Smooth Muscle Cell-Specific Knockout of Androgen Receptor: A New Model for Prostatic Disease

Michelle Welsh, Lindsey Moffat, Alan McNeilly, David Brownstein, Philippa T. K. Saunders, Richard M. Sharpe, and Lee B. Smith

Medical Research Council Centre for Reproductive Health (M.W., L.M., A.M., P.T.K.S., R.M.S., L.B.S.), and University of Edinburgh (D.B.), The Queen’s Medical Research Institute, Edinburgh, EH16 4TJ, Scotland, United Kingdom

Androgen-driven stromal-epithelial interactions play a key role in normal prostate development and function as well as in the progression of common prostatic diseases such as benign prostatic hyperplasia and prostate cancer. However, exactly how, and via which cell type, androgens mediate their effects in the adult prostate remains unclear. This study investigated the role for smooth muscle (SM) androgen signaling in normal adult prostate homeostasis and function using mice in which androgen receptor was selectively ablated from prostatic SM cells. In adulthood the knockout (KO) mice displayed a 44% reduction in prostate weight and exhibited histological abnormalities such as hyperplasia, inflammation, fibrosis, and reduced expression of epithelial, SM, and stem cell identity markers (e.g. p63 reduced by 27% and PTEN by 31%). These changes emerged beyond puberty and were not explained by changes in serum hormones. Furthermore, in response to exogenous estradiol, adult KO mice displayed an 8.5-fold greater increase in prostate weight than controls and developed urinary retention. KO mice also demonstrated a reduced response to castration compared with controls. Together these results demonstrate that prostate SM cells are vital in mediating androgen-driven stromal-epithelial interactions in adult mouse prostates, determining cell identity and function and limiting hormone-dependent epithelial cell proliferation. This novel mouse model provides new insight into the possible role for SM androgen action in prostate disease. (Endocrinology 152: 3541–3551, 2011)

The prostate, like much of the male reproductive tract, depends on androgen-driven stromal-epithelial interactions for normal structure and function (1–5) but the underlying mechanisms remain unclear. Classical experiments by Cunha and and Donjacour (5) and Chung and Cunha (6) have highlighted the direct role for androgen action via the stromal cells in inducing normal prostate development. However, much of the evidence for this comes from tissue recombination studies using neonatal stroma and epithelium; therefore, understanding of the role for stromal androgen action in the normal adult prostate remains limited. Perturbation of these androgen-driven stromal-epithelial interactions is also implicated in prostatic diseases such as benign prostatic hyperplasia and prostate cancer (PCa) (7, 8); therefore, greater understanding of the role for stromal AR in normal adult prostate homeostasis could uncover new insight into how androgens mediate prostate disease and so provide new opportunities for intervention. Although this is challenging to address directly in humans, the advent of genetic modification in mice provides an exciting tool with which to advance our fundamental understanding of prostate development and function.

The mouse prostate consist of ducts and glands lined by a secretory epithelial layer that lies above a layer of p63-positive basal cells (9), which have been proposed to contain one of the two stem cell populations identified in the mouse prostate (10–13). Prostatic ducts are surrounded
by a thin smooth muscle (SM) layer and an outer heterogeneous stromal layer. From puberty, under the control of androgens, luminal epithelial cells secrete several well-characterized products that contribute to the seminal fluid, such as serine peptidase inhibitor Kazal type 3 (Spink3) and prostate β defensin 1 (Pbd1/Defb37) (14). Androgens modulate gene transcription by binding to the androgen receptor (AR), which is expressed in prostatic stromal and epithelial cells, but not in the basal stem cells (15, 16). Adult prostates also express both estrogen receptors (ER) α and β (17), and disruption of the androgen-estrogen balance in mice leads to abnormal prostate histology and carcinoma (18). Interestingly, androgens are thought to mediate different effects in each cell compartment; for example, prostatic epithelial AR is essential for the epithelial secretory function observed in the prostate of mature mice (19) and inhibits proliferation (20) whereas stroma AR is thought to determine the fate of the epithelium, regulate epithelial cell apoptosis, and promote proliferation (21, 22). However, because prostatic stroma is composed of many cell types including immune cells and fibroblasts, exactly which cell types mediate these effects is unclear. For example, it is unknown whether the epithelium relies on interactions with the entire stromal compartment, or whether signaling from a subset of the stroma, such as the SM cells or the stromal fibroblasts, is sufficient to mediate normal epithelial cell identity and function. Recent generation of a SM cell AR knockout (KO) mouse model (termed PTM-ARKO (23)) has provided an exciting opportunity to investigate the role for SM-specific androgen action in normal adult prostate function as well as its possible role in prostate disease.

