sca\text{-}1^+$) cells that may have Wnt signaling than the VP.

As prostate stem and progenitor cells express Sca-1 [31,33], we determined the fraction of Sca-1 expressing cells that may contain an active Wnt pathway (GFP^+) (Table IIb and Figs. 4 and 5B). A small fraction of Sca-1 expressing cells (Fig. 4, cells in upper right and upper left quadrants express Sca-1) displayed Axin2 expression suggesting that a subpopulation of progenitor cells in the prostate may utilize Wnt signaling. Comparison of the proximal with remaining regions indicated that the DP contained significantly more Sca-1 expressing cells with Axin2 expression (3.4 ± 1.2% in the pDP and 2.9 ± 1.5% in the rDP) compared to the VP (1.0 ± 0.6% in the pVP and 0.2 ± 0.1% in the rVP) (P <0.01 and 0.01, respectively). Furthermore, the rLP (0.9 ± 0.3%) contained significantly more Sca-1 expressing cells with Axin2 expression than the rVP (P <0.01). This indicates that there may be differences in the number of primitive cells utilizing Wnt signaling among the regions of the individual lobes.

Wnt signaling has a role in homeostasis of stem cells and also functions in more differentiated cells. As the regenerating ability of the prostate resides in the Sca-1 expressing population [31], we determined the fraction of cells that expressed Axin2 and also co-expressed Sca-1 and noted no significant differences in the regional expression of this population of cells (Table IIc and Figs. 4 and 5C). However, significant numbers of Axin2 expressing cells lacked Sca-1 expression, suggesting that not all Wnt active cells are progenitor cells.

Suppression of GSK3β Increases the Growth of Primary Prostate Epithelial Cells

Much work has demonstrated that activation of the Wnt-signaling pathway contributes to maintenance and/or self-renewal of stem cells in different tissues [2,7,14,39,41]. The binding of Wnt proteins to their frizzled receptors, results in altered gene expression and cellular functions with one of the major regulators of the Wnt pathway being GSK3β [42,43]. In an unstimulated cell, GSK3β phosphorylates β-catenin to promote its degradation. After stimulation GSK3β phosphorylation is inhibited, β-catenin is stabilized and available to activate transcription. In order to determine if GSK3β had an effect on cell proliferation, we mimicked the effects of an active Wnt signal in prostate cells by using a chemical inhibitor of GSK3β or its kinase inactive inhibitor analog, methylBIO [37]. The BIO compound and not methylBIO has been shown to decrease β-catenin phosphorylation and degradation [37], and is used to activate intracellular Wnt-signaling molecules by bypassing Wnt ligand binding [44–47]. Although consequences of the inhibition of GSKβ are not definitive proof of Wnt signaling as this protein also acts in other pathways, we determined the effect of BIO on the growth of primary prostate cells. Cells from the proximal and remaining regions of ducts from individual lobes were therefore cultured in the presence of BIO (1 μM) or methylBIO (1 μM) and an approximate twofold (P <0.01)

increase in number was noted in BIO treated cells (Table III). This indicates that stimulation of Wnt signaling may result in an increase in the number of prostatic epithelial cells and is consistent with in vivo models showing increased proliferation when Wnt signaling is activated [14,16,38].

CONCLUSIONS

We demonstrate here that Axin2 expression, strongly suggestive of Wnt signaling, is present in all lobes of the mouse prostate and that this expression/Wnt activity is androgen dependent. These data support the idea of interplay between Wnt and androgen signaling. On the one hand, activation of the Wnt-signaling pathway has been shown to enhance or activate different steps in the androgen receptor-signaling pathway. Stimulation of LNCaP cells with Wnt3a can induce ligand independent nuclear accumulation of androgen receptor and androgen receptor-mediated transcription [48,49]. Buttyan and co-workers [50] demonstrated that three LEF1/TCF binding elements lying upstream of the human androgen receptor promoter confer robust transcriptional activation to the promoter after activation of the Wnt-signaling pathway. On the other hand, androgen receptor signaling is also able to alter the Wnt-signaling pathway, albeit usually in an inhibitory manner. Studies demonstrate that androgen receptor activation downregulates transcription from β-catenin inducible promoters [51,52] by using β-catenin at androgen response elements rather than TCF response elements [53,54]. These studies demonstrate a complex balance between Wnt and androgen receptor signaling and imply that there is likely a complex interaction between Wnt and androgen signaling in the different lobes of the mouse prostate. In our system, androgen deprivation results in decreased Wnt activity. Indeed, androgens are known to stimulate Wnt signaling in prostate cells through phosphorylation of GSK3β [55] and direct interaction with β-catenin [56,57]. The resulting lack of Wnt signaling we see after castration could be explained by any of these scenarios where removal of androgens would result in decreased Wnt signaling.

