Androgen receptor and immune inflammation in benign prostatic hyperplasia and prostate cancer

Both benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are frequent diseases in middle-aged to elderly men worldwide. While both diseases are linked to abnormal growth of the prostate, the epidemiological and pathological features of these two prostate diseases are different. BPH nodules typically arise from the transitional zone, and, in contrast, PCa arises from the peripheral zone. Androgen deprivation therapy alone may not be sufficient to cure these two prostatic diseases due to its undesirable side effects. The alteration of androgen receptor-mediated inflammatory signals from infiltrating immune cells and prostate stromal/epithelial cells may play key roles in those unwanted events. Herein, this review will focus on the roles of androgen/androgen receptor signals in the inflammation-induced progression of BPH and PCa.

Keywords: • androgen receptor • benign prostate hyperplasia • immune inflammation • metastasis • prostate cancer

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Part I: benign prostatic hyperplasia
Epidemiology of benign prostatic hyperplasia

Benign prostatic hyperplasia (BPH) is the most common male benign proliferative disease. The prevalence rate of BPH is more than 20% after men reach 50 years, and increases gradually up to 90% at 90 years. The incidence of BPH in Asians and Caucasians is similar, but the incidence of prostate cancer (PCa) in Asians is lower than in Caucasians [1]. A high incidence of BPH was also reported in African Americans and Latinos, too [2–4]. As the life span increases in most countries, BPH may become a more common and problematic disease for males in their late middle age and beyond.

BPH is clinically characterized by lower urinary tract symptoms (LUTS) [5]. While α-1 adrenergic receptor blockers (A1B) used for BPH treatment showed a significant statistical improvement in not only LUTS but also sexual functions [6], they may induce other adverse events such as retrograde ejaculation and hypotension [7].

Nocturia is a common and bothersome urologic symptom of BPH. It was reported that men less than 60 years old with nocturia were more likely to develop coronary heart disease later in life than men without nocturia, and that men over 60 years with nocturia were more likely to die from coronary heart disease than older men without nocturia [8]. Another study also reported that nocturia may increase mortality risk with increasing number of voiding episodes nightly [9].

Together, development of BPH may not only influence the quality of life, it may also be viewed as a life-threatening disease.

Age usually correlates well with both prostate volume and the transitional zone (TZ) volume [10,11], and even though testosterone levels decrease in aged men, serum dihydrotestosterone (DHT) levels in the BPH patients was significantly higher than those in the normal males [12,13]. Similar conclusions were also obtained in a recent large cross-sectional study including 505 men showing higher serum DHT levels and DHT/testosterone ratios were associated

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with larger prostate volume and higher incidence of BPH [14]. However, the detailed mechanisms by which BPH develops or has a higher incidence depending on age/race remain unclear.

Androgen receptor expression in BPH

Androgen concentration and/or androgen receptor (AR) activity linked to BPH incidence has been documented since 1895 [15]. There was little difference in AR expression between the peripheral zone and TZ hyperplastic nodules [16], and an early study revealed that AR is expressed almost exclusively in the epithelial cell nuclei, with little in the stromal cells [17]. In contrast, another study found AR was expressed in both epithelial and stromal cells of hyperplastic nodules, with higher expression in BPH epithelial cells [18]. Addition of the synthetic androgen mibolerone to human prostate epithelial cells increased AR expression with enhanced cell growth [19]. Interestingly, another study indicated that AR is evenly distributed between the epithelia and stroma of BPH, and BPH tissues contain 2.2-times higher DHT than normal prostate tissues and BPH stroma shows 2- to 3-times higher 5-α reductase activity than the epithelia [20]. As BPH consists of 88% stromal cells and 9% epithelial cells, it may be reasonable to believe that stromal cells may also play important roles for the BPH development [21]. Importantly, a recent study showed that the periurethral area of the TZ, site of the primary BPH nodule, has the highest levels of both androgens and AR compared with the other regions, suggesting this region may be responsible for the growth-promoting processes of BPH that result in the urinary obstruction [22]. Objective quantification of AR using quantitative pathology device applied to human BPH tissues revealed that the number of AR-positive cells and the intensity of AR staining in both epithelial and stromal cells in BPH were increased compared with normal prostate tissue and that the size of the nucleus in epithelial and stromal cells in BPH was smaller than those of normal prostate tissue. These results indicate that while the exposure of BPH cells to androgen decreases with aging, BPH cells have greater sensitivity to androgens [23].

Using prolactin transgenic (Pb-PRL-tg) mouse BPH model, loss of AR in both stromal fibroblasts and smooth muscle cells (dARKO/Pb-PRL-tg) resulted in the development of smaller prostates with less proliferative indices and mice displayed better urination function and normal bladder volume compared with wild-type Pb-PRL-tg mice. Mechanism dissection suggested that prolactin-induced hyperplastic prostate growth involved the epithelial-stromal interaction via epithelial autonomous prolactin/prolactin receptor

signals to regulate granulocyte colony-stimulating factor and granulocyte/macrophage colony stimulating factor production in a paracrine manner to facilitate stromal cell growth with elevated STAT3 activity.

Together, results from this BPH mouse model clearly suggest that the stromal AR could function through epithelial-stromal interacting signals to promote the BPH growth [24].

Targeting androgen/AR signals with androgen-deprivation-therapy to suppress BPH

BPH patients who received medical castration with luteinizing hormone-releasing hormone (LH-RH) analog showed a marked decrease of prostatic volume after 2–3 months, and this volume reduction correlated well with a relief of LUTS [25]. However, some studies with the LH-RH analog buserelin showed no improvement in LUTS despite decreased prostate size [26,27]. But, the LH-RH analog leuprolide could shrink prostate size and concomitantly improve LUTS [28,29]. However, some critical problems including the complete loss of erectile function and sexual activity during leuprolide treatment may occur [30].