Materials and Methods

Breeding of transgenic mice

Mice in which AR has been ablated from the SM cells of the testis and seminal vesicles (SV) have been described by us previously (23, 24), using male mice expressing Cre recombinase and green fluorescent protein (GFP), both under the control of a SM myosin-heavy chain promoter (MH) (25). These mice also expressed Cre recombinase in the SM cells of the prostate, therefore providing a novel opportunity to investigate the role for androgen action in the prostate SM. The Cre-positive (AR<sup>flox</sup> positive) male offspring are termed PTM-ARKO or KO, whereas the AR<sup>flox</sup> positive Cre negative male littermates were used as controls (termed control), as previously reported (23). All mice were bred under standard conditions of care and use under licensed approval from the United Kingdom Home Office. Stud male MH mice and PTM-ARKO male offspring were genotyped for the presence of Cre using PCR as previously described (23).

Recovery of reproductive tissues

Male mice were culled at embryonic day (e)17.5 and at postnatal days (d) 35, 50, 100, and 200, and prostates were recovered, weighed, and processed as previously reported (23). Each prostate lobe [ventral (VP), anterior (AP), and dorsolateral (DLP)] was isolated and weighed separately. The Bar Harbor consensus report (26) states that no mouse prostate lobe is more relevant to human prostate disease than another; therefore the VP was examined in further detail as a representative lobe. VP were stained with hematoxylin and eosin and examined for histological abnormalities such as hyperplasia, edema, and hypertrophy, by an expert mouse pathologist.

Hormone implant study

Control (n = 5) and PTM-ARKO (n = 5) male mice (d130–d150) were implanted with a 1-cm SILASTIC implant (Dow Corning Corp., Midland, MI) containing 100 mg testosterone (T): 10 mg estradiol (E2) (Sigma-Aldrich, Poole, UK), as previously reported in PTM-ARKO (24); this treatment regime was originally reported by Ricke et al. (27). Empty sham implants were used as a treatment control. Implants were left in situ for 13 wk after which mice were culled, blood was collected, and VP were recovered.

Castration studies

Both testes were surgically removed from adult control (n = 9) and PTM-ARKO (n = 4) male mice (d 130–d 150) under isofluorane. After 7 d, mice were culled, blood was collected, and VP were recovered.

Immunohistochemical analysis

Mice were examined by immunohistochemistry for the presence of Cre in VP SM cells, identified by immunoeexpression of α-smooth muscle actin (SMA), using a previously published method (23, 24). Fluorescent immunostained sections were mounted in Mowiol mounting medium (Calbiochem, San Diego, CA) and fluorescent images were captured using a Zeiss LSM 510 Meta Axiovert 100M confocal microscope (Carl Zeiss Ltd., Welwyn, UK). Adult VP from PTM-ARKO and control males were also stained for green fluorescent protein as a marker for Cre expression, e-cadherin, SMA, pan-cytokeratin, phosphohistone H3, Sox9, and p63. Sections were deparaffinized and rehydrated and antigen was retrieved as previously detailed (24) before using a specific polymer high-contrast program on a Bond-X automated immunostaining machine (Vision Biosystems, Newcastle, UK). Optimal antibody concentrations are detailed in supporting information Table 1. All kits were purchased from Vision Biosystems, and images were captured using a Provis microscope (Olympus Optical Co., Tokyo, Japan) equipped with a Kodak DCS330 camera (Eastman Kodak, Rochester, NY). To ensure reproducibility of results, representative VP from at least three animals at each age were used, and sections from PTM-ARKO and control littermates were processed in parallel on the same slide on at least two occasions. Appropriate negative controls were included to ensure that any staining observed was specific. All antibodies used showed only minor nonspecific staining.