We found no difference in Axin2 expression (Wnt activity) between the proximal and remaining regions in the individual lobes of the prostate. However, we noted that the pLP had significantly more Axin2 expressing cells than the pDP, and the pDP had significantly more Axin2 expressing cells than the pVP. No significant differences in the fraction of Axin2 expressing cells among remaining regions were noted. Our observation that the pLP and pDP may contain the highest fraction of Wnt active cells may be relevant in light of the fact that the lobes of the prostate are associated with different functions. For example, Berquin et al. [58] used DNA array analysis to show that the gene expression pattern in the mouse dorso-lateral lobe, and not the ventral lobe, was closest to that of the human prostate peripheral zone. This supports the hypothesis that these prostate compartments are more similar to each other than they are to the VP [59–61]. As Wnt-signaling pathways are active in carcinogenesis [17,62,63], our data suggesting more Wnt activity in the LP and DP may result in a greater susceptibility of these lobes to the development of prostate cancer [50,64,65]. This may indicate that the LP and DP may serve as better models than the VP for studying prostate carcinogenesis as these lobes may be more closely related to the peripheral zone in which human prostate cancer primarily develops.

The cells in the murine prostate with prostate regenerating potential reside within the Sca-1 expressing population [31,33]. We show that a significant fraction of Axin2 expressing cells co-express Sca-1 and are therefore progenitor cells. These findings are similar to those of the hair follicle in which the strongest Wnt activity occurs in progenitor cells as they leave the cell cycle and begin terminal differentiation [66,67]. In addition, Wnt signaling in the brain is present in proliferating neural progenitors rather than in the most primitive neural stem cells [41]. Thus our data suggest that Wnt signaling in the prostate may be present in

progenitor cells where it is likely to promote both proliferation and differentiation to a mature prostate epithelial cell. Indeed we show that the activation of Wnt signaling may result in increased cell numbers consistent with a role for Wnt in proliferation in the prostate.

While Wnt signaling has a role in regulating transcription, it is also associated with changes in cell adhesion [68,69] by promoting association of cytoplasmic β-catenin with cadherins to stabilize extracellular protein interactions between cells [20,68,70]. Therefore signals leading to Axin2 expression could also be related to changes in cell adhesion in addition to or independent of a role in transcriptional activation. It is therefore possible that the higher Axin2 expression observed in the LP could in part be involved in mediating different cell–cell contacts not observed in the VP and DP. Because progenitor cells are longer-lived than more differentiated cells, it is possible that primitive cells remain in their niche by establishing contacts with neighboring cells and extracellular matrix to facilitate their maintenance in their niche. Indeed Wnt signaling has been shown to be important for maintenance of cells in their normal location and migration within many tissues. Active Wnt signaling in the SW480 human colon carcinoma cell line leads to increased E-cadherin expression [71] suggesting a role for Wnt signaling in cell adhesion. Additionally, overexpression of a stable form of β-catenin in intestinal epithelium results in augmentation of E-cadherin at adherens junctions which slows migration of primitive crypt cells up the villus during their progression to a more differentiated phenotype [72]. It is therefore possible that Wnt signaling in the prostate is important for cell adhesive properties of prostate progenitors and that this confers greater growth potential on the cells.

While there have been several reports of alterations in Wnt signaling being associated with prostate cancer [50,65,73], a role for Wnt signaling in prostate homeostasis is not documented. Here we show that all lobes of the prostate may contain cells with androgen-dependent Wnt signaling. Furthermore, these cells have the characteristics of prostate progenitor cells and may use the Wnt-signaling pathway for their expansion and differentiation. Knowledge of the role of Wnt signaling in the normal prostate may facilitate an understanding of the processes involved in carcinogenesis as Wnt signaling is involved in the development of cancer [17,62,63,65]. It furthermore contributes to an understanding of the signals involved in the biology of prostate progenitor cells.

