Androgen Receptor Roles in the Development of Benign Prostate Hyperplasia

Kouji Izumi,* Atsushi Mizokami,† Wen-Jye Lin,* Kuo-Pao Lai,* and Chawnshang Chang*†

From the George H. Whipple Laboratory for Cancer Research,* and the Departments of Pathology and Laboratory Medicine,† Urology,‡ and Radiation Oncology (Wilmot Cancer Center),§ University of Rochester Medical Center, Rochester, New York; the Department of Integrative Cancer Therapy and Urology,∥ Kanazawa University, Kanazawa, Japan; and the Sex Hormone Research Center,¶ China Medical University and Hospital, Taichung, Taiwan

Benign prostate hyperplasia (BPH) is the most common male benign proliferative disease, and approximately 8 million patients are estimated to visit physicians with the diagnosis of primary or secondary BPH.¹ The incidence of gross enlargement of the prostate gland has been reported as 40% in 70-year-old men, and microscopic foci of the prostate gland are present in up to 80% of these men.² BPH patients often have lower urinary tract symptoms, and need to be treated with surgery or medication. Although transurethral resection of the prostate is the most common surgical treatment for BPH worldwide, the procedure can lead to complications (e.g., bleeding, urethral stricture, incontinence) and may have limitations for people of advanced age.³⁻⁵ Although α-blockers are frequently prescribed for treatment of BPH and have a quick onset of action, within 3 to 5 days,⁶ these drugs alone fail to shrink BPH volume and are often insufficient to eliminate symptoms.⁷ In contrast, 5-α reductase inhibitors (5-ARIs), which suppress testosterone conversion into dihydrotestosterone (DHT), have greater efficacy in reducing BPH volume, and clinical data indicate that the combination of 5-ARIs with α-blockers leads to the best symptomatic response to date.⁸⁻⁹

The above clinical data suggest that androgen/androgen receptor (AR) signaling plays key roles in development of BPH and that targeting androgen/AR signaling could be a major therapeutic approach for BPH. Nevertheless, the...
detailed mechanisms of androgen/AR signaling, and especially the pathogenic roles of AR in BPH, are still unclear.

In this review, we focus on the roles of AR in promoting prostate stromal cell growth and prostate epithelial cell growth with increased epithelial—mesenchymal transition (EMT). We also discuss how AR regulates development of BPH through the inflammatory environment with macrophage infiltration, and address potential new therapeutic approaches, such as targeting AR with the newly identified AR degradation enhancer ASC-J9 and/or targeting specific inflammatory cytokines downstream of AR.

Clinical Evidence of Androgen/AR Roles in Development of BPH

Basic Evidence for Androgen/AR Roles Supporting Clinical Effects

The hormone dependency of development of BPH is well documented, and androgen concentration or AR activity in BPH patients has been a subject of study since 1895. One study in the 1980s found little difference in AR expression in prostate tissues isolated from either BPH patients or unaffected control subjects. However, the serum concentration of testosterone and DHT may change with age, with reduced serum testosterone in healthy old men, relative to that in healthy younger men. In contrast, DHT levels are elevated, and serum DHT in BPH patients is significantly higher than that in unaffected men at a similar age. Similar conclusions were also obtained recently in a large cross-sectional study that included 505 men aged 40 to 79 years (mean, 58 years); higher serum DHT levels and DHT/testosterone ratios were associated with larger prostate volume and higher prevalence of BPH.

Tang et al found no difference in AR expression between the peripheral zone (PZ) and the transitional zone (TZ) in BPH. In other studies, although nuclear AR expression was detected in both epithelial and stromal cells of hyperplastic nodules, higher nuclear AR expression was detected in prostate epithelial cells than in stromal cells. Others found higher 5-α reductase activity in stromal cells than in epithelial cells, with AR distributed evenly between epithelial and stromal cells. Importantly, the primitive BPH nodules found in the periurethral area of the TZ had higher concentrations of androgens and higher nuclear AR expression than those in other prostate regions. This finding suggests that androgen/AR signaling may play important roles in promoting the proliferation of epithelial and stromal cells in the periurethral area of the TZ, thus leading to development of BPH with urinary obstruction (Figure 1). Clinical Effects of Antiandrogens and 5-ARIs on BPH