A double-blind, randomized trial for BPH patients with the anti-androgen flutamide (750 mg/day for 6 months) showed significantly decreased prostate volume by 35% compared with controls within 6 months [31]. Bicalutamide (Casodex™) at a dosage of 50 mg/day also obtained improvement in symptom scores compared with placebo patients [32]. A randomized multicenter study showed that flutamide reduced prostate volume and increased early peak flow rate; nevertheless, urinary symptoms were not improved in the flutamide group [33]. Hence, androgen-deprivation therapies with surgical or medical castration or with anti-androgens are no longer used as a standard treatment for BPH.

Targeting androgen/AR signals with 5-α reductase inhibitors to suppress BPH

5-α reductase inhibitors (5-ARI) that suppress testosterone conversion into DHT have better efficacy to reduce BPH volume. The efficacy and safety profiles of finasteride, a 5-ARI for 5-α reductase type 2, showed a 20% reduction in prostate volume with significant improvement in urinary symptoms [34,35]. However, finasteride failed to reduce DHT in stromal cells of TZ even though 5-α reductase type 2 is expressed in both prostate stromal and epithelial cells. In contrast, using another 5-ARI, dutasteride, that inhibited both 5-α reductase type 2 and type 1 showed a better decrease of DHT (98.4 ± 1.2%) than finasteride (70.8 ± 18.3%) [36–38]. The study of efficacy and safety with dutasteride showed long-term treatment in BPH patients resulted

in continuing improvements in LUTS and flow rate with reductions in prostate volume [39,40].

A randomized controlled trial of A1B in BPH patients showed the improved maximum flow rate with decreased International Prostate Symptom Score (IPSS), and no significant adverse events [41–43]. However, these A1B drugs alone cannot decrease prostate volume and may not be able to eliminate symptoms completely [44].

A combined therapy with dutasteride and the A1B, tamsulosin, was significantly superior to either monotherapy at reducing the relative risk of BPH progression and symptoms [45], especially for those patients with prostate volumes of 30–58 ml [46], suggesting that the benefit of combination therapy is greatest in patients with moderate BPH. Another treatment may be needed for patients with larger BPH, but A1B alone may be sufficient to alleviate LUTS in patients with smaller BPH [47]. Regardless of these positive effects, 5-ARI treatment can also produce adverse sexual events including decreased libido, erectile dysfunction and ejaculation problems [48–50]. There is a wide variation of the reduction in prostate volume after 5-ARI treatment, and downregulation level of androgen-regulated genes such as *TMPRSS2*, *KLK2*, *KLK4* and *PSA* accounts for the variation. It was reported that the most important predictor of the response to 5-ARI was *AR* level itself [51].

Interestingly, using tadalafil, a phosphordiesterase type 5 inhibitor (PDE5I), was able to improve the erectile dysfunction with improved LUTS and maximum flow rate [52–55], and was approved by the FDA for BPH treatment in 2011 [56] even though PDE5I cannot totally reduce prostate volume.

Together, Table 1 summarizes clinical trials to suppress BPH. While targeting both androgen/AR and α-1 adrenergic receptor signals definitely contribute to suppress the BPH development, further detailed mechanism dissections should allow us to develop better medicines with better efficacies and reduced side effects for suppressing BPH.

AR-independent immune inflammation in BPH

In addition to the androgen/AR signals that may influence BPH development, inflammation is another key factor linked to BPH development. Early studies indicated that bacterial and noninfectious inflammation is a common finding in BPH [57,58]. T cells and macrophages have been thought to be major components of the infiltrating cells in BPH inflammation. Macrophages accumulate in the lumen and glandular epithelial layers of damaged prostatic glands and T cells, but not B cells, also accumulate in large numbers in the glandular epithelial layers and around the glands [58].

In the examination of inflammation using 282 BPH samples, 81% had T-cell markers (CD3) and 82% had macrophage markers (CD163), and patients with higher inflammation level had larger prostate volumes and stronger symptoms [59].

There are evidences showing the relationship between T cells infiltration and BPH development. The chemokine interleukin (IL)-8 mediated stromal cell proliferation through the autocrine/paracrine manner with inflammation, consistent with its marked secretion in BPH stromal cells associated with induction of T cells derived inflammatory cytokines [60]. Overexpression of IL-15 and IL-17 in BPH also may be the cause of the proliferation and the activation of T cells in BPH [61,62]. A kind of positive feedback system among IFN-γ, IL-17, IL-6 and IL-8 in BPH inflammation activates T cells resulting in stromal cell proliferation [63]. BPH tissues contain IL-2 and IFN-γ, which stimulate the proliferation of BPH stromal cells, but not that of normal prostate stromal cells [64]. Thus, as T cells infiltration may lead to the development of BPH, the detailed mechanisms still remain to be examined.

