Androgen sensitivity of prostate epithelium is enhanced by postnatal androgen receptor inactivation

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Submitted 9 January 2009; accepted in final form 13 April 2009

Simanainen U, McNamara K, Gao YR, Handelsman DJ. Androgen sensitivity of prostate epithelium is enhanced by postnatal androgen receptor inactivation. Am J Physiol Endocrinol Metab 296: E1335–E1343, 2009. First published April 14, 2009; doi:10.1152/ajpendo.00017.2009.—Postnatal inactivation of epithelial androgen receptor (AR) in prostate epithelial AR knockout (PEARKO) mice results in hindered differentiation but enhanced proliferation of epithelial cells. As this resembles the precancerous proliferative atrophy of human prostates with undifferentiated but intensively replicating epithelial cells, we utilized the PEARKO mice to characterize the epithelial response to castration-induced involution with a focus on identifying the potential role of stromal AR and responsiveness of the androgen-deprived epithelia to the aromatizable androgen testosterone (T) or its nonaromatizable metabolite dihydrotestosterone (DHT). PEARKO and littermate control mice were orchidectomized at 8 wk of age and treated 2 wk later with subdermal implantation of 1-cm Silastic tubing filled with T or DHT for a week. Following castration, the prostatic involution and epithelial apoptosis did not significantly differ between control (intact AR) and PEARKO (only stromal AR) males, demonstrating that prostate epithelial involution following castration is mediated primarily via stromal AR-dependent apoptotic signals. Androgen replacement (T/DHT) for 7 days induced significant growth and epithelial proliferation in all prostate lobes in both control and PEARKO, but full regrowth was observed only in controls treated with T. In PEARKO, prostatic androgen (T and DHT) treatment induced significant epithelial cell “shedding” into the lumen, with T treatment resulting in acinar disorganization, cyst formation, and aberrant epithelial structures, described as a “gland within a gland.” These data suggest that epithelial AR inactivation during postnatal prostate development sensitizes prostate epithelial cells to paracrine signaling mediated by stromal AR activity leading to indirectly androgen-induced epithelial hyperproliferation and formation of epithelial hyperplastic cysts by aromatizable androgens.

ANDROGENS ARE INDISPENSABLE for prostate growth and functional differentiation (4), as the prostate does not develop without androgen receptor (AR)-mediated androgen action (11, 25). Following androgen ablation by chemical or surgical castration, the prostate undergoes intense apoptosis mainly in the epithelial cells, peaking at 3 days postcastration and resulting in major involution of the gland (14). Yet, androgen replacement following castration induces epithelial proliferation and redifferentiation resulting in regrowth of the prostate (35, 36).

At the tissue level, the mature prostate is composed of stroma and epithelia. Classical tissue recombination experiments proved that androgens induce mouse prostate development and epithelial proliferation indirectly via paracrine signals originating from activation of AR in the fetal stroma (mesenchyme), whereas epithelial AR activity is not required during fetal life (5). By contrast, in mature mice, castration causes regression of both prostate stroma and epithelium. We previously demonstrated that prostate epithelial AR plays a role in a postnatal prostate development and cellular maturatation. Using a Cre-LoxP system to create mice with AR inactivation selectively in the postnatal prostatic epithelium (30), these prostate epithelial AR knockout (PEARKO) males displayed diminished epithelial functional differentiation and atrophy despite a significant increase in epithelial proliferation. These atrophic, undifferentiated, and highly proliferative epithelia in the PEARKO males are comparable to the atrophic, intensively replicating epithelial lesions observed in early prostate cancer (6, 7, 10).

Natural prostate development as well as regrowth after castration share the requirement of androgens for morphological and functional development (4). Androgen action via the AR is also essential for development of late-life human prostatic pathologies like prostate cancer and benign prostate hyperplasia (13, 21). However, estrogenic action is also relevant, as prostatic abnormalities develop in aromatase-deficient mice (20, 27), and exposure to aromatizable androgens is required to develop experimental prostate cancer (9, 24). Prostate cancer responds to castration or chemical androgen ablation by extensive apoptosis of epithelial cells, but eventually emerges as hormone refractory prostate cancer.