Quantification of VP cell composition

The proportion of VP composed of epithelium, SM, stroma, and lumen was quantified stereologically in d 100 control and
PTM-ARKO VP immunostained for SMA by grid point counting using stereology equipment and sampling procedures previously described (23, 24). SM thickness and epithelial cell height were also quantified in d 100 control and KO VP, as previously described (24).

Analysis of cell mitosis and apoptosis in VP

VP from control and KO adults were immunostained for phosphohistone H3 and positive or negative mitotic cells were counted in the epithelial compartment, as previously described (24). The number of positive cells in the epithelial compartment was then divided by the total number of epithelial cells visible in section to calculate the epithelial proliferation index. Apoptotic cells were identified in d 12 and d 100 VP from PTM-ARKO and control mice using terminal deoxynucleotide transferase-mediated dUTP nick end labeling, as previously described (28). Quantification was deemed unnecessary because few apoptotic cells were identified at either age.

Determination of deletion of AR exon 2

RNA was isolated and quantified from frozen VP, and cDNA was prepared, as previously described (23). RT-PCR was performed as previously described (23) on cDNA synthesized from VP from PTM-ARKO and controls. Testes from ARKO mice were used as a positive control for AR deletion because ARKO mice have no prostate.

Quantitative analysis of gene expression

Quantitative PCR was performed on d 100 PTM-ARKO and control VP for the genes listed in Table 2, using previously described methodology (23). The expression of each gene was related to 18S, an internal positive control, and expression was corrected for proportional changes in cell compartments.

Statistical analysis

Data were analyzed using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, CA) using a one-tailed unpaired t test, one-way ANOVA or two-way ANOVA, as appropriate, followed by Bonferroni post hoc tests. Values are expressed as mean ± SEM. Normality was confirmed using KS normality test.

Results

Identification of SM AR deletion in VP from PTM-ARKO mice

We have already described mice with AR ablation from the SM cells of the testis and SV (23, 24); these mice express Cre recombinase and GFP under the control of a SM myosin-heavy chain promoter (25). Within the prostate, GFP (Fig. 1A, arrow) (and therefore Cre) was expressed exclusively in the SM layer in KO mice at d 100. RT-PCR for AR exon 2 revealed that control prostates only expressed the full-length [wild-type (WT)] AR transcript whereas KO prostates expressed both the WT and the smaller KO AR transcripts, demonstrating that AR was deleted from a proportion of prostate cells (Fig. 1B). ARKO testis cDNA, the positive control, only expressed the smaller KO transcript because AR mice have no prostate.

Table 1. Immunohistochemistry antibody details

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antibody source</th>
<th>Dilution</th>
<th>Detection system</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA/AR</td>
<td>Sigma</td>
<td>1:1000</td>
<td>Tyramide 488</td>
</tr>
<tr>
<td>SMA</td>
<td>Sigma</td>
<td>1:50</td>
<td>Goat antirabbit Alexa 546</td>
</tr>
<tr>
<td>AR</td>
<td>Santa Cruz</td>
<td>1:100</td>
<td>Bond automated polymer system</td>
</tr>
<tr>
<td>GFP</td>
<td>Abcam</td>
<td>1:1000</td>
<td>Bond automated polymer system</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>Sigma</td>
<td>1:4000</td>
<td>Bond automated polymer system</td>
</tr>
<tr>
<td>SMA</td>
<td>Sigma</td>
<td>1:1000</td>
<td>Bond automated polymer system</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>BD Bioscience</td>
<td>1:400</td>
<td>Bond automated polymer system</td>
</tr>
<tr>
<td>Sox9</td>
<td>Chemicon</td>
<td>1:4000</td>
<td>Bond automated polymer system</td>
</tr>
<tr>
<td>p63</td>
<td>Santa Cruz</td>
<td>1:1200</td>
<td>Bond automated polymer system</td>
</tr>
</tbody>
</table>