The macrophage's role in BPH also have been actively studied. DNA microarray analyses revealed that the macrophage inhibitory cytokine-1 (MIC-1) gene was significantly downregulated in specimens from patients with symptomatic BPH [65]. Sixty-four percent of BPH samples showed downregulated MIC-1 gene expression, whereas mRNA level of MIC-1 was high in six of six normal prostate adenoma samples. Only one of seven BPH samples with a glandular predominant pattern showed MIC-1 gene downregulation, however, 15 of 24 BPH samples with a mixed type or stromal predominant pattern showed MIC-1 gene downregulation. The downregulation of the MIC-1 gene may induce gland destruction with inflammatory infiltrates, and replacement of the stromal component subsequently in symptomatic BPH [66]. Roles of CC chemokine ligand (CCL) 2, which is also called as macrophage chemoattractant protein-1, in BPH were also investigated. Stromal cells secreted CCL2 and both stromal cells and epithelial cells express CCR2, which is the CCL2 receptor. CCL2 stimulated the proliferation of epithelial cells, but not stromal cells, and a specific CCL2 antagonist suppressed this effect. Conditioned media from stromal cells stimulated the proliferation of epithelial cells as well, an effect completely inhibited by the CCL2 antagonist. The inflammatory cytokines IL-1β, IFN-γ and IL-2 enhanced the secretion of CCL2 from both stromal cells and epithelial cells. In addition, CCL2 levels correlated with the macrophage marker CD68 [67].

Table 1. Randomized controlled trials of androgen/androgen receptor signaling-related agents for benign prostatic hyperplasia.						
Agent	Category	n	Dosage	TPVR (vs placebo)	Year	Ref.
Leuprolide	LH-RH analog	50	3.75 mg, every 4 weeks	-34.5 vs -2.6%	1993	[30]
Flutamide	Anti-androgen	43	750 mg, daily	-35 vs 0%	1991	[31]
Bicalutamide	Anti-androgen	60	50 mg, daily	-26.4 vs -3.7%	1993	[32]
Flutamide	Anti-androgen	372	250–750 mg, daily	-27 (750 mg) v.s. -2%	1996	[33]
Finasteride	5-α reductase inhibitor	1645	1–5 mg, daily	-19 (5 mg) vs -3%	1992	[35]
Dutasteride	5-α reductase inhibitor	4325	0.5 mg, daily	-25.7 vs +1.7%	2002	[40]
Dutasteride + tamsulosin	5-α reductase inhibitor	4844	0.5 + 0.4 mg, daily	-27.3 vs +4.6% (vs tamsulosin alone)	2010	[45]
Cetrorelix	LH-RH antagonist	140	5–10 mg, weekly	-18.7 (10 mg) vs -5%	2008	[77]
LH-RH: Luteinizing hormone-releasing hormone; TPVR: Total prostate volume reduction rate.						

AR-dependent immune inflammation in BPH

Importantly, more and more reports linked the androgens/AR signals to inflammation to impact the BPH progression. Recently, a study of immune inflammation in 105 BPH specimens revealed that the group with strong immune inflammation had larger prostate volumes, higher AR expression levels and higher serum prostate-specific antigen (PSA) levels [68].

A study investigating the interaction of infiltrated macrophages and stromal cells in BPH showed that mouse stromal cells (mPrSC) could recruit mouse macrophages (RAW264.7) that may result in promoting the proliferation of stromal cells [69]. Mechanism dissection found a significant increase of CCL3 expression in both mPrSC cells and RAW264.7 cells, and neutralizing CCL3 antibody could reduce the migration of RAW264.7 cells toward mPrSC cells and macrophage-enhanced mPrSC cell proliferation [69]. The *in vivo* Pb-PRL-tg mouse BPH model also confirmed the increased macrophages number and CCL3 expression in BPH and targeting stromal AR via deletion of the stromal fibromuscular AR in the Pb-PRL-tg mouse BPH model reduced the infiltrated macrophage number and CCL3 expression level in prostate. Moreover, human clinical immunohistochemical analysis also showed the higher number of infiltrated macrophages into the stroma and the higher expression of CCL3 in human BPH prostates compared with normal prostates. Stromal AR could promote BPH development via enhancing the recruitment of infiltrating macrophages with increased CCL3 expression that resulted in increased stromal cell proliferation [69]. Interestingly, a study indicating opposite effects of androgen/AR signals showing DHT can regulate the immune system in BPH with suppressing inflammatory cytokines from stromal cells was also reported [70].

Another study of the interaction of infiltrated macrophages and epithelial cells in BPH showed that human epithelial BPH-1 cells could recruit human monocyte/macrophage THP-1 cells toward BPH-1 cells in the coculture system. Recruited THP-1 cells subsequently enhanced BPH-1 cell growth and epithelial-mesenchymal transition (EMT) tendency of BPH-1 cells. EMT of epithelial cells in prostate ducts was also reported to contribute to the BPH development by increasing stromal cells as a result [71]. Mechanism dissection revealed that TGF-β2 expression in BPH-1 cells was increased in the coculture system and TGF-β2 neutralizing antibody could suppress THP-1-mediated cell growth and EMT in BPH-1 cells. Overexpression of AR in BPH-1 cells promoted THP-1 macrophage migration with induction of EMT gene expression in BPH-1 cells. When human BPH-1/THP-1 cells were replaced with mouse epithelial mPrE cells and RAW264.7 cells in the coculture system, almost the same results were obtained [72].

Future perspective of BPH treatments

Selective androgen receptor modulators (SARM) may provide alternative therapeutic agents for BPH. S-40542, which is an SARM, showed a concentration-dependent AR antagonistic action [73]. BPH model rats were repeatedly treated with S-40542, and results indicated that S-40542 had little effect on the serum testosterone and luteinizing hormone with decreased prostate volume in the BPH rats [74].

LH-RH antagonist cetrorelix had inhibitory effects on the BPH-1 cell proliferation that may function through the modulation the IGF-I, IGF-II, FGF-2, EGF, adrenergic receptors and STAT3 signals [75]. Importantly, several inflammatory cytokines in rat prostate tissues were also suppressed after cetrorelix treatment [76], and Phase II study of administered cetrorelix showed rapid improvement in IPSS and maximum urinary flow rate [77].