Castrated BPH patients showed a marked decrease of prostatic volume after 2 to 3 months, and this volume reduction correlated well with a relief of lower urinary tract symptoms. A double-blind, randomized trial for BPH patients with the antiandrogen flutamide (750 mg/day for 6 months) showed significantly decreased prostate volume by 35%, compared with controls, within 6 months. However, these androgen-deprivation therapies with surgical or medical castration or with antiandrogens are no longer used as a standard treatment because of adverse reactions and have been replaced by the 5-ARIs, which have better efficacy in suppressing development of BPH. A study of BPH patients treated for 1 year with finasteride, a 5-ARI for 5-α reductase type 2, showed a significant decrease in the size of the periurethral area, as well as total prostate gland size, suggesting that suppression of testosterone conversion into DHT could lead to a significant decrease in the size of the prostate. The efficacy and safety profiles of finasteride in BPH patients over a 12-month period also showed a 74% reduction in serum DHT levels, a 21% reduction in prostate volume, and a significant improvement in urinary obstructive symptoms. However, finasteride treatment led to a 55% decrease of DHT in epithelial cells of the TZ, with little effect on stromal cells, even though 5-α reductase type 2 is expressed in both stromal and epithelial cells in the prostate. In contrast, another 5-ARI, dutasteride, inhibiting both type 2 and type 1 of 5-α reductases (the latter expressed predominantly in epithelial cells), showed a greater decrease in DHT (98.4 ± 1.2%) than did finasteride (70.8 ± 18.3%).

Taken together, these clinical studies suggest that androgen/AR may contribute to the increase of prostate volume, and 5-ARIs with suppression of testosterone conversion into DHT are effective medicines for reducing BPH size.

Limitations of Current 5-ARIs Targeting Testosterone Conversion into DHT for Treatment of BPH

Although 5-ARIs have been shown to reduce the volume of BPH, these may have some limitations. The Combination of Avodart and Tamsulosin (CombAT) study, with 4844 BPH patients, showed that combined therapy with both dutasteride and the α-blocker tamsulosin was significantly superior to either monotherapy in reducing the relative risk of BPH progression and symptoms. Later analysis, however, found that combined therapy was better than dutasteride monotherapy in men with prostate volumes of ≧30 to <58 mL, but not in men with a prostate volume of <30 or ≥58 mL. The benefit of combination therapy is thus greatest only in patients with larger BPH volumes, and α-blockers alone may be sufficient to alleviate lower urinary tract symptoms in patients with smaller BPH volumes; however, transurethral resection of the prostate may be needed for patients with the largest BPH volumes (approximately 60 mL). Importantly, 5-ARIs have been reported to produce significant adverse sexual reactions, including decreased libido, erectile dysfunction, or ejaculation problems. In addition, 5-ARIs may not be able to suppress completely the intraprostatic DHT in BPH patients, and the remaining DHT may still be able to transactivate AR and lead to development of BPH.
AR Roles in Promoting Prostate Epithelial Cell Growth with Enhanced EMT in Development of BPH

Epithelial AR Influences Cell Growth

Bello et al.\textsuperscript{43} using the human prostate epithelial cell line RWPE-1, found that addition of the synthetic androgen mibolerone increased AR expression level and enhanced cell growth, and Silva et al.\textsuperscript{44} found that DHT can promote human primary prostate epithelial cell growth. These \textit{in vitro} cell-line data suggest that androgen/AR signaling may play positive roles in promoting prostate epithelial cell proliferation.

Wu et al.\textsuperscript{45} generated conditional knockout AR mice that lack AR only in prostate epithelial cells (pes-ARKO) and found that the pes-ARKO mice developed larger but less differentiated prostate with increased cell death, compared with wild-type littermates. Further analyses of each individual cell type within the epithelium in pes-ARKO mice revealed that loss of epithelial AR increased the proportion of CK5\textsuperscript{+} basal epithelial cells but decreased the proportion of CK8\textsuperscript{+} luminal epithelial cells, suggesting that luminal epithelial AR may play survival roles and that basal epithelial AR may play suppressor roles in maintaining homeostasis of prostate epithelial cells.\textsuperscript{45} It would be interesting to learn whether AR in epithelial cells of human BPH has roles similar to those in the normal prostate.