Table 2. Taqman primer details

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 18 (CK18)</td>
<td>agatgacaccaacatcacaaggg</td>
<td>cttccagacacttggacactcttct</td>
</tr>
<tr>
<td>Sox9</td>
<td>cagcaagacttgccggtaag</td>
<td>tccgacagaggtctcttcttc</td>
</tr>
<tr>
<td>Nkx3.1</td>
<td>cagctgactgagccgggtccagagg</td>
<td>aaactctgatagcggagaggaagg</td>
</tr>
<tr>
<td>Pten</td>
<td>accttcctgcctgctggtggccactg</td>
<td>cttgatctgcctgctgccgacatt</td>
</tr>
<tr>
<td>Cytokeratin 14 (CK14)</td>
<td>ggcctccgagaggcgtctggtgac</td>
<td>gacgagaagagctggaagagaa</td>
</tr>
<tr>
<td>Desmin</td>
<td>gcctctacccgtcagacttcgta</td>
<td>agttggaggtttggtgagtttccac</td>
</tr>
<tr>
<td>Smooth muscle actin (SMA)</td>
<td>accttcctgcctgctggtggccactg</td>
<td>gcctggaagacttgcgtggctggtgagattgagga</td>
</tr>
<tr>
<td>Probasin</td>
<td>ggtgctctctctgtggttcctttc</td>
<td>atgctggaggttcctttccgccttctcttcc</td>
</tr>
<tr>
<td>Spermine binding protein (SBP)</td>
<td>cctttcttccagggcagtcttcagggta</td>
<td>gcaagacagaggtctggtgtaagga</td>
</tr>
<tr>
<td>Defensin β2 (Pros Def)</td>
<td>gccttgacagagaggtctggtgtaagga</td>
<td>agttggaggtttggtgagtttccac</td>
</tr>
<tr>
<td>Serine peptidase inhibitor Kazal type 3 (Spink3)</td>
<td>gccttgacagagaggtctggtgtaagga</td>
<td>gccttgacagagaggtctggtgtaagga</td>
</tr>
<tr>
<td>Cyclooxygenase 1 (Cox-1)</td>
<td>gccttgacagagaggtctggtgtaagga</td>
<td>gccttgacagagaggtctggtgtaagga</td>
</tr>
</tbody>
</table>

Endocrinology, September 2011, 152(9):3541–3551 endo.endojournals.org 3543
from all cells (Fig. 1B). Immunohistochemistry demonstrated that AR was absent in most SM cells in KO prostates (identified by SM actin expression) (Fig. 1C). AR continued to be expressed in the prostate epithelial and non-SM stromal cells in KO as in controls (Fig. 1C). Deletion of AR can be identified in the PTM-ARKO urogenital sinus from e 17.5 (24). Serum T and E2 concentrations are normal at all ages, based on measurements in a total of 93 mice measured in five different assays (23, 24). External sexual development was normal in these males (23, 24), and their reproductive tracts formed normally.