Although the PEARKO model has given insight into cell- and tissue-specific AR action in the postnatal prostate, the specific mechanisms involving AR in prostate involution and androgen-mediated regrowth are not known. In the present study, we utilized the PEARKO mouse model with highly proliferative prostate epithelia to characterize the in vivo role of epithelial and stromal AR in the castration-induced involution of the prostate as well as in the acute prostate regrowth and epithelial proliferation after replacement with either aromatizable or nonaromatizable androgens.

MATERIALS AND METHODS

Mice. Prostate epithelia-specific exon 3 deletion of AR [Tg(Pbsn-cre × ARIEx3)], denoted PEARKO, were produced and genotyped as previously described (30). F1 generation of experimental PEARKO and littermate control males were used for all experiments. All animal procedures were approved by the Animal Welfare Committee of the Sydney South West Area Health Service within relevant National Health and Medical Research Council guidelines for animal experimentation.

Experimental design. At 8 wk of age, both control and PEARKO male mice were castrated or sham operated through scrotal incision under ketamine-zylazine anesthesia. Two weeks postoperation, the castrated males were implanted subdermally with Silastic tubing implants (id 1.47 mm, od 1.95 mm) filled with ~10 mg of crystalline DHT or T, whereas the sham-operated, intact males did not receive...
implants. The 1-cm T and DHT implants produce stable supraphysiological blood levels of T and DHT and restore sex accessory glands to normal weights when implanted into mice (31). The experiment was terminated at 3 wk postcastration/sham operation.

Sample collection. Mice were killed by cardiac exsanguination under ketamine-xylazine anesthesia. Serum was stored frozen at −20°C. Individual prostate lobes were dissected free of periprostatic fat and connective tissue and weighed separately. Separate lobes were fixed in Bouins solution for 4 h at room temperature for histology.

Histology and stereology. Serial 5-µm sections were cut from fixed, paraffin-embedded prostate lobes. Every 10th section was stained with hematoxylin and eosin. CASTGRID v. 1.10 (Olympus Alberta, Edmonton, Alberta, Canada) software generated counting frames and a point grid. Sections were mapped manually for tissue boundaries and sampling conducted at uniform random intervals along x- and y-axes. At least 100 counts per tissue compartment (stroma, lumen, and epithelia) were obtained. Point counts were combined to get a reference volume for each lobe, and relative volumes for each tissue compartment were determined. Absolute volume estimates were obtained by multiplying the relative volume of each compartment by the weight of the organ (20).

Detection of proliferation and apoptosis. a Cell proliferation was determined using proliferating cell nuclear antigen (PCNA) kit (Zymed, San Francisco, CA) according to the manufacturer’s instructions, including microwave-induced antigen retrieval in 10 mM citrate buffer, pH 6.0 for 15 min. For quantitative analysis of epithelial proliferating cell index, CASTGRID v. 1.10 stereological software was used to generate counting frames after tissue boundaries were manually mapped. Two random sections including distal and proximal ends of anterior lobe were stained and counted for PCNA negative and positive cells. Sampling was conducted at uniform random intervals along x- and y-axes. At least 300 cells were counted (30).

Apoptosis was detected by in situ detection of TUNEL staining of nuclear DNA break using an ApopTaq staining kit (Chemicon, Boronia, Australia). Analyses were performed on 5-µm-thick dewaxed paraffin sections.

RNA extraction and RT-PCR. Total RNA was extracted using a RNeasy Plus Mini Kit (Qiagen, Doncaster, Australia) and cDNA synthesized with Omniscript reverse transcriptase (Qiagen) from 250 ng of total RNA, respectively, using oligo(dT) (Invitrogen Australia Pty, Mount Waverley, Australia). Final reverse transcription reactions were diluted 1:5 for storage at −20°C (30).

Real-time RT-PCR. Quantitative real-time RT-PCR analyses for selected paracrine signaling factors Fgf7, Fgf10, Fgf receptor (Fgfr)1, Fgfr2, Igf-1, and Bmp7, as well as β-actin (housekeeping gene), were performed on cDNA using SABioscience’s RT2 Real-Time PCR kits (Jomar Bioscience Pty, SA, Australia) and a Rotor-Gene 2000 System (Corbett Research, Mortlake, Australia) as previously described (30).