Furthermore, targeting IL or IFN-γ to suppress lymphocytes may also lead to new therapies to battle BPH. For example, CCL2 antibody and CCR2 antagonist have been reported to inhibit the proliferation of prostate epithelial cells, and hexanic lipidosterolic extract of serenoa repens was also reported to reduce CCL2 mRNA levels in both BPH-1 and prostate stromal cell line (WPMY-1) cells [67,78]. Addition of neutralizing CCL3 antibody in the coculture system of mPrSC and RAW264.7 cells resulted in a significant reduction of the migration of RAW264.7 cells toward mPrSC cells and macrophage-induced mPrSC cell proliferation.

Importantly, the newly identified AR degradation enhancer, ASC-J9®, that could selectively degrade AR proteins in various cells with little influence on serum testosterone and normal sexual activity/fertility could also suppress stromal AR-mediated enhancement of RAW264.7 infiltration, mPrSC cell growth and CCL3 induction [69,79–82]. Interestingly, ASC-J9 also could downregulate AR expression in AR-over-expressed BPH-1 cells. The consequences of such downregulation of AR expression in BPH-1 cells could lead to reduced migration of macrophage THP-1 cells to BPH-1 cells. This reduced migration following treatment with ASC-J9 then resulted in suppression of the sphere growth and induction of EMT markers in BPH-1 cells during coculture [72].

In summary, androgen/AR signals, especially those involved in inflammation, may play key roles to enhance cell growth in both stromal and epithelial cells for the promotion of BPH development (see cartoon in Figure 1). Targeting both AR and immune inflammation may become a reasonable therapeutic approach for treatment of BPH.

Part II: PCa
Epidemiology of PCa
PCa is the most prevalent cancer in males and a second leading cancer accounting for 10% of estimated male deaths in the United States [83]. The 5-year survival rate
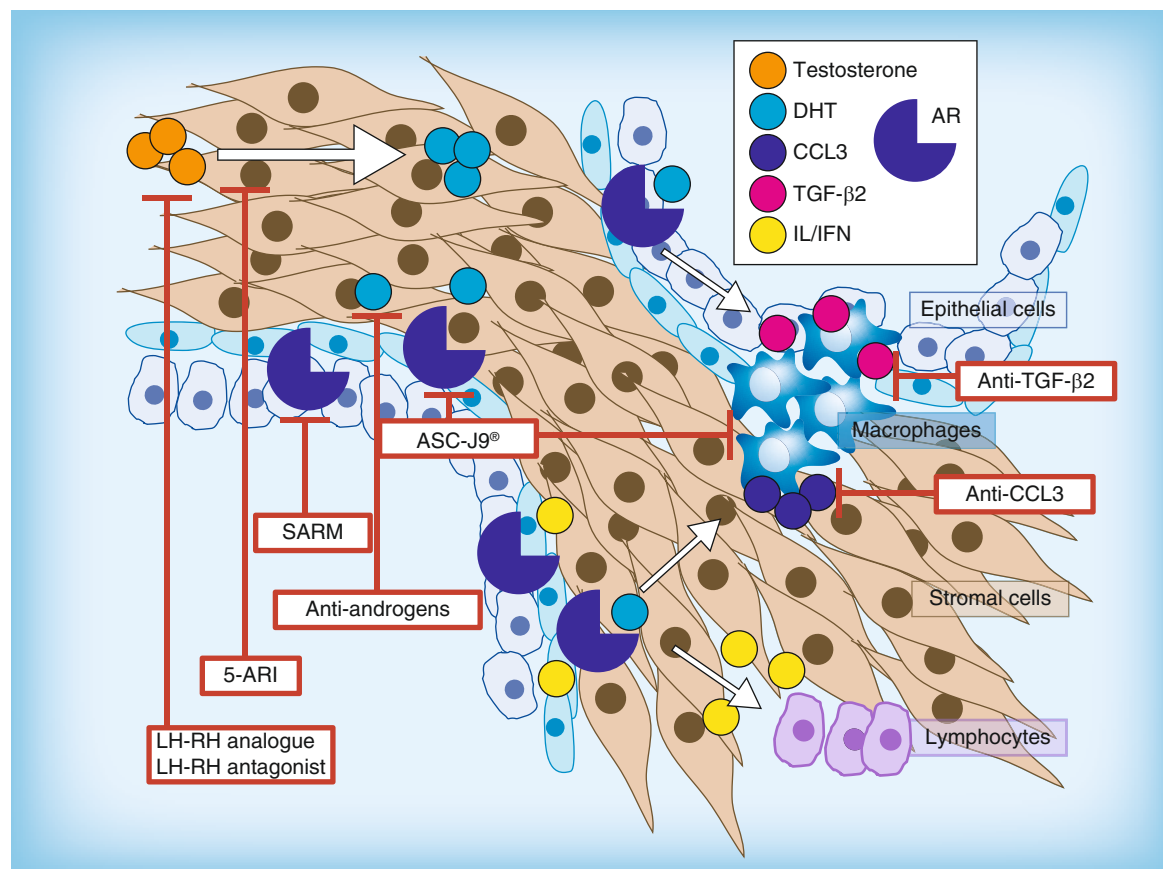