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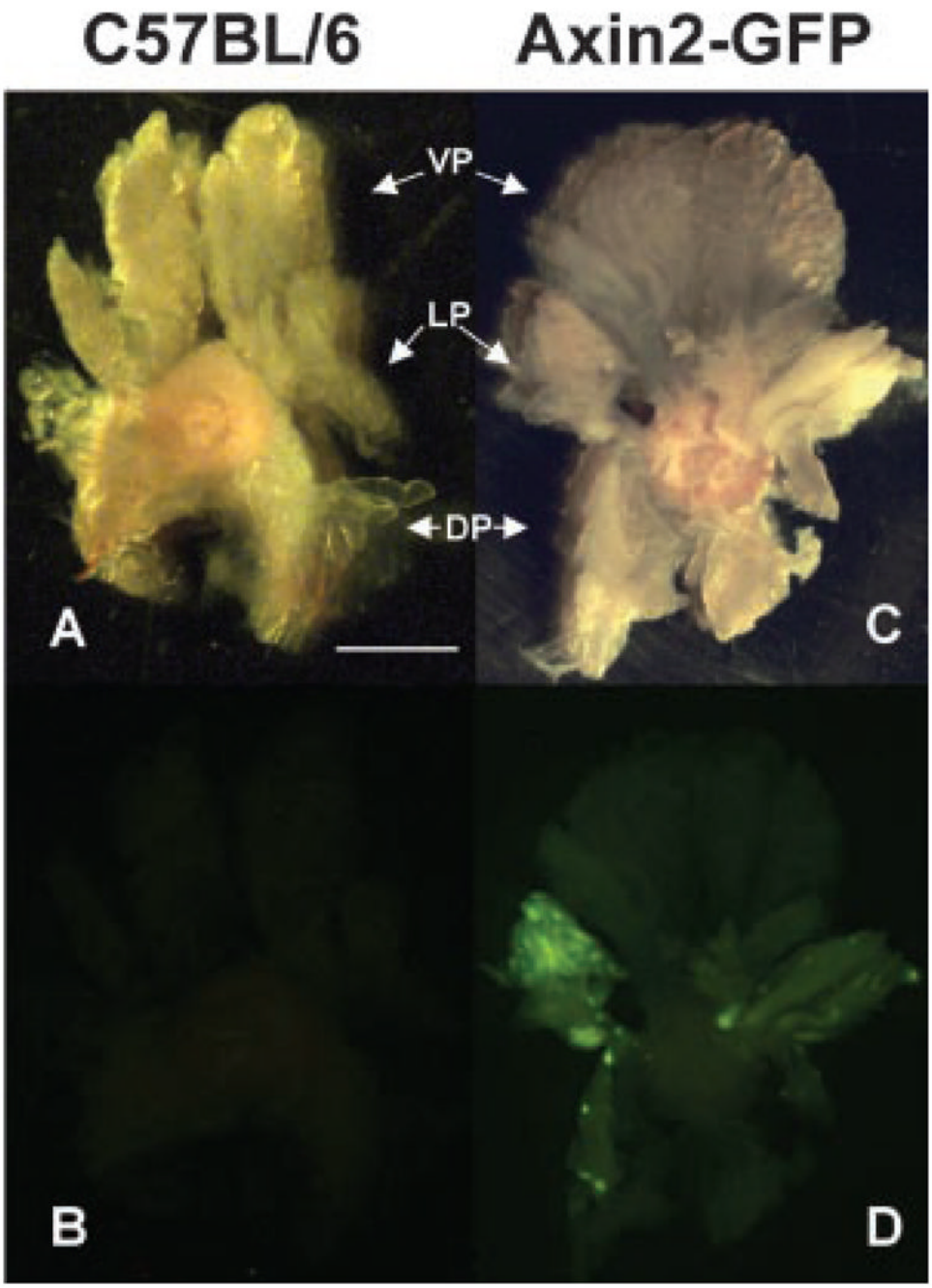

Fig. 1.

Axin2 is differentially expressed in lobes of the prostate from Axin2-GFP mice. Prostate glands from six Axin2-GFP mice were analyzed for GFP indicative of Axin2 expression. **A** and **B** represent brightfield and fluorescence images respectively of a control C57BL/6 prostate. **C** and **D** represent brightfield and fluorescence images, respectively, of an Axin2-GFP prostate. The LP has higher levels of Axin2 than the VP and DP (D). Scale bar: 2mm.