Statistics. Statistical analysis for body and organ weights was performed using two-way analysis of variance (ANOVA) with treatment and genotype as the main factors and the least significant difference (LSD) method as a post hoc test. Three-way ANOVA, with lobe, treatment, and genotype and LSD method as a post hoc test was used for stereology data. Statistical analyses were performed using SPSS software (SPSS, Chicago, IL). In case of significant main factor interactions, the simple effects within the main factor were compared by one-way ANOVA with the LSD method as a post hoc test. P < 0.05 was considered statistically significant. Data are expressed as means ± SE unless otherwise specified.

RESULTS

Body weights and organ weights. Treatment affected body weight significantly (P < 0.001) by two-way ANOVA. Although body weights were comparable for intact control and PEARKO males (30 ± 0.3 g for control vs. 31 ± 0.4 g for PEARKO), castration significantly (P < 0.001) reduced body weights to 90 ± 1.8% of intact in control and to 90 ± 2.0% of intact in PEARKO to a similar extent (P = 0.29). Androgen replacement returned the body weight to the intact level in both control and PEARKO (data not shown), and there was no difference between effects of T and DHT (data not shown).

The weight of each prostate lobe depended on the genotype (P < 0.001) and treatment (P < 0.001), with significant (P < 0.001) interaction of these two factors by two-way ANOVA, indicating that effect of treatment on prostate lobe weight was highly dependent on the genotype. Within genotype or treatment, the intact sham-operated PEARKO males had significantly smaller anterior (AP; P < 0.001) and dorsolateral prostate (DLP; P < 0.001), but unchanged weight of ventral prostate (VP; P = 0.148) compared with intact controls (Fig. 1). Within a treatment, castration induced a significant (P < 0.001 for all lobes) reduction in weight of all prostate lobes compared with intact, resulting in comparable (P = 0.11) prostate weights in control and PEARKO (Fig. 1). Three weeks postcastration, the weights for AP, DLP, and VP in control

Fig. 1. Prostate weights (mg) in intact and castrated (21 days) prostate lobes of control (C, open bar) and prostate epithelial androgen receptor (AR) knockout (PEARKO, filled bars) male mice, demonstrating significantly reduced anterior (AP) and dorsolateral prostate (DLP) weights in intact PEARKO males compared with controls, whereas ventral prostate (VP) weight was unaffected. Castration significantly reduced prostate weights to comparable levels in control and PEARKO. Two-way ANOVA was used to assess effect of treatment (P < 0.001), genotype (P < 0.001), and interaction of treatment and genotype (P < 0.001). Values are means ± SE; n = 9 (control) and 7 (PEARKO) in intact and 9 for both control and PEARKO in castrated group. *Control and PEARKO values significantly different within treatment (ANOVA, P < 0.05); †significantly different (ANOVA, P < 0.05) from respective intact within genotype.
were 16 ± 1, 23 ± 1, and 14 ± 1% and in PEARKO 15 ± 3, 20 ± 2, and 17 ± 3% of intact control, respectively, as a fraction of precastration weights.

Androgen replacement (T/DHT) for 7 days induced significant (P < 0.001 for all lobes) regrowth as indicated by weight of all prostate lobes for both control and PEARKO. However, the magnitude of regrowth following T was dependent on the genotype, such that, while T induced nearly a full regrowth of all prostate lobes in control, only ~60% regrowth was observed in PEARKO compared with intact PEARKO (Fig. 2). DHT failed to restore the prostate weights to intact level in both control and PEARKO (Fig. 2).

Histological and stereological analysis of prostate. Examination of hematoxylin- and eosin-stained sections for intact AP, DLP, and VP in control mice demonstrated a glandular epithelial duct surrounded by loose stroma and the glandular lumen filled with eosinophilic secretion (Fig. 3). In contrast to control, the intact PEARKO males demonstrated disorganized epithelial layers (Fig. 3). Three weeks postcastration of mice, the prostate glandular epithelium was fully regressed, with flattening of the epithelial cells and loss of cytoplasm. The epithelium was mostly cuboidal, and the cellular density of stroma was increased compared with sham treated (Fig. 3). The AP of PEARKO males demonstrated dilated lumen of major duct with flattened epithelia that was not observed in control
males. The dilated lumen of AP in PEARKO was filled with thick, eosinophilic fluid.