**Prostate weight, morphology, and histology**

AP, DLP, and VP weights were all found to be reduced at d 200 (Fig. 2A) in KO males. Because the Bar Harbor consensus report (26), states that no mouse prostate lobe is more relevant to human prostate disease then another, further detailed examination was focused upon the ventral prostate as a representative lobe. Further investigation revealed that VP weights were significantly reduced at d 50 and d 100, but not at d 35, compared with controls (Fig. 2B). Note that there was no significant reduction in VP weight in adult males expressing either MH Cre or AR<sup>fl</sup> alone compared with MH Cre-negative, AR<sup>fl</sup>-negative males ($P = 1.0$ and $P = 0.73$, respectively); this demonstrates that the phenotype observed in the KO animals results from AR ablation rather than from expression of Cre recombinase or AR<sup>fl</sup> per se. Histological abnormalities and atrophy were seen in the stromal and epithelial cells in adult KO VP, but not in controls (Fig. 2C and Supplemental Fig. 1A published on The Endocrine Society’s Journals web site at http://end.endojournals.org). The phenotype of individual acini varied within each KO VP, as in controls, but all KO VPs were similarly affected, displaying signs of elongated epithelial folding, epithelial cell hypertrophy and intracellular edema, and diffuse epithelial cell hyperplasia leading to overcrowding and desquamation (d 100 in Figs. 2C and 3B and d 35 in Supplemental Fig. 1A). This hyperplasia was confirmed quantitatively at d 100 (Fig. 3A) but not at d 35 (Supplemental Fig. 1B). Epithelial cell height was unchanged in d 100 KO VP compared with controls (Fig. 3B) but lumens were smaller in KO VP (d 100 in Fig. 2C and d 35 in Supplemental Fig. 1); this was confirmed quantitatively at d 100 (Fig. 3A) but not at d 35 (Supplemental Fig. 1B). Stromal fibrosis was observed in d 100 KO VP, characterized by eosinophilic staining around fibroblasts (Fig. 2C). Diffuse stromal hyperplasia was evident in KO VP (d 100 in Fig. 2C and d 35 in Supplemental Fig. 1) and was confirmed quantitatively at d 100 (Fig. 3A) but not at d 35 (Supplemental Fig. 1B); this was mostly composed of fibroblasts and immune cells. However, the proportion of SM in KO VP was unchanged compared with controls at d 100 (Fig. 3, A and C) and d 35 (Supplemental Fig. 1B). Considerable infiltration of neutrophils, leukocytes, and monocytes was observed in the glands and stroma of most KO VP at d 35 and d 100 (Supplemental Fig. 1, asterisk; and Fig. 2C). The epithelial mitotic index did not differ significantly in KO VPs at d100 compared with controls (data not shown) and few apoptotic cells were identified in KO or control VP.
Expression of VP cell identity markers

E-cadherin was expressed in all luminal epithelial cells in control VP at d 100 whereas in KO VP, E-cadherin expression was absent from some acini (Fig. 4 A). Sox9 was expressed in luminal and basal epithelial cells in d 100 control VP but was absent from some luminal epithelial cells in d 100 KO VP (Fig. 4B). p63 was expressed in basal epithelial cells in control and KO VP at d 35 and d 100 (Fig. 4, C and D), but significantly fewer p63 positive cells were evident in KO VP at d 100 (Fig. 4, D and E) but not at d 35 (Fig. 4C). Similarly, a significant reduction in relative mRNA expression of luminal epithelial cell markers cytokeratin 18 (CK18), Sox9, Nkx3.1, and Pten and the basal epithelial cell marker cytokeratin 14 was found in d 100 KO VP compared with controls (Fig. 4F). Relative mRNA expression of the SM cell genes desmin and SMA was also significantly reduced in d 100 KO VP (Fig. 4F). Notably there was no change in expression of any of these markers at d 35 (Supplemental Fig. 1C). Relative mRNA expression of ERα or β was similar in d 100 KO and control VP (data not shown).

Expression of genes encoding epithelial secretory proteins

mRNA expression of Defensin β2 (Pros Def), serine peptidase inhibitor Kazal type 3 (Spink3), and Cyclooxygenase 1 (Cox-1) was significantly reduced in d 100 KO VP, compared with controls (Fig. 4F); conversely, Probasin and spermine-binding protein (SBP) mRNA expression was unchanged (Fig. 4F). Expression of these genes was not altered in KO VP at d 35 (Supplemental Fig. 1C).

FIG. 2. Gross morphology and histology of PTM-ARKO prostates. A, Quantification of prostate lobe weights at d 200. B, Quantification of VP weight in control and KO mice at d 35–d 200. Values are means ± SEM (n = 3–14 mice). *, P < 0.05; **, P < 0.01, compared with controls. C, Hematoxylin and eosin (H&E) staining of d 100 KO and control VP. Histological abnormalities can be seen in KO VP with epithelial cells (E) detaching from the basement membrane (*) and sloughing off into the lumens (L). Hyperplasia of the stroma (S) is also obvious (arrow).
Effect of treatment with exogenous T and E2

