Review Article
Androgen action in prostate function and disease

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Abstract: Benign prostatic hyperplasia (BPH) is an enlargement of the prostate gland that is frequently found in aging men. Androgens are essential for the development and differentiated function of the prostate, as well as for proliferation and survival of prostatic cells. In man, dog and rodent, there are age-related decreases in serum testosterone. Despite the lower serum testosterone levels, benign prostatic hyperplasia increases with age in men and dogs, while age-dependent prostatic hyperplasia develops in the dorsal and lateral lobes of the rat prostate. The possible mechanisms that lead to prostate hyperplasia have been extensively studied over many years. It is clear that androgens, estrogens and growth factors contribute to the condition, but the exact etiology remains unknown. Prostate cancer (CaP) represents a significant cause of death among males worldwide. As is the case of BPH, it is clear that androgens (testosterone and dihydrotestosterone) and their metabolites play important roles in the disease, but cause-effect relationships have not been established. Androgen deprivation therapy has been used for decades, primarily in the metastatic stage, to inhibit androgen-dependent prostate cancer cell growth. Androgen deprivation, which can be achieved by targeting hormone biosynthesis or androgen receptor activation, results in symptom amelioration. However, most patients will develop hormone refractory cancer or castration-resistant prostate cancer (CRPC). Prostatic epithelial cells demonstrate enormous plasticity in response to androgen ablation. This characteristic of prostatic epithelial cells may give rise to different populations of cells, some of which may not be dependent on androgen. Consequently, androgen receptor positive and negative cells might co-exist within CRPC. A clear understanding of this possible cellular heterogeneity and plasticity of prostate epithelial cells is necessary to develop an optimal strategy to treat or prevent CRPC.

Keywords: Prostate, BPH, CRPC, androgens, aging, Leydig cell

Tribute to Donald S. Coffey, Ph.D.

This review is dedicated to the memory of Dr. Donald S. Coffey, whom we and countless others have been fortunate to have had as a dear friend and mentor. Much of our early interest in the role of testosterone in prostate function and disease came from interacting and working with Dr. Coffey (Don) in the context of a Program Project grant on benign prostatic hyperplasia (BPH) that he led for many years. Don freely shared his ideas and enthusiasm with all those involved with the grant, and particularly with young investigators just starting out. Working with and around Don was simply thrilling, always enriching, and never dull!! After the completion of the Program Project grant, our interest in testicular function and prostate health and disease, which had been stimulated by interactions with Don, continued for many years. We and many, many others will never forget the influence of this great man, or the consistent joy in interacting with him.

Introduction

Testicular androgens are essential for the formation and functioning of the prostate throughout life, and in particular for the proliferation and survival of cells within the gland. Testosterone is produced during the fetal and adult periods by two distinct populations of testicular cells, the fetal Leydig cells and the adult Leydig cells, respectively. The high levels of testosterone produced by the fetal Leydig cells decline postnatally, coincident with the loss in the num-

bers of these cells. Then, during postnatal weeks 2 and 3 in the rat, the fetal Leydig cells are gradually replaced by adult Leydig cells, and testosterone gradually increases to high levels with the pubertal transition to adulthood. During the pre-pubertal and pubertal periods, the conversion of testosterone (T) to dihydro-testosterone (DHT) within the prostate is considered by many investigators to stimulate the growth of the prostate to its adult size. Thereafter, a balance between prostatic cell proliferation and cell death is reached such that there is no further growth of the prostate. During aging, serum testosterone levels decrease in some species, including man, dog, and rat. Despite the lower serum testosterone levels, aging often is associated with increased prostatic cell proliferation relative to cell death, an imbalance that can lead to prostatic hyperplasia or cancer in men and dogs. The possible reason(s) for the imbalance is (are) uncertain. Clearly however, it is not only the serum testosterone concentration that determines whether or not there is abnormal prostate growth.

Our major objectives in this review are to discuss the role(s) considered to be played by androgens and androgen signaling in BPH and prostate cancer (CaP), with an emphasis on early work that led to current thinking.