The stereological analysis demonstrated significant changes in relative proportions of stroma, lumen, and epithelia (Table 1). Two-way ANOVA demonstrated that the volumes of stroma, lumen, and epithelia were significantly dependent on lobe ($P < 0.001$), genotype ($P < 0.05$), and treatment ($P < 0.001$). In addition, all three factors (lobe, genotype, and treatment) had significant ($P < 0.003$) interaction for relative epithelia, while genotype and treatment had significant ($P < 0.002$) interaction for relative stroma.

Within intact control and PEARKO males, relative volume (relative to stroma and lumen) of epithelia was increased in AP and DLP of PEARKO males compared with controls (Table 1). As a consequence, the increase in epithelial volume lead to significantly increased epithelial-to-stromal ratios in PEARKO males compared with controls (Fig. 4). The relative volumes of stroma, lumen, and epithelia in VP were similar between control and PEARKO (Table 1), leading to unchanged epithelial/stromal ratios (Fig. 4).

Table 1. Stereological comparison of stroma, lumen, and epithelial volumes of AP, DLP, and VP in intact and castrated (21 days) as well as castrated (14 days) and T- or DHT-treated (7 days) control and PEARKO males

<table>
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<th>Line</th>
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Main effects

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Values are means ± SE; $n = 4–5$ for control (CON) and prostate epithelial androgen receptor knockout (PEARKO) mice in all treatment groups. Volumes are presented as %total volume (100%) of prostate lobe. AP, anterior prostate; DLP, dorsolateral prostate; VP, ventral prostate; T, testosterone; DHT, dihydrotestosterone. Three-way ANOVA was used to assess main effect of lobe analyzed ($P < 0.001$), treatment ($P < 0.001$), and genotype ($P < 0.05$) and possible interactions among these factors. Significant interactions for main factors occurred for relative epithelia between all 3 factors ($P < 0.01$), whereas line and treatment had significant ($P < 0.002$) interaction for relative stroma. *CON and PEARKO volumes significantly ($P < 0.05$) different within treatment. †Volume after treatment significantly ($P < 0.05$) different from Sham volume within genotype.

Within a treatment, the castration reduced the relative volume of the epithelia and lumen and increased that of stroma (Table 1), resulting in significantly ($P < 0.05$) reduced epithelial/stromal ratios in each lobe of both control and PEARKO males (Fig. 4). In castrated males, the epithelial/stromal ratios did not differ between control and PEARKO males for each lobe (Fig. 4).

Following androgen replacement (T and DHT) in the control prostate, the epithelial layers became organized, and the epithelium was characterized by predominantly columnar luminal cells (Fig. 3). By contrast, in PEARKO males, androgen treatment induced significant epithelial cell “shedding” into the lumen (Fig. 5). The shedding was more pronounced after T treatment and resulted in acinar disorganization and cyst formation (Fig. 5). Furthermore, abnormal epithelial structures, resembling a “gland within a gland,” were detected in DLP of PEAR KO males following T but not DHT treatment (Figs. 5 and 6). The inner epithelial layer was mainly separated from the outer epithelial layer but remained connected to the outer epithelia by a connective “bridge” (Figs. 5 and 6). Approximately one-half of the cells in both the outer and inner layers of the glandular

Fig. 4. Prostate stromal-to-epithelial ratio (stromal and epithelial volumes analyzed by stereology) in intact, castrated (21 days) or control (C; open bar) and PEARKO (P; filled bars) males after castration (14 days) and T or DHT treatment (7 days). Three-way ANOVA was used to assess effect of lobe analyzed ($P < 0.001$), treatment ($P < 0.001$), and genotype ($P < 0.01$) and the interaction of these main factors: lobe, genotype, and treatment ($P < 0.05$); $n = 4–5$ for both control and PEARKO values, different within treatment (ANOVA, $P < 0.05$); *significantly different (ANOVA, $P < 0.05$) from respective intact within genotype.
Fig. 5. Comparison of prostate lobe AP (A–E), DLP (F–J), and VP (K–O) histology of DHT- and T-treated (castration for 14 days and androgen treatment for 7 days), control, and PEARKO males. In controls (A, B, F, G, K, L; ×10 magnification), epithelial layers became organized, and epithelium was characterized by predominantly columnar luminal cells compared with castrated (Fig. 3). In PEARKO, androgen (T and DHT) treatment induced significant epithelial cell “shedding” into the lumen ([C, D, M, N; ×10], and E, and O [×20]; arrows), resulting in severe acinar disorganization and cyst formation following T treatment. T treatment induced abnormal hyperplastic epithelial proliferation resulting in “gland-within-a-gland” structures [I [×10], J [×20]; arrows].
epithelia were PCNA positive, demonstrating that the cells were in G1 and G/S phases of cell cycle proliferating.