Figure 1. Androgen/androgen receptor signaling and immune inflammation in benign prostatic hyperplasia.
Androgen/AR signaling plays key roles to enhance cell growth in both stromal and epithelial cells. Some inflammatory cytokines are associated with androgen/AR signaling and contribute to benign prostatic hyperplasia development. Medical treatments targeting androgen/AR signaling and androgen/AR signaling-associated cytokines are shown as red outlined boxes and red bars.
AR: Androgen receptor.
For color images please see online www.future-science.com/doi/full/10.4155/CLI.14.77
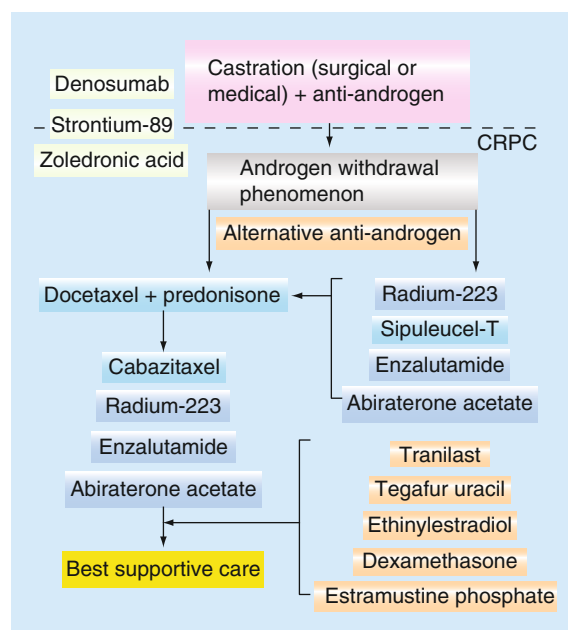
Figure 2. Treatment strategy of advanced prostate cancer. Androgen-deprivation therapy with combined androgen blockade (surgical or medical castration plus anti-androgen) is the standard therapy for advanced prostate cancer. If the patient has skeletal metastasis, addition of treatments targeting skeletal metastasis (green) is considered. In the castration-resistant phase, after confirmation of androgen withdrawal phenomenon, docetaxel, which is a conventional standard therapy, or recently developed therapies proven by randomized controlled Phase III studies, are applied according to the condition of the prostate cancer (blue). Before these standard therapies, alternative anti-androgen therapy may be considered. The best supportive care is applied after the sequence of standard therapies. However, treatments with less evidence may be applied before the best supportive care (orange).
CRPC: Castration-resistant PCa
For color images please see online
www.future-science.com/doi/full/10.4155/CLI.14.77

for localized PCa is almost 100% in the United States. However, the 5-year survival rate for metastatic PCa is decreased to 28% [84], with 80% of these patients developing skeletal metastases with osteoblastic changes [85–87]. Advanced metastatic PCa is usually treated with androgen-deprivation therapy (ADT) with various anti-androgens. However, most cases treated with ADT unfortunately progress into castration-resistant PCa (CRPC) within 1–2 years [88,89] with a variety of adverse events [90–92]. Various therapeutic approaches in the treatment of CRPC are summarized in Figure 2 [93–105].

AR expression in PCa
ADT constitutes the major therapeutic approach to reduce or prevent androgens from binding to AR [106]. AR is present in most primary and metastatic PCa

tumors regardless of stage and grade [107–109], and AR expression was correlated with ADT response and survival [110,111]. AR expression was markedly increased (9- to 11-fold) in metastatic lesions of CRPC relative to untreated or ADT-treated primary cancers [88]. Interestingly, AR activity was also reported to be high in local, untreated PCa and decreased in CRPC after ADT [112]. The reduced AR in CRPC was also reported in the study using PCa biopsy specimens, and PSA values in these CRPC patients were also decreased after ADT [113]. However, PCa patients with low PSA levels (≤ 2.5 ng/mL) were found more likely to have seminal vesicle invasion with worse outcomes than their counterparts with higher PSA levels [114]. These differential results indicate that androgen/AR signals in CRPC are complicated and may not always be able to be used to determine PCa status.

AR signals alter metastasis in CRPC
Early studies reported important AR roles in PCa progression before or after ADT [102,103,115–117]. Suppression of androgen synthesis, including adrenal and intratumoral *de novo* androgen synthesis, impacts CRPC progression [118,119]. Increased AR protein levels due to gene amplification or altered mRNA expression in CRPC was also reported [120–122]. AR mutations that alter androgen and anti-androgen sensitivity in advanced PCa prior to ADT and after ADT in CRPC may also influence the progression of CRPC [123–126]. Splice variants of AR and changing ratios of AR to AR coregulators to enhance AR signals in CRPC under the castration levels of androgens were also reported [88,127,128].

Newly redeveloped ADT with abiraterone acetate, an inhibitor of androgen biosynthesis in the adrenal system, prolonged the overall survival among patients with metastatic CRPC [94,95]. Similarly, new ADT with enzalutamide significantly prolonged the survival of patients with metastatic CRPC after chemotherapy in a randomized Phase III study [96]. Another new ADT with ARN-509 could also decrease the PSA in 90% of patients in a Phase I study [129]. However, several reports found that ADT with anti-androgens might enhance PCa metastasis even though they could suppress primary tumors with decreased PSA in some PCa patients [130,131]. Further mechanism dissection indicated that enzalutamide significantly enhanced PCa cell invasion via enhancing the TGF-β1/Smad3/MMP9 pathway or altering the infiltrating macrophages and CCL2-STAT3 signals [130,131].

These results indicated that therapeutic suppression of androgen/AR signals may elicit unwanted signals that may promote the surviving PCa cells to be more invasive. The genetic ablation of AR in prostate epithelial cells promoted the development of invasive PCa [132]. Targeting

AR may suppress cell deaths via anoikis and entosis that may potentially lead to increased metastasis [133].