Epithelial Cells Contribute to Development of BPH via Epithelial Cell—Stromal Cell Interaction

Clinical studies showing better efficacy of dutasteride than finasteride in suppressing BPH\textsuperscript{33,34} suggest that prostate epithelial cells may play positive roles in promoting development of BPH. In two studies from the 1990s, epithelial cell content was significantly greater in the TZ than in the PZ,\textsuperscript{46} and epithelial cells were only 9.0\% of BPH cells.\textsuperscript{47} However, epithelial cells may still play important roles in enhancing stromal cell growth via epithelial cell—stromal cell interaction. In coculture of primary human BPH stromal fibroblasts and epithelial cells, cell growth of cocultured cells was significantly increased, compared to separate culture,\textsuperscript{48} suggesting that epithelial cells may have a supportive role in the growth of stromal cells in development of BPH. On the other hand, stromal androgen/AR signaling may be able to enhance the expression and/or release of growth factors that act on epithelial cells.\textsuperscript{49,50} Thus, prostate epithelial and stromal cells may each support proliferation of the other cell type through growth factors in a paracrine manner.

Epithelial Cells Contribute to Increased Stromal Cell Population via EMT and So Contribute to Development of BPH

EMT is a physiological process in which epithelial cells acquire the motile characteristics of mesenchymal (stromal) cells.\textsuperscript{51} EMT is characterized by decrease of epithelial markers (eg, E-cadherin) and increase of mesenchymal and transcriptional factors (eg, N-cadherin, vimentin, and Snail).\textsuperscript{52} EMT is associated mainly with cancer progression, including migration, invasion, and metastasis. Cancer cells undergoing EMT may acquire invasive properties, entering the surrounding stromal cells and creating a favorable

---

**Figure 1** Schematic of prostate structure. Most cases of prostate cancer occur in the peripheral zone (PZ), but BPH is caused by enlargement of the transitional zone (TZ), especially in the periurethral area. The urethra penetrates the periurethral area, sandwiched between left and right adenoma and flattened in BPH.
microenvironment for cancer progression and metastasis. Interestingly, Alonso-Magdalena et al\textsuperscript{12} found that EMT may also contribute to development of BPH. Although they found little evidence of proliferation in clinical human BPH stromal cells, they found that 0.7% of basal cells and 0.4% of luminal cells were positive for the proliferation markers Ki-67 and PCNA in the epithelium of some ducts.\textsuperscript{12} Importantly, they found that regions of the ductal epithelium had lost their polarization and expressed little E-cadherin.\textsuperscript{12} Others reported that Snail2/Slug is the important transcriptional factor for TGF-\(\beta\)-1-induced EMT of prostate epithelial cells in BPH.\textsuperscript{54} These results suggest that EMT in epithelial cells may also promote development of BPH (Figure 2).

**AR Promotes Development of BPH by Influencing Infiltrating Immune Cell Migration toward Prostate Epithelial Cells**

Inflammation with infiltrating macrophages influences epithelial cells and may contribute to development of BPH. A study investigating the association between immune inflammation and AR expression in human BPH specimens from prostatectomy showed that the total prostate volume was significantly larger in specimens with infiltrated inflammatory cells including B or T lymphocytes than in those without infiltrated inflammatory cells, suggesting that the immune inflammatory process may also contribute to development of BPH.\textsuperscript{55} A recent study by Lu et al\textsuperscript{56} also showed higher expression of the human macrophage marker CD68 in human BPH specimens, further confirming that infiltrated immune cells may be able to contribute to development of BPH.

Using coculture of human BPH-1 epithelial cells with human monocyte/macrophage THP-1 cells, Lu et al\textsuperscript{56} found that BPH-1 cells have the capacity to recruit more THP-1 cells. They found that recruited THP-1 cells subsequently enhanced BPH-1 cell growth in a three-dimensional culture system, with increase of EMT in BPH-1 cells, and they demonstrated that increased TGF-\(\beta\)-2 expression in BPH-1 cells and the addition of TGF-\(\beta\) neutralizing antibody suppressed THP-1 macrophages-mediated cell growth and EMT in BPH-1 cells. Importantly, they found that addition of AR in BPH-1 cells induced EMT gene expression in BPH-1 cells and promoted THP-1 macrophage migration during coculture with THP-1 cells. Addition of the AR degradation enhancer ASC-J9 also suppressed THP-1 macrophage-mediated cell growth in stably transfected AR BPH-1 cells.\textsuperscript{56} Similar data were also obtained when human BPH-1/THP-1 cells were replaced with mouse epithelial mPrE cells and mouse macrophage RAW264.7 cells in their coculture system (Figure 3).\textsuperscript{56}

Taken together, these results demonstrate that epithelial AR may be able to enhance epithelial cell growth and EMT induction by influencing recruitment of infiltrating immune cells, which could then enhance development of BPH.