Within the androgen treatments, both DHT and T failed to restore the relative volumes of stroma, lumen, and epithelia from castrated level to the intact level in AP and DLP of PEARKO males, whereas the intact relative volumes were restored by androgens in control males (Table 1 and Fig. 4). In PEARKO, the relative volume of stroma remained significantly (P < 0.05) higher, and the volumes of epithelia and lumen were significantly (P < 0.05) lower than the respective intact volumes (Table 1). Although the epithelial/stromal ratios in AP and DLP of PEARKO males were slightly increased from the castrated level, the ratio remained significantly (P < 0.01) lower compared with intact PEARKO (Fig. 4). Both T and DHT restored the relative volumes of stroma, lumen, and epithelia in VP of both control and PEARKO (Table 1), leading to intact levels of stromal to epithelial ratios (Fig. 4).

**Epithelial proliferation and apoptosis.** The AP was studied in further detail with analysis of PCNA and Tunel positivity to examine the role of epithelial proliferation and apoptosis in androgen replacement-induced restoration of the prostate morphology and the unusual formation of epithelial acinar cysts in PEARKO males.

Figure 7 shows representative examples of PCNA-positive staining of AP in intact animals as well as from DHT- and T-treated control and PEARKO males. As before, in intact PEARKO males, the epithelial proliferation was significantly increased in AP compared with control.

Following 7 days of androgen replacement, both T and DHT treatment activated prostate epithelial proliferation in control and PEARKO males (Fig. 7) compared with androgen-ablated tissue (data not shown). However, epithelial proliferation remained significantly higher in androgen-treated PEARKO epithelia compared with androgen-treated control (Fig. 7). Two-way ANOVA demonstrated that the epithelial proliferation was significantly dependent on genotype (P < 0.01) and treatment (P < 0.001).

Figure 8 shows representative examples of in situ TUNEL staining of nuclear DNA breaks in AP of intact control and PEARKO males. TUNEL staining demonstrated very low levels of apoptosis in the intact AP of both control and PEARKO males (Fig. 8). The level of apoptosis increased in both control and PEARKO following castration for 3 wk. Similarly to intact mice, only a few apoptotic cells were detected in epithelia following androgen (DHT/T) treatment (data not shown).

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**Fig. 6.** Representative photo of hematoxylin and eosin (H&E)-stained (A) epithelial gland-within-a-gland structure detected in DLP of PEARKO males after castration (14 days) and following T treatment (7 days). Inner epithelial cells were still proliferating, as demonstrated by positivity to proliferating cell nuclear antigen [proliferating cell nuclear antigen (PCNA; B)].

**Fig. 7.** PCNA immunostaining was performed to detect proliferative cells in AP of control (A, B, C) and PEARKO (D, E, F) of intact (A, D) or castrated (14 days) and DHT- (B, E) or T- (C, F) treated males. PEARKO males demonstrated significantly increased proliferation in prostate of intact males but also after androgen treatment. Arrowheads point to PCNA-positive epithelial cells. G: percentage of epithelial cells staining positive for PCNA in AP (proximal and distal ends included) as analyzed by stereology. Two-way ANOVA demonstrated that epithelial proliferation was significantly dependent on genotype (P < 0.01) and treatment (P < 0.001). Values are means ± SE; n = 4. *Control and PEARKO values significantly different within treatment (ANOVA, P < 0.05); †significantly different (ANOVA, P < 0.05) from respective intact within genotype.