AR-mediated immune inflammation influences CRPC cell invasion

It has been shown that castration-induced B cells infiltration may influence the PCa cells to become castration-resistant [134]. AR expression in PCa stem/progenitor cells was low and these cells showed high self-renewal ability after castration. The number of PCa stem/progenitor cells also increased after ADT. Moreover, loss of androgen/AR signals increases B-cell progenitors and precursors and induces B-cell development [81,135]. These effects of loss of androgen/AR signals may promote CRPC cell invasion after ADT. PCa stem/progenitor cells have relatively lower expression of microRNA (miR) 331, which is an important suppressor of a key proto-oncogene ERBB2, and overexpression

of AR in the PCa stem/progenitor cells increased the expression of miR331 [135]. Another study using microarray analyses found miR-21, miR-32, miR-99a, miR-99b, miR-148a, miR-221 and miR-590–5p were differentially expressed in CRPC compared with BPH and further study showed miR-148a-enhanced proliferation of PCa cells and was regulated by androgen/AR signal [136]. Intriguingly, miR-148a could promote osteoclastogenesis through the activation of peripheral blood mononuclear cells [137]. Deterioration of PCa bone metastasis may be attributed to the effect of miR-148a.

ADT-treated patients had a significantly increased number of CD3(+) and CD8(+) T lymphocytes as well as CD68(+) macrophages. An elevated number of CD56(+) natural killer cells was related with a lower risk of PCa progression, while a high density of CD68(+) macrophages was associated to an increased risk of PSA progression [138]. A study reported that
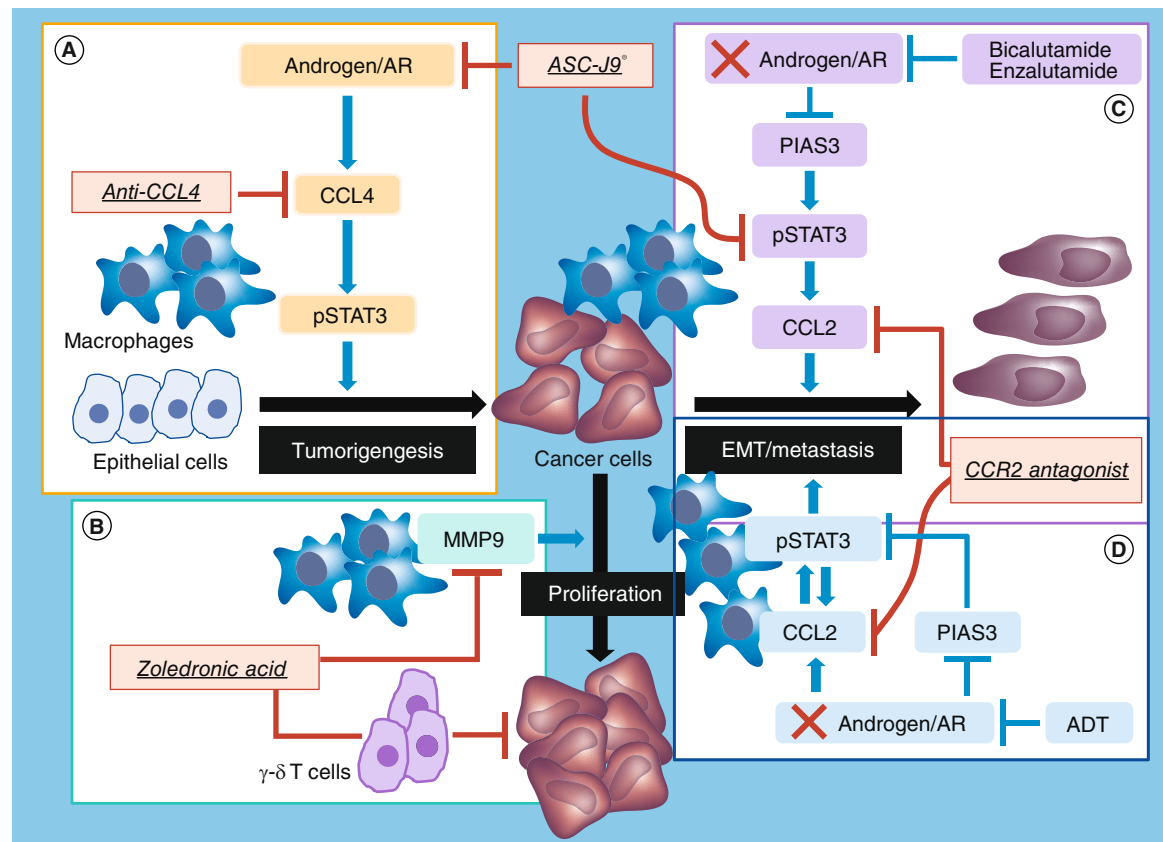
Figure 3. Interaction of prostate cancer cells and macrophages. (A) Androgen receptor-CCL4-pSTAT3 signaling is a key regulator during prostate tumorigenesis. ASC-J9 and anti-CCL4 showed antitumorigenesis effects. **(B)** Migrated macrophages express a variety of pro-angiogenic and immunosuppressive mediators including MMP9. Zoledronic acid selectively suppressed the expression of MMP9 and could effectively drive the proliferation of activated γ-δ T lymphocytes and then inhibit cancer progression. **(C)** Bicalutamide or enzalutamide promoted macrophage migration to PCa cells that consequently led to enhanced PCa cell invasion via activation of PIAS3-pSTAT3-CCL2 signaling. ASC-J9 suppressed both macrophage migration and subsequent PCa cell invasion. **(D)** Androgen-deprivation therapy induced EMT in PCa via the activation of CCL2/CCR2-STAT3 signaling in the interaction of PCa cells and macrophages. CCR2 antagonists block CCL2 in both **(C)** and **(D)**. The combination of ADT and anti-CCL2/CCR2-STAT3 signaling may be a better therapeutic approach to battle PCa progression and metastasis.
ADT: Androgen-deprivation therapy; AR: Androgen receptor; PCa: prostate cancer.