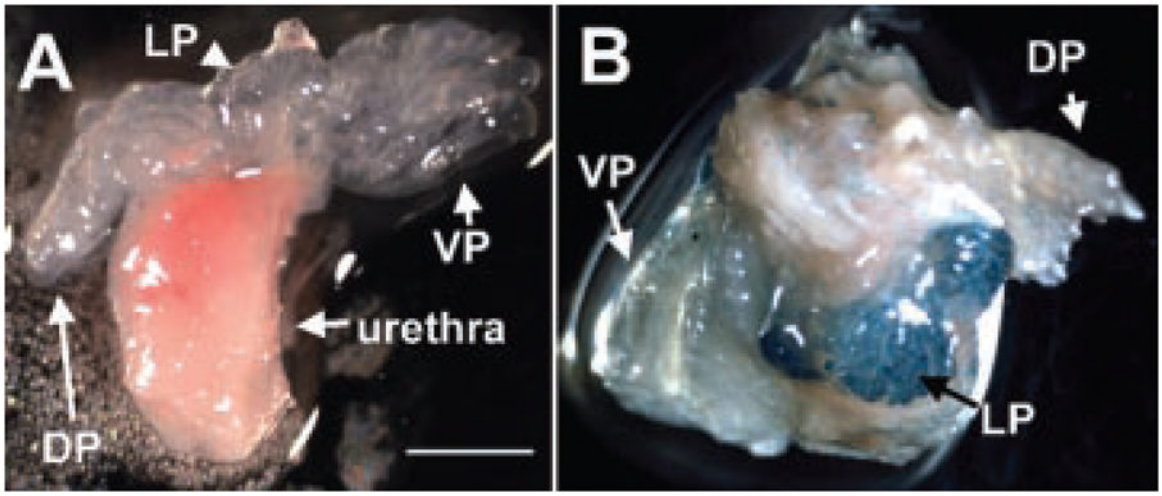

Fig. 2.

Axin2 is differentially expressed in lobes of the prostate from Axin$^{+/lacZ}$ mice. Prostate glands from three Axin2$^{+/+}$ and Axin2$^{+/lacZ}$ mice were analyzed for β-galactosidase activity indicative of Axin2 expression. **A** and **B** represent prostate lobes (VP, LP, and DP) from Axin2$^{+/+}$ and Axin2$^{+/lacZ}$ mice, respectively. The LP has higher levels of Axin2 than the VP and DP (B). Scale bar: 2mm.

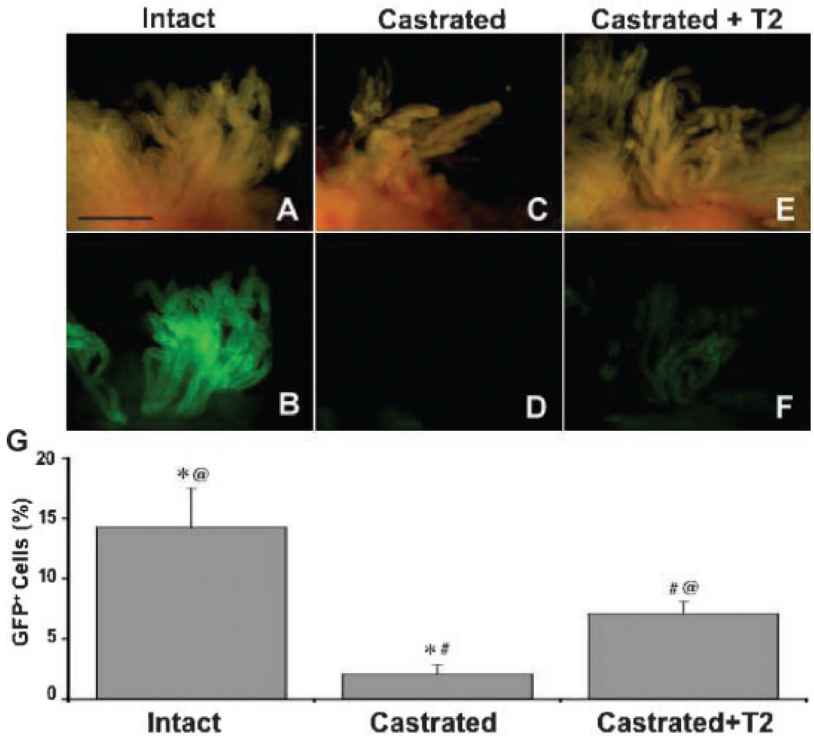

Fig. 3.

Axin2 expression is positively regulated by androgens. Five Axin2-GFP mice were castrated (**C** and **D**) or castrated then given daily testosterone injections for 7 days (six mice) (castrated +T2) (**E** and **F**) and GFP fluorescence compared to that noted in intact Axin2-GFP mice (**A** and **B**). A,C, and E represent brightfield images of the LP and B,D, and F represent the corresponding fluorescence images. Scale bar:1mm. (**G**) FACS was performed to determine the incidence of Axin2-GFP positive cells in the LP from four intact Axin2-GFP mice, three castrated Axin2-GFP mice, and two Axin2-GFP mice castrated then given daily testosterone injections for 7 days. $^{*}P<0.03$, $^{\#}P<0.03$, $^{@}P=0.22$.