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**Fig. 8.** Representative photo of in situ TUNEL staining of epithelial gland-within-a-gland structure detected in DLP of PEARKO males after castration (14 days) and following T treatment (7 days). Inner epithelial cells were still proliferating, as demonstrated by positivity to proliferating cell nuclear antigen [proliferating cell nuclear antigen (PCNA; B)].
mRNA expression. Gene expression analysis of selected paracrine signaling factors demonstrated that the mRNA expression of Bmp7 and Igf-1 were significantly dependent on genotype (two-way ANOVA, \( P < 0.05 \)) and treatment (two-way ANOVA, \( P < 0.001 \)) (Fig. 9). Expressions of Fgf7, Fgf10, Fgfr1, and Fgfr2 were not significantly affected by genotype or treatment (two-way ANOVA, \( P > 0.05 \); data not shown).

**DISCUSSION**

Prostate diseases like benign prostatic hyperplasia and prostate cancer are androgen-dependent diseases (3, 16), and the most widely used treatment for prostate cancer involves chemical or surgical castration to withdraw androgens (33). In an endeavor to identify the mechanisms of androgen-dependent prostate diseases and to enable the development of better targeted therapies, it is necessary to understand the cell-specific mechanisms of androgen action in the prostate.

In the present work, we utilized the PEARKO model of prostate epithelial androgen deficiency (30) to examine the specific role of epithelial AR in prostate involution after castration and regrowth after androgen replacement. The PEARKO prostate exhibits undifferentiated and atrophic but highly proliferative epithelia (30) that resembles the atrophic but proliferative lesions frequently observed in early prostate cancers (10). Furthermore, as previous research suggests that aromatizable androgens are required to induce epithelial pathogenesis in rodents (9, 24), both aromatizable T and its nonaromatizable metabolite DHT were used. We demonstrated that postnatal inactivation of prostate epithelial androgen action and thereby epithelial differentiation influences that organ's structural response to castration induced involution as well as its regeneration after androgen replacement. In addition, the loss and/or disruption of normal epithelial differentiation due to epithelial AR inactivation in PEARKO prostates sensitizes the prostate epithelium to acute T-induced epithelial hyperplasia and formation of cystic lesions.

We demonstrated that castration and inactivation of AR-dependent signaling in both stromal and epithelial compartments of prostate resulted in significant epithelial apoptosis and drastic involution of the prostate in both control and PEARKO males. While this signifies that the stromal AR still has a major and persisting influence on the intact PEARKO prostate, it provides the first in vivo evidence that androgen-dependent epithelial apoptosis after castration is mediated via stromal-mediated, AR-dependent apoptotic signals. This is consistent with and extends to postnatal life the finding in ex vivo tissue...
recombination experiments where epithelial apoptosis occurred in recombinants with fetal wild-type stroma and tfm epithelia (lacking AR) grown under the kidney capsule of nude mice (17). However, the results demonstrate a difference in hormonal regulation of AP between rat and mouse, because a low level of apoptosis (vs control) was detected in rat epithelial cells of AP upon castration for 3 days (1, 2), whereas we clearly demonstrated an increased level of apoptosis in mouse AP epithelial cells 3 wk after castration.

Despite the epithelial AR inactivation, the postnatal prostate structural development and branching morphogenesis in PEARKO males was normal (30). However, after 3 wk of castration, histological findings in the AP of PEARKO demonstrated not only significantly reduced epithelial infolding and dilation of central lumen, but a dilated lumen filled with thick eosinophilic fluid and lined with low cuboidal epithelia. As the secretary activity of the prostate is highly androgen dependent, (4) and dilatation of lumen was largely confined to the main ducts of AP, it is interpreted that the fluid may not be a normal secretary product of epithelial cells, but the mechanism of this increase in fluid accumulation is not clear. However, this suggests that defects of prostate epithelial and structural development in PEARKO males could change the prostate response to castration via abnormal epithelial to stromal signaling. Analysis of suggested paracrine signaling factors (19, 26), Bmp7 and Igf-1 demonstrated differences in AP of control and PEARKO males, serving as possible candidates for the affected epithelial-to-stromal signaling. Expression of FGF7, FGF10, or FGF receptors 1 and 2 was similar between control and PEARKO, demonstrating that the stromal-to-epithelial FGF signaling remains intact in the PEARKO prostate. In addition, candidates like Wnt and Sonic hedgehog signaling are suggested to be involved in the stromal and epithelial communication during prostate development and androgen-induced regrowth following castration (8, 19, 29, 34) and serve as other possible candidates to be further analyzed in the present model.