To assess the prostate response to estrogens without causing involution, we treated adult mice with exogenous T plus estradiol (T+E2) for 13 wk, as previously described (24). All comparisons were made to age-matched control and KO males treated with ‘empty’ sham implants for 13 wk. T+E2 treatment resulted in normal serum T concentrations and 400-fold increased serum E2 concentrations, compared with untreated mice (24). VP recovered from T+E2-treated controls and KO displayed a significant increase in weight compared with VP from untreated males. Interestingly, VP from T+E2-treated KO males increased by 213% and became significantly larger than VP from T+E2-treated control males, which only increased by 25% (Fig. 5A). All T+E2-treated KO males developed marked urinary retention, resulting in two deaths; no T+E2-treated controls developed urinary retention. VP from T+E2-treated control and KO mice both displayed stromal and epithelial hyperplasia (Fig. 5B) but this was more pronounced in KO. Epithelial hypertrophy was also seen in T+E2-treated control and KO VP, but this was more dramatic in KO. Squamous metaplasia was seen in T+E2-treated control and KO VP (Fig. 5B).

Prostatic response to castration

Seven days after castration, VP weight was reduced by 63% in control males and by 51% in KOs, resulting in similar sized VP in both controls (0.0035 g ± 0.004) and KO (0.0028 g ± 0.004).

Discussion

It is well established that stromal androgen action is vital for normal prostate development, but its role in adult prostate homeostasis, and thus prostatic diseases such as PCa and BPH, is
less clear. Furthermore, exactly how and via which stromal cell type androgens act has also remained unclear. The current study investigated the role for SM androgen action in mediating normal adult prostate function, cell identity, and homeostasis using mice in which prostatic SM AR was ablated. These mice had significantly smaller prostates in adulthood, which exhibited histological abnormalities, altered gene expression, inflammation, and fibrosis. These changes could not be explained by changes in serum T or E2 concentrations. Furthermore, KO prostates displayed exaggerated epithelial cell proliferation in response to high concentrations of exogenous E2, resulting in urinary retention in all KO. These results demonstrate that AR signaling via the SM is vital in mediating normal androgen-driven stromal-epithelial interactions in the adult prostate, determining prostate homeostasis, cell identity, and function, and for limiting hormone-dependent prostatic-epithelial cell proliferation. These changes may provide some explanation for the role for SM androgen action in facilitating prostate disease development and/or progression and thus highlights androgen-dependent SM signaling as a possible new therapeutic target in prostate disease.