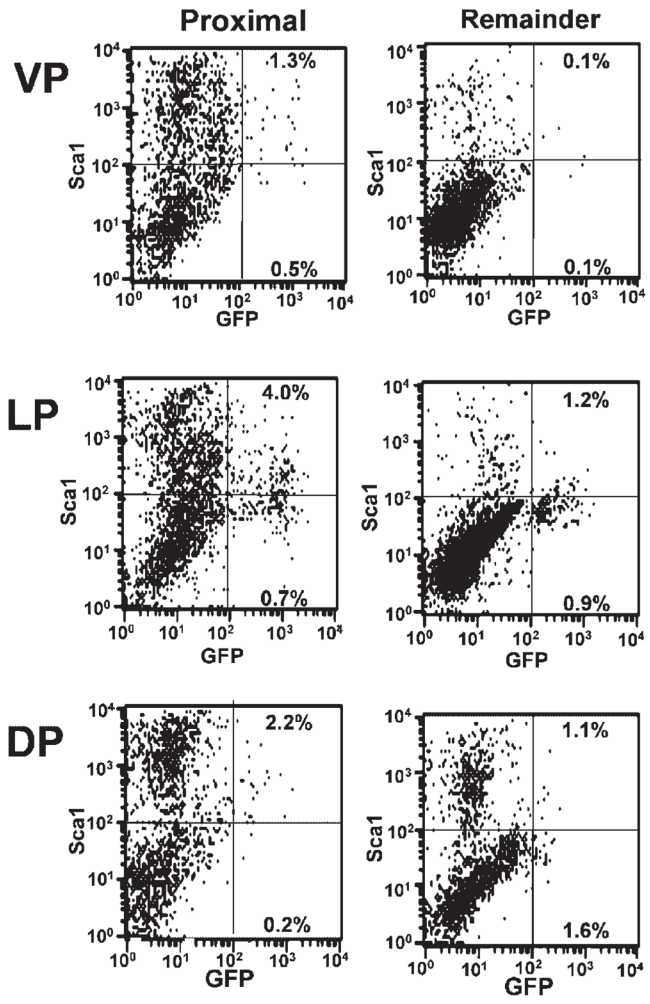

Fig. 4.

Quantitation of Axin2-GFP and Sca-1 expressing cells. FACS analysis was performed to determine the incidence of Axin2-GFP and Sca-1 expressing cells in the proximal and remaining regions of the VP, LP, and DP in five mice. A representative dot plot indicating Wnt activity (GFP^+) and Sca-1 expression in cells from different regions of each lobe is depicted.