We observed that in castrated control mice, a supraphysiological dose of T restored prostate weight and morphology more completely that did a supraphysiological dose of DHT, consistent with previous reports (35, 36). Although the main circulating androgen is T, which can bind directly to AR and induce androgenic response (12), T is reduced to the more potent androgen DHT via the 5α-reductase pathway within the prostate (15), representing an androgen amplification mechanism within the prostate. By contrast, DHT is not able to undergo further amplification of androgenic potency (18). However, either T or DHT was unable to restore the prostate weight in PEARKO males despite the significantly higher level of epithelial cell proliferation in PEARKO prostate. This led us to hypothesize that it could be due to increased prostate epithelial apoptosis in PEARKO males. In contrast, we demonstrated that epithelial apoptosis is very low in AP of both control and PEARKO after androgen replacement. In contrast, significantly elevated epithelial proliferation in androgen-treated PEARKO males resulted in increased epithelial cell shedding. Furthermore, formation of abnormal cystic foci was detected in PEARKO prostates following castration and T replacement. Similar epithelial shedding and acinar disorganization were reported in prostate of Stat5 knockout mice (23). Stat5 transcription factors are involved in proliferation, differentiation, and oncogenesis (37), and it was recently demonstrated that liganded AR enhances nuclear accumulation of Stat5 by direct physical interaction of AR and Stat5α/β proteins (32). Therefore, it is hypothesized that the mutated AR in our model is incapable of interactions with Stat5 and thereby reduces the Stat5 activity, leading to the similar phenotype observed in Stat5 knockout mice. Furthermore, the results suggest that the cystic formation is influenced by estrogenic signaling, as the cysts were more prominent following T treatment with potential estrogenic action than upon DHT treatment.

Cystic regeneration of glandular epithelia is frequently evident in benign prostate hyperplasia (22). Furthermore, the T treatment in DLP of PEARKO males induced hyperplastic formation of “gland within a gland,” which is also associated with hyperplasia (22). These results suggest that inactivation of epithelial AR increases prostate susceptibility to acute T-induced epithelial hyperplasia and could, at least partly, explain the inability to restore the epithelial volume in PEARKO males. This increased susceptibility to T-induced epithelial hyperplasia is in agreement with a previous report demonstrating that delay in postnatal prostate maturation sensitizes mouse prostate to epithelial abnormalities (28). Therefore, this PEARKO prostate model of epithelial AR deficiency serves as a model to analyze the acute response of epithelial cells to androgenic induction of prostate epithelial hyperplasia.

In conclusion, our results provide the first in vivo evidence of stromal AR-dependent apoptotic signaling in the mature prostate and suggest that postnatal inactivation of epithelial AR, leading to abnormal prostate differentiation, modifies the prostate response to castration-induced structural involution as well as androgen-induced restoration of the prostate. However, further studies are warranted to elucidate whether these defects are due to cellular changes during castration or are due to changes in epithelial signaling in the mature prostate. We also demonstrated that epithelial AR inactivation during postnatal prostate development sensitizes prostate epithelial cells to androgen-induced epithelial hyperproliferation and formation of epithelial hyperplastic cysts. These mechanisms are suggested to be attributable to the persistence of paracrine signaling mediated by stromal AR. It is emphasized that identification of the specific mechanisms involved in prostate response to androgens after castration may allow us to devise new strategies to impede abnormal prostate growth and especially the androgen-independent growth of the prostate cancer.

ACKNOWLEDGMENTS

We thank Dr. Fen Wang for the supply of the Probasin Cre [Tg(Pbsn-cre)] mice and Dr. Jeffrey Zajac for collaboration in providing the ARflox mice.

GRANTS AND FELLOWSHIPS

This study was supported by a Cancer Institute NSW Early Career Fellowship (U. Simanainen), the University of Sydney Cancer Fund, and the National Health and Medical Research Council.

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