Leydig cell development and steroidogenic function

Virilization of the male urogenital system depends upon the testosterone produced by the fetal Leydig cells. In the mouse, the fetal Leydig cells form from the differentiation of steroidogenic factor 1 (SF-1; NR5A1) - positive cells [1, 2]. In the rat, the fetal Leydig cells begin to produce testosterone by gestational day 15.5, initially independent of luteinizing hormone (LH) and later in response to LH [3, 4]. Late in fetal life, the fetal Leydig cells begin to regress, with only few persisting in the adult [5-8]. Early in the postnatal period, the fetal Leydig cells begin to be replaced by the forming adult Leydig cells [1, 9]. The latter cells, which arise from stem cells that are present in the neonatal testis [10-13], produce high levels of testosterone in response to LH.

Age-related reductions in serum levels of testosterone [hypogonadism] can occur in both young and aging men. Indeed, significant de-cline in serum testosterone levels affects millions of American men [14, 15] including 20-50% of men over age 60 and approximately 15% of men who are among the couples who seek infertility-related medical appointments [16-18]. There are many other men who also present with what is referred to as "low T", including those with sickle cell disease and spinal cord injury [19]. In some men, reduced serum testosterone results from reduced serum LH and thus reduced stimulation of the Leydig cells (hypogonadotropic hypogonadism) [18]. In most hypogonadal men, however, serum LH either does not change or increases, indicative of primary testicular deficiency of testosterone biosynthesis (primary hypogonadism) [16, 17]. Whether in aging or young men, reduced serum testosterone is associated with a number of metabolic and quality-of-life changes, including decreased lean body mass, bone mineral density, muscle mass, libido and sexual function, increased adiposity, osteoporosis and cardiovascular disorders, and altered mood [17, 19].

Exogenous administered testosterone, known as testosterone replacement therapy (TRT), often is prescribed to reverse symptoms of low testosterone. A host of methods that are relatively easy to use and produce constant testosterone concentrations are available by which to do this. However, recent studies suggest that there may be increased risk of cardiovascular disease in older men after TRT [20-22]. There also are reports suggesting that exogenous testosterone treatment might increase the risk of CaP [23].

Benign prostatic hyperplasia

Of the hundreds of mammalian species, all of which have prostate glands, humans and dogs in particular develop BPH and CaP [24]. In the human, different anatomic regions within the prostate have different rates of BPH and carcinoma. Thus, the transition zone has a high incidence of BPH and a low incidence of carcinoma, whereas the peripheral zone has a high incidence of carcinoma and a low incidence of BPH [25, 26]. BPH in humans is characterized primarily by stromal hyperplasia [27, 28]. In dogs, BPH takes the form of overgrowth or hyperplasia of both the epithelial and stromal compartments throughout the gland. Despite the differences between dog and man, Coffey and others argued that there are sufficient simi-

larities between the two to regard the dog as an appropriate model for the human [29-35]. This was a central tenant of the Coffey Program Project grant. We later discovered that prostatic hyperplasia also occurs in the dorsal and lateral lobes, but not the ventral lobe, of the aged Brown Norway rat prostate, with some similarities to both dog and man [36].

In both man and dog, the development of BPH requires functioning testes, advancing age, and the involvement of hormonal factors [37-39]. Walsh and Wilson [40] and others [41, 42] demonstrated that BPH could be induced in young castrated dogs by administering androgens and estrogens concomitantly. In a long-term study conducted by Don Coffey, Larry Ewing and colleagues [29], it was reported that whereas 100% of intact control, aging beagles developed BPH, restoration of serum testosterone levels after castration of young dogs resulted in only 50% of the aging dogs developing BPH. This study made it clear that the testes, but not just the testosterone that they produce, are important for the development of canine BPH.

Androgens are essential for development and differentiated function of the prostate, as well as for proliferation and survival of cells within the gland. The issue of how BPH is initiated is made more complex by the fact that this condition occurs with aging as there is a gradual decline in the mean serum testosterone concentration in aging dogs and men. With the decline in testosterone, there is decline in the ratio of testosterone to 17β-estradiol in the serum, which may be critical in the pathogenesis of BPH [29]. There also may be altered sensitivity of the prostate to serum testosterone. Hyperplasia and/or hypertrophy of the prostate in the face of decreasing serum testosterone concentrations also might mean that the changes that are responsible for prostatic hyperplasia may be initiated early in adult canine life, and that testosterone later in life is permissive, not causative. Alternatively, as suggested by Coffey and others, there may be metabolic changes within the prostate that favor BPH. For example, it has been suggested that there may be increased production of the active androgen 5a-DHT [43, 44]. Indeed, prostatic DHT levels have been reported in many studies to be several-fold greater in hyperplastic tissue compared with normal prostate in both man [45-49] and dog [50, 51], providing evidence

for such a metabolic shift. It should be noted however, that contrary to most studies, there are reports that there are no significant difference in prostatic DHT concentration between dogs with histologically normal prostates and those with spontaneous BPH [52], and that steroid hormone treatment regimens resulting in elevated prostatic DHT concentrations do not always result in high prostatic weight [53].