We have previously shown that AR is ablated from SM cells of the male reproductive tract from e 17.5 in KO mice (23, 24). As with the SV (24), this model offers a unique opportunity to investigate androgen action in postnatal SM cells, without preventing prostate formation or its prepubertal growth. Absence of any effect on formation is probably due to Cre recombinase not being expressed until after the prostate has already been programmed to form (29). Furthermore, serum hormone concentrations and AR expression in prostatic non-SM cells were normal in these KO, so any prostatic phenotype must result from ablation of SM AR. Control prostates greatly increase in weight between d 35 and d 200 but PTM-ARKO prostates did not, resulting in adult KO prostates being approximately 50–60% of normal adult weight. In contrast, ablation of epithelial AR (PEARKO mice) only caused a 20% reduction in prostate weight (19). Interestingly, castration of PTM-ARKO resulted in a smaller reduction in prostate weight than in controls. This is consistent with tissue recombination studies, which suggested that stromal, not epithelial, AR is important in mediating the castration response (30). Together these studies show that SM AR is essential for normal androgen-driven growth of the postpubertal prostate, with epithelial AR appearing to be less critical.
The adult prostate is normally growth quiescent, despite high androgen concentrations (7), but growth can be reactivated via unknown mechanisms in prostate disease leading to enlarged prostates and urinary retention. Tissue recombination studies indicate that epithelial cell proliferation depends on stromal, rather than epithelial, androgen action (30). Interestingly, epithelial cell proliferation was not significantly altered in our KO VP, suggesting that SM AR does not regulate epithelial cell proliferation under normal hormonal conditions. Estrogens have also been shown to stimulate prostate cell proliferation with prostates normally expressing both AR and ER (17, 18, 31); therefore, we tested whether exogenous estrogens could elicit the same response in the absence of SM AR. Mice were treated with exogenous T/H11001E2, which resulted in normal serum T but hugely elevated serum E2 concentrations compared with untreated control or KO mice (24). This regime increased prostate weight in both controls and KO, demonstrating that lack of SM AR does not prevent E2-driven prostate growth. However, the response was considerably enhanced in KO (213% increase in VP size) compared with controls (25% increase), such that KO VP became significantly larger than T+E2-treated control VP. This is opposite to the SV response to E2 in KO, because E2 increased control, but not KO, SV size (24). The T+E2-induced increase in VP size was associated with pronounced urinary retention in all treated KO males. This suggests that SM AR may normally limit adult prostate proliferation and growth and that, in its absence, prostate homeostasis and cell numbers are poorly controlled if the hormonal environment is altered. Interestingly, SM AR expression is often lost in men with aggressive metastatic PCa (32) whereas circulating estrogen concentrations are either stable or slightly increased in aging men whereas T concentrations fall, leading to an androgen-estrogen imbalance similar to, although not as pronounced as, that induced by our T+E2 treatments. Our present findings suggest that this hormonal imbalance and loss of SM AR expression could permit uncontrolled prostate cell proliferation and so facilitate the development and/or progression of prostate disease in aging men. This is not surprising given that stromal AR is suggested to regulate epithelial cell proliferation and apoptosis (7, 30). Loss of SM AR may thus be one of the ways in which the epithelium is released from its normal tight control by the stroma and so allows PCa to proceed. This is in agreement with the hypothesis that SM-epithelial interactions are perturbed in PCa, resulting in adverse consequences for both compartments and disease progression (33). PTM-ARKO mice provide a new tool with which to investigate this and its mechanisms.

Despite the lack of increased epithelial cell proliferation in untreated adult PTM-ARKO prostates, apparent stromal and epithelial overcrowding or hyperplasia was observed at d 35 and increased with age. This suggests that the epithelial hyperplasia in KO VP reflects similar cell numbers in a smaller space rather than increased proliferation per se. This results in an increased proportion of epithelial and stromal cells and increased infolding, overcrowding, and desquamation of the epithelium in adult KO prostates, suggesting that SM AR is important for maintaining normal adult prostate structure and homeostasis. Furthermore, we also demonstrated that SM AR is important in maintaining adult prostate cell identity because expression of several cell identity markers was
reduced in KO prostates in adulthood, but not at d 35. This highlights a progressive prostatic phenotype in these mice, suggesting that absence of SM AR expression, directly or indirectly, leads to less differentiated prostates. This is similar to observations in cancer-associated reactive stroma in which SM-marker expression is reduced, associated with a poorer patient prognosis (34), and is consistent with studies showing that stromal androgen action determines and maintains the fate of the epithelium (7, 30).

Given these observations, it is not surprising that epithelial cell identity was also altered in PTM-ARKO prostates. For example, E-cadherin expression was lost from some acini in adult KO prostates. E-cadherin normally mediates cell adhesion in prostatic luminal epithelium and maintains epithelial differentiation and homeostasis (35). Aberrant or decreased expression of E-cadherin is associated with epithelial-mesenchymal transition (36) and larger more aggressive prostate tumors (37). The loss of E-cadherin from KO epithelial cells may explain the desquamation of epithelial cells and could be a direct consequence of the loss of SM AR or could be indirect due to the loss of SM identity. Epithelial stem cell markers Nkx3.1 and p63 were also both reduced in adult KO VP. Nkx3.1 is androgen dependent (38), regulates prostate epithelial cell differentiation, and marks a luminal stem cell that is castrate resistant (13), whereas p63 is expressed in basal stem cells. Our results suggest that SM AR might regulate expression of stem cell markers and/or the stem cell niche, possibly by altering the production of growth factors (39). Pten expression is also reduced in these mice, and studies in mice and humans have associated loss of Pten (40), Nkx3.1 (41), or p63 (42) with initiation of PCa; however, PCa per se was not identified in KO mice. Further investigations are required to understand the role of SM AR, and the associated changes, on PCa development and progression.