Table 2. Effects of androgen/androgen receptor signal in the induction of inflammation.				
Pathological subject	Leading actors	Key mediator	Year	Ref.
BPH	Stromal cell Macrophage	CCL3	2012	[69]
BPH	Epithelial cell Macrophage	TGF-β2	2012	[72]
PCa (tumorigenesis)	Stromal cell Macrophage	CCL4	2012	[80]
PCa (progression)	Cancer stem cell B cell	AKT/PI3K	2013	[81]
PCa (progression)	Cancer cell Macrophage	CCL2	2013	[113]
PCa (progression)	Cancer cell Macrophage	STAT3	2013	[130]
PCa (tumorigenesis)	Epithelial cell Macrophage	CCL4	2013	[141]
BPH: Benign prostate hyperplasia; PCa: Prostate cancer.				

CCL2 might enhance PCa growth/metastasis *in vivo* by increasing the recruitment of tumor-associated macrophages (TAM) and focused on the roles of CCL2 in infiltrating macrophages that enhance PCa progression/metastasis [139]. These results suggest a significant role for inflammatory cells in promoting castration-resistance and metastasis of PCa cells.

A previous study linked the AR function in macrophages-associated inflammation showing that the deficit of AR in mice tends to create an immunosuppressive microenvironment that may alter the inflammatory responses during PCa progression [82,140]. Recent studies also found targeting AR with AR-siRNA in macrophage (THP-1) cells or PCa cells induced the migration of macrophages to PCa (C4–2 and LNCaP) cells that resulted in enhanced PCa cell invasion [113]. Mechanism dissection found that targeting AR with AR-siRNA in either macrophages or PCa cells induced the expression of CCL2 and CCL2-dependent STAT3 activation that enhanced the EMT signaling to promote the PCa cell migration. Pharmacologic interruption of CCL2/CCR2-STAT3 signaling, using anti-CCL2 antibody or CCR2 antagonist, suppressed the EMT and PCa cell migration. Knocking out macrophage AR in the TRAMP mouse model leads to promote the PCa metastasis via induction of CCL2 and macrophage infiltration. Combined therapy targeting AR with AR-siRNA in PCa cells and CCR2 antagonist results in better suppression of PCa growth and reduction of metastasis than targeting AR alone in a xenografted model with orthotopic injection of TRAMP-C1 mouse PCa cells. Clinical data also showed that PCa patients'

outcome was much worse when their PCa tissues had CCL2-positive staining as compared with those PCa patients whose tissues were CCL2-negative in human PCa tissue microarray analysis. In addition, the increased expression levels of CCL2 and pSTAT3 in CRPC were found in biopsy specimens [113].

Together, these results may provide a novel therapeutic approach to better battle PCa progression and metastasis at the castration-resistant stage via the combination of targeting AR with AR-siRNA and anti-CCL2/CCR2-STAT3 signaling.

A study, using human (C4–2B/THP1) and mouse (TRAMP-C1/RAW264.7) PCa cells–macrophages coculture systems, reported the currently used anti-androgens, bicalutamide or enzalutamide, promoted macrophage migration to PCa cells that consequently led to enhanced PCa cell invasion [130]. In contrast, the ASC-J9® treatment suppressed both macrophages migration and subsequent PCa cell invasion. Mechanism dissection showed that bicalutamide and enzalutamide reduced the AR-mediated PIAS3 (endogenous protein inhibitor of activated STAT3) expression and enhanced the pSTAT3-CCL2 pathway. Addition of CCR2 antagonist reversed the bicalutamide/enzalutamide-induced macrophage migration and PCa cell invasion. In contrast, ASC-J9 could regulate pSTAT3-CCL2 signaling via two pathways: an AR-dependent pathway via inhibiting PIAS3 expression and an AR-independent pathway via direct inhibition of the STAT3 phosphorylation/activation. These findings were confirmed in the *in vivo* mouse model with orthotopically injected TRAMP-C1 cells [130].

AR-mediated immune inflammation influenced PCa tumorigenesis & progression

Macrophages also influenced the prostate tumorigenesis. Clinical sample surveys confirmed the number of macrophages was significantly increased in high-grade prostatic intraepithelial neoplasia (PIN) or PCa lesions compared with those in benign prostate tissues [141]. Infiltrating macrophage cells were associated with the PIN lesion in the wild type AR/PTEN(+/-) mice, meanwhile, infiltrating macrophage cells were significantly reduced in the scARKO/PTEN(+/-) mice, which have selective AR deletion in stromal cells. Targeting AR with AR-siRNA in PTEN(+/-) stromal cells showed the levels of CCL3, CCL4, CXCL2 and IL-10 were significantly reduced and mechanism dissection showed liganded AR could induce the IL-1β mediated CCL4 promoter activation [80]. Persistent coculturing of immortalized prostate epithelial cells with macrophages, without adding any carcinogens, induced prostate tumorigenesis and that induction involved the alteration of signaling of macrophage AR-inflammatory chemokine CCL4-pSTAT3 activation as well as EMT and downregulation of p53/PTEN tumor suppressors. Moreover, *in vivo* studies showed that PTEN(+/-) mice lacking macrophage AR developed fewer PIN lesions, supporting the important role of macrophage AR during prostate tumorigenesis. CCL4-neutralizing antibody effectively inhibited macrophage-induced prostate tumorigenic signaling and targeting AR using ASC-J9 decreased CCL4 expression, and xenografted tumor growth *in vivo*. CCL4 upregulation was associated with increased Snail expression and downregulation of p53/PTEN in high-grade PIN and PCa. This study elucidated that the AR-CCL4-pSTAT3 axis is a key regulator during prostate tumorigenesis and highlighted the important roles of infiltrating macrophages and inflammatory cytokines for the prostate tumorigenesis [141].