**AR Roles in Prostate Stromal Cell Growth That Promote Development of BPH**

**Stromal AR Contributes to Development of BPH**

It is well known that prostate cancer occurs predominantly in the PZ, whereas BPH typically develops in the TZ.\textsuperscript{57} A study in the 1990s reported that BPH is comprised of 88.4% stromal cells and 9.0% epithelial cells,\textsuperscript{57} and it is therefore reasonable to think that stromal cells may play a major role in development of BPH. Jiang et al\textsuperscript{57} found differences between stromal cells in the TZ and those in the PZ with regard to AR expression, DHT response, and cytokine expressions, and they demonstrated that stromal cells in the TZ, but not the TZ, are more supportive for prostate cancer epithelial cell growth; however, little is known about whether stromal cells in the TZ also contribute to development of BPH.\textsuperscript{57} In other studies, DHT concentration and IGF-II activity were highest in the periurethral area of the TZ in BPH,\textsuperscript{58} and basic fibroblast growth factor (FGF) expression in stromal cells, but not in epithelial cells, was reduced in BPH patients after finasteride treatment.\textsuperscript{59} These results indicate that stromal cells in the TZ may play an important role in development of BPH via modulation of androgen/AR signaling and expression of growth factors and cytokines.

Yu et al\textsuperscript{60} established the stromal smooth-muscle selective AR knockout (SM-ARKO) mouse model and found decreased epithelial cell proliferation, with little change of apoptosis and differentiation. They also found decreased IGF-I expression in the prostates of SM-ARKO mice and primary PS-1 stromal cells with AR knockdown, suggesting that AR in stromal smooth-muscle cells may also contribute to development of BPH via regulation of IGF-I signaling. Yu et al\textsuperscript{61} also established the stromal fibroblast selective AR knockout (FSP-ARKO) mouse model, and found that loss of AR in the stromal fibroblasts decreased proliferation.
of epithelial cells with increased apoptosis, which may involve the suppression of IGF-I and FGFs.

Lai et al. established the double stromal AR knockout (dARKO) mouse model with selectively deleted AR in both stromal fibroblasts and smooth-muscle cells, and found that the size of the anterior prostate lobes was significantly reduced, compared with those from wild-type littermate controls. Decreased proliferation and increased apoptosis of the prostate epithelium in the dARKO mouse anterior prostate was also observed. Lai et al. further confirmed these phenotypic changes with newly established immortalized prostate stromal cells (PrSC) from wild-type and dARKO mice. Mechanism dissection studies on the PrSCs from these mice again suggested that IGF-I may play key roles in mediating positive roles of stromal AR in normal prostate growth.

The above in vivo mouse models showing stromal AR can promote normal prostate growth were recently further extended to a prolactin transgenic (Pb-PRL tg) mouse BPH model. Mice with loss of AR in both stromal fibroblasts and smooth-muscle cells (dARKO/Pb-PRL tg) developed smaller prostates with a lower proliferative index and exhibited better urination function and normal bladder volume, compared with wild-type/Pb-PRL tg mice (Lai et al, unpublished data). Mechanism dissection suggested that prolactin-induced hyperplastic prostate growth involved the epithelial-stromal interaction through epithelial autonomous prolactin/prolactin receptor action to regulate granulocyte colony-stimulating factor production in a paracrine manner to facilitate stromal cell growth by elevating STAT3 activity (Lai et al, unpublished data). The results indicated that stromal AR could modulate such epithelial—stromal interacting signals, resulting in promotion of hyperplastic prostate growth (Lai et al, unpublished data). Furthermore, therapeutic approaches targeting stromal AR with ASC-J9 reduced prostate size and suppressed stromal cell proliferation (Lai et al, unpublished data).

Taken together, results from various stromal ARKO mouse models suggest that stromal AR may play crucial roles in promoting stromal cell growth in both normal prostate and in development of BPH.

**Stromal AR Promotes Development of BPH by Influencing Infiltrating Macrophage Migration toward Prostate Stromal Cells**

The potential influence of inflammatory infiltrating cells on stromal cell growth during development of BPH has also been addressed. Wang et al. found that the number of infiltrated macrophages increased in stroma of human BPH specimens, and that mouse stromal mPrSC cells had the capacity to recruit more infiltrating macrophage RAW264.7 cells in a coculture system. The consequent increase of macrophage infiltration toward stromal cells could promote the proliferation of stromal mPrSC cells.