In the rat, as in dog and man, spontaneous as well as hormonally-induced hyperplasia can develop [36, 54-56]. In both young and old Brown Norway rats, exogenously administered testosterone resulted in age- and lobe-specific overgrowth of the ventral, dorsal, and lateral lobes. In the case of old rats, both hyperplasia and hypertrophy were seen in the dorsal and lateral lobes of untreated control rats, as well as in rats treated with testosterone. Thus, despite the lower serum testosterone levels in old rats, age-dependent and spontaneous prostatic hyperplasia developed in the dorsal and lateral lobes of the prostate, though not in the ventral lobe [55]. The lobe-specific, age-dependent hyperplasia was enhanced by the administration of testosterone [54]. Castration led to decreased weight of all prostate lobes, but less rapidly and to a lesser magnitude in the dorsal and lateral lobes compared to the ventral lobe. Moreover, less cell death occurred in the prostates of old than young rats in response to castration. These studies revealed marked differences in cell death and survival among the different rat prostatic lobes in response to castration, and suggested an age-dependent response of apoptosis to reduced androgen. The lower rates of cell death observed for the dorsal and lateral lobes, and particularly so with increasing age, appear to be important components of the age-dependent and lobe-specific overgrowth observed for these lobes. Moreover, the age-dependent decline in apoptotic cell death observed in the prostates of old rats suggests that prostate cells develop an altered sensitivity to androgen as a function of age, and that survival of these cells is less dependent on androgen (**Figure 1**).

What causes the imbalance of cell death and cell proliferation that leads to prostatic hyperplasia?

As indicated above, androgens are essential for the proliferation and survival of cells within the
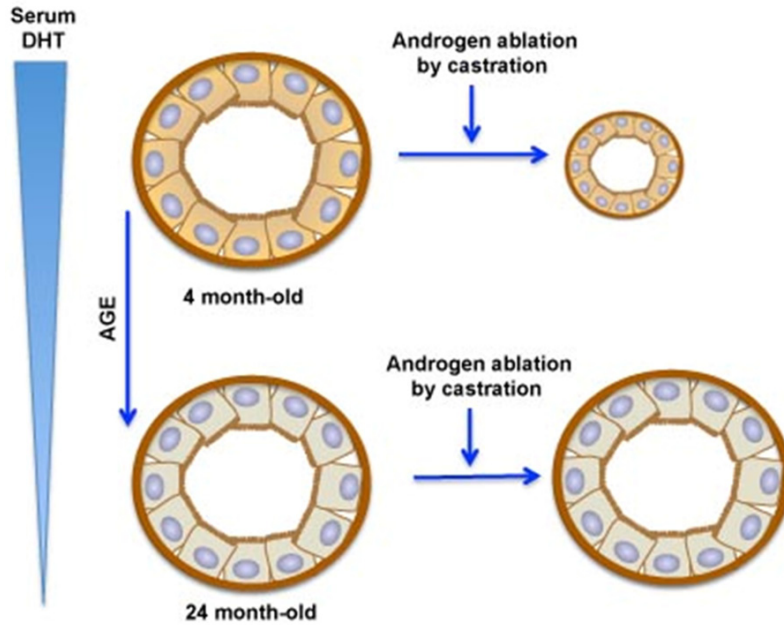
Figure 1. Age-dependent changes in androgen sensitivity in the dorsolateral lobe of Brown Norway rat prostatic acini. Prostatic epithelial cells of young adults (age 4 months) are androgen-dependent, and therefore castration reduces the size of the dorsolateral lobe. However, epithelial cells of the aging dorsolateral prostate become relatively insensitive to changes in androgens, and therefore are relatively unaffected by castration or by normally occurring androgen reductions.

prostate gland. Despite the