TAM influences diverse processes such as angiogenesis, tumor cell proliferation and metastasis during cancer progression. Co-inoculation of male athymic nude mice with PC-3 PCa cells and U937 promonocytic cells enhanced tumor growth and increased tumor angiogenesis [142]. The recruitment of macrophages into PCa promoted PCa growth and metastasis via CCL2 [143]. The increased number of TAMs shortened PCa specific survival time and increased microvessel density, cell proliferation and Gleason score in PCa samples from transurethral resection of the prostate [144]. The higher TAM count in prostate biopsy samples was associated with higher serum PSA levels, higher Gleason scores, higher clinical T stages and predicted PSA failure or progression of PCa after ADT [145]. Zoledronic acid, an aminobisphosphonate clinically approved for treatment of symptomatic skeletal events such as bone pain, pathological bone fractures and spinal cord compression, has recently been shown to have immunomodulatory properties that can be exploited in cancer immunotherapy. PCa cells recruit macrophages, which in turn express a variety of proangiogenic and immunosuppressive mediators including MMP9. Zoledronic acid selectively suppressed the expression of MMP-9 and could effectively drive the proliferation of activated γ-δ T lymphocytes and then inhibit cancer progression [146].

Thus, immune inflammation including TAMs may play key roles from the beginning of PCa initiation to later metastatic stages and CRPC via regulating AR signaling and/or its modulated inflammatory cytokines/chemokines (Figure 3). A summary of effects of androgen/AR signal on the induction of inflammation are shown in Table 2.

Future perspective of PCa treatments

ADT with anti-androgens enhances macrophages-associated inflammatory cytokines-AR signals to promote PCa metastasis. A combinational ADT therapy with additional drugs to target those inflammatory cytokines is needed to block PCa progression. As CCL2 is the most promising target because it promotes tumor growth by angiogenesis, macrophage infiltration, tumor invasion and distant metastasisan anti-CCL2 antibody, carlumab, with high affinity and specificity for human CCL2, has been exploited. However, in a Phase II study it did not block the CCL2/CCR2 axis or show antitumor activity as a single agent in metastatic CRPC [147]. To block the CCL2/CCR2 axis, other treatment strategies such as CCR2 antagonist or targeting downstream of the CCL2/CCR2 axis may be needed. Alternatively, using a newly developed AR degradation enhancer, ASC-J9, to simultaneously suppress AR and those macrophages-associated inflammatory cytokines with less toxicity or side effects may become a new therapy to better battle PCa in the future.

Executive summary

Androgens-mediated inflammatory signals in benign prostatic hyperplasia & prostate cancer

- Both benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are very frequent diseases in middle-aged to elderly men.
- The common biological feature of BPH and PCa is that androgen/androgen receptor (AR) signaling clearly contributes to enlargement of their lesions and blockade of this signaling usually succeeds to ameliorate their symptoms or shrink the size of lesions.
- Both stromal and epithelial AR in prostate contributes to BPH progression via epithelial-stromal interacting signals.
- The interaction of infiltrated macrophages and stromal cells in BPH promotes the proliferation of stromal cells via androgen/AR signaling induced CCL3.
- The interaction of infiltrated macrophages and epithelial cells in BPH promotes epithelial cells growth and epithelial-mesenchymal transition tendency of epithelial cells via androgen/AR signaling.
- Although advanced metastatic PCa is usually treated with androgen-deprivation therapy (ADT) composed of medical or surgical castration with or without anti-androgen as a first line treatment, most cases treated with ADT alone unfortunately develop castration-resistant PCa within a few years after starting ADT.
- Castration-resistant PCa still remains AR dependent, meanwhile therapeutic suppression of androgen/AR function elicits unwanted signals that favor the progression of surviving PCa cells to the advanced stage.
- PCa stem/progenitor cells with low AR expression showed high self-renewal ability and the number of PCa stem/progenitor cells increased after ADT.
- Loss of androgen/AR signals increases B-cell progenitors and precursors and induces B-cell development and castration-induced B cells infiltration may influence the PCa cells to become castration-resistant.
- Anti-androgens promote macrophage migration to PCa cells that consequently lead to enhanced PCa cell invasion via activation of PIAS3-pSTAT3-CCL2 signaling.
- Suppression of androgen/AR signaling in both PCa and macrophages inhibits PCa growth but promotes PCa metastasis via CCL2/CCR2-pSTAT3 signaling.
- Interaction of prostate epithelial cells and macrophages also induces prostate tumorigenesis *via* activation of AR-CCL4-pSTAT3 signaling.
- The combination of suppressing androgen/AR signaling and anti-inflammation signaling may be a better therapeutic approach to battle BPH development and PCa progression (both cell growth and metastasis).

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