Mechanism dissection further revealed a remarkable increase of expression of the cytokine CCL3 in both mPrSC...
stromal cells and RAW264.7 macrophages in a coculture system, and addition of neutralizing CCL3 antibody resulted in a significant reduction of the migration of macrophage RAW264.7 cells toward stromal cells and a significant reduction of macrophage-enhanced stromal mPrSC cell proliferation during coculture.65

These in vitro coculture data were further confirmed using an in vivo prolactin-induced BPH mouse model in a study showing increased macrophage numbers and CCL3 expression, compared with those from wild-type littermate controls.65 Importantly, targeting stromal AR via deletion of the stromal fibromuscular AR in the prolactin-induced BPH model reduced the number of infiltrated macrophages and reduced CCL3 expression in prostate tissues. These findings confirm in vitro coculture data and support the key role of prostate stromal AR in controlling CCL3 expression, which could affect macrophage infiltration and prostate stromal cell growth during development of BPH.65 Moreover, human clinical immunohistochemical analysis also revealed higher expression of CCL3 in human BPH prostates, compared with normal prostates.65

Taken together, the various findings described above lead to the conclusion that stromal AR may promote development of BPH by enhancing the recruitment of infiltrating macrophages with increased CCL3 expression, thereby increasing stromal cell growth (Figure 3).65

Targeting AR with ASC-J9 as a New Therapeutic Approach to Treatment of BPH

The conclusion that AR in both stromal and epithelial cells may play key roles in promoting development of BPH suggests that targeting AR could provide a potential therapeutic approach for BPH. Alternatively, targeting AR-regulated downstream genes (eg, TGFβ2 or CCL3) could provide a potential therapeutic approach. Indeed, Lu et al and Wang et al65 reported that treatment with ASC-J9 suppressed epithelial and stromal cell growth, respectively. Importantly, injection of ASC-J9 into prolactin-induced BPH mice also led to suppression of development of BPH. Early studies have demonstrated well the efficacy of ASC-J9 in treating several AR-related diseases, including prostate cancer,66–68 liver cancer,69,70 and bladder cancer,71 as well as promoting wound healing,72 with little change in serum testosterone concentration and less toxicity or adverse effects in sexual function or fertility.66–73 ASC-J9 thus has potential as a candidate drug targeting AR for treatment of BPH. Further in vivo or clinical studies of this agent for treatment of BPH may be expected.

Conclusions

Androgen/AR signaling plays key roles in enhancing cell growth in both stromal and epithelial cells, thus promoting development of BPH. Although BPH is comprised largely of stromal cells, epithelial cells may still play an important role in development of BPH via epithelial cell—stromal cell interaction or EMT. One such mechanism with positive roles of AR involves increased recruitment of infiltrating macrophages, with modulation of some cytokines. Targeting AR may therefore provide a reasonable therapeutic approach for treatment of BPH. ASC-J9, which offers improved efficacy in targeting AR and reduction in number or severity of adverse effects, has the potential to become a next-generation therapy for BPH patients.

References

16. Hammond GL, Kontturi M, Vihko P, Vihko R: Serum steroids in liver cancer,69,70 and bladder cancer,71 as well as promoting wound healing,72 with little change in serum testosterone concentration and less toxicity or adverse effects in sexual function or fertility.66–73 ASC-J9 thus has potential as a candidate drug targeting AR for treatment of BPH. Further in vivo or clinical studies of this agent for treatment of BPH may be expected.

Conclusions

Androgen/AR signaling plays key roles in enhancing cell growth in both stromal and epithelial cells, thus promoting development of BPH. Although BPH is comprised largely of stromal cells, epithelial cells may still play an important role in development of BPH via epithelial cell—stromal cell interaction or EMT. One such mechanism with positive roles of AR involves increased recruitment of infiltrating macrophages, with modulation of some cytokines. Targeting AR may therefore provide a reasonable therapeutic approach for treatment of BPH. ASC-J9, which offers improved efficacy in targeting AR and reduction in number or severity of adverse effects, has the potential to become a next-generation therapy for BPH patients.
peripheral zone of the prostate? A preliminary study. BJU Int 2007, 100:1091—1096


64. Begley LA, Kasina S, MacDonald J, Macoska JA: The inflammatory microenvironment of the aging prostate facilitates cellular proliferation and hypertrophy. Cytokine 2008, 43:194–199