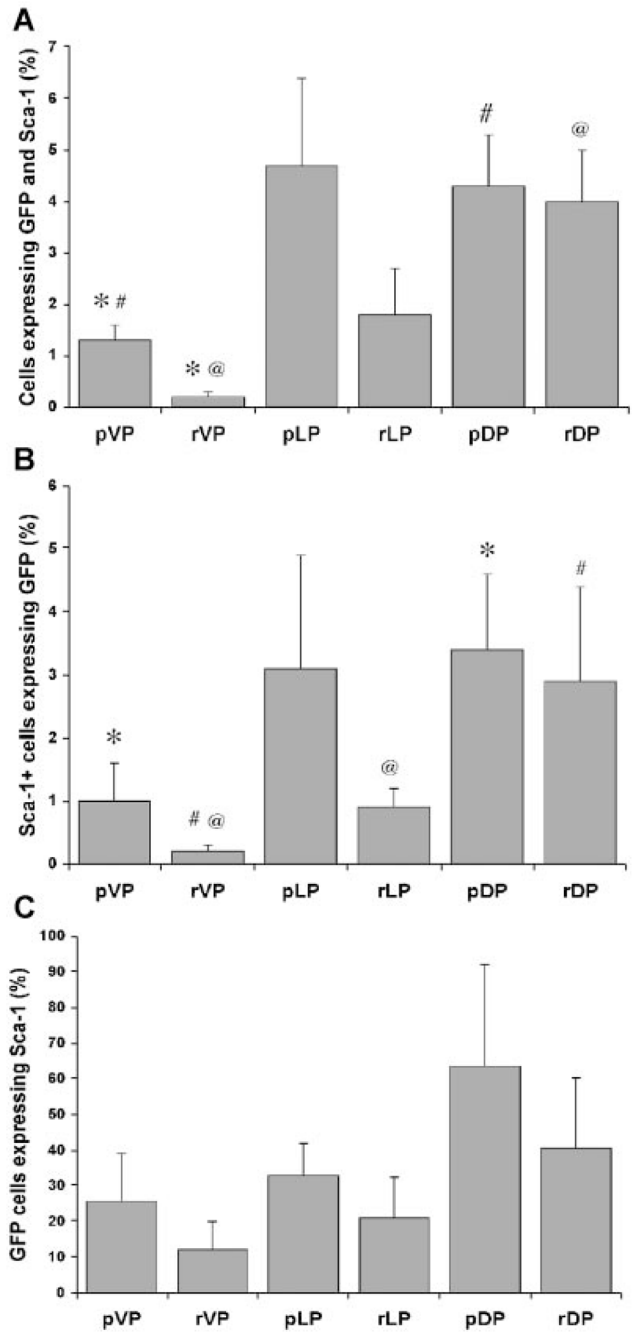

Fig. 5.

The relationship between Axin2-GFP and Sca-1 expression in different regions of prostate lobes. FACS analysis was performed to determine the incidence of Axin2-GFP and Sca-1 expressing cells in the proximal and remaining regions of the VP, LP, and DP. Each bar represents the average expression of the indicated antigens from five mice. Each data point represents the average expression of the indicated antigens from five individual mice. (**A**) $^{*}P < 0.02$, $^{\#}P < 0.03$, and $^{@}P < 0.02$; (**B**) $^{*}P < 0.01$, $^{\#}P < 0.01$, and $^{@}P < 0.01$.

TABLE I

Axin2-GFP Expression by Cells in Different Regions of Prostate Lobes

Region and lobe	[a] GFP$^+$ cells (%)
pVP	4.7 ± 1.0*
rVP	1.9 ± 0.3
pLP	12.3 ± 3.0$^\#$
rLP	9.2 ± 4.0
pDP	6.8 ± 1.4$^{*,\#}$
rDP	6.8 ± 1.5

p, proximal; r, remainder.

[a] Each data point represents the average expression of the indicated antigens from 12 individual mice.

$^*P<0.01$.

$^\#P<0.01$.

TABLE II

The Relationship Between Axin2-GFP and Sca-1 Expression in Different Regions of Prostate Lobes

(a) Region and lobe	[a] Cells expressing GFP and Sca-1 (%)
pVP	1.3 ± 0.3 [*,†]
rVP	0.2 ± 0.1 [*,‡]
pLP	4.7 ± 1.7
rLP	1.8 ± 0.9
pDP	4.3 ± 1.0 [†]
rDP	4.0 ± 1.0 [‡]
(b) Region and lobe	[a] $Sca\text{-}1^+$ cells expressing GFP (%)
pVP	1.0 ± 0.6 [§]
rVP	0.2 ± 0.1 [§]
pLP	3.1 ± 1.8
rLP	0.9 ± 0.3 [§]
pDP	3.4 ± 1.2 [§]
rDP	2.9 ± 1.5 [§]
(c) Region and lobe	[a] GFP cells expressing Sca-1 (%)
pVP	25.7 ± 13.4
rVP	12.2 ± 7.9
pLP	33.0 ± 9.0
rLP	21.1 ± 11.4
pDP	63.6 ± 28.5
rDP	40.7 ± 19.7

p, proximal; r, remainder.

[a] Each data point represents the average expression of the indicated antigens from five individual mice.

[*] $P<0.02$.

[†] $P<0.03$.

[‡] $P<0.02$.

[§] $P<0.01$.

TABLE III

Suppression of GSK3β Increases the Number of Primary Prostate Epithelial Cells[a]

Region and lobe	MethylBIO (cells)*	BIO (cells)*
pVP	4,723 ± 342	7,980 ± 626
rVP	6,516 ± 742	11,724 ± 1,315
pLP	9,019 ± 1,171	17,804 ± 1,844
rLP	2,651 ± 349	6,081 ± 830
pDP	2,958 ± 437	8,225 ± 1,013
rDP	3,568 ± 457	10,963 ± 1,021

p, proximal; r, remainder.

[a]Each data point represents the average number of cells from 12 replicates.

*$P < 0.01$ for each region cultured.