Abnormal infiltration of immune cells was seen in the stroma and glands of KO VP at both d 35 and in adulthood, but it is unclear whether this is a primary effect of the absence of SM AR or is secondary to the histological abnormalities observed in these mice. Some of the histological and gene expression changes discussed above may be induced indirectly by the inflammation seen in this model. For example, fibrosis was observed in the stromal compartment at d 100 in KO VP, which is a common consequence of inflammation and/or aberrant changes in growth factors (26). Inflammatory mediators secreted by stromal cells attract immune cells and induce proliferation of both the stroma and the epithelium (43); therefore, further investigation is required to identify how SM AR signaling drives prostate fibrosis and inflammation and their relationship to prostate homeostasis, gene expression, and disease.

Based on the histological and differentiation abnormalities discussed above, it is not surprising that VP lumen volume and epithelial cell-secretory function was impaired in adult KO. For example, expression of secretory genes Spink 3, prostate ß defensin 1, and Cox-1 in adult KO VP was reduced, whereas no change was seen in the mRNA expression of probasin or SBP. This suggests that prostate epithelial secretory function is not globally impaired in the KO mice but that secretion of specific products is affected. This may be an indirect effect of the epithelial cell abnormalities discussed above, such as loss of epithelial cell identity, or the inflammation observed in these mice because it is believed that epithelial, rather than stromal, AR mediates epithelial secretory function (44).

In conclusion, we have demonstrated an essential role in vivo for AR signaling via the SM cells in mediating normal androgen-driven stromal-epithelial interactions in the mouse adult prostate, determining cell identity and function and limiting hormone-dependent epithelial cell proliferation. These results differ from those recently reported by Yu et al. (45) who ablated AR from the SM of the AP and demonstrated reduced epithelial cell proliferation and infolding and little effect on differentiation. These differences in effects are most likely due to the use of different Cre and AR flox models; for example, the SM22-Cre line used by Yu et al. appears to target only the AP whereas we show that the MHC-Cre targets all prostate lobes. The PTM-ARKO mouse model therefore provides a unique tool for identifying the underlying molecular mechanisms of SM androgen action in the prostate without preventing prostate formation. Further investigations with these mice could provide new insight into the role for SM AR in human adult-onset prostate diseases.

Acknowledgments

We thank Karel De Gendt and Guido Verhoeven (Leuven Catholic University, Leuven, Belgium) for providing the AR flox mice and Michael Kotlikoff (Cornell University, Ithaca, New York) for the smMHC-Cre mice. We thank Axel Thomson (University of Edinburgh, Edinburgh, UK) for advice with the hormone treatments and Mark Fisken (University of Edinburgh, Edinburgh, UK) and the members of the imaging facility and assay laboratory for technical assistance.

Address all correspondence and requests for reprints to: Dr. Michelle Welsh, University of Edinburgh, MRC HRSU, QMRI, 47 Little France Crescent, Edinburgh, United Kingdom, EH16 4TJ. E-mail: m.welsh@beatson.gla.ac.uk.

This work was supported by the United Kingdom Medical Research Council.
Present address for M.W.: The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Glasgow G61 1BD, Scotland, United Kingdom.


References

7. Cunha GR, Hayward SW, Wang YZ 2002 Role of stroma in carci
nogenesis of the prostate. Differentiation 70:473–485
24. Gravalik D, Halvorsen OJ, Haukaas SA, Akslen LA 2007 A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent impor-

43. Begley LA, Kasina S, MacDonald J, Macoska JA 2008 The inflammatory microenvironment of the aging prostate facilitates cellular proliferation and hypertrophy. Cytokine 43:194–199
44. Donjacour AA, Cunha GR 1993 Assessment of prostatic protein secretion in tissue recombinants made of urogenital sinus mesenchyme and urothelium from normal or androgen-insensitive mice. Endocrinology 132:2342–2350

Become a member of The Endocrine Society and receive discounts on color and page charges as well as all the other benefits!
www.endo-society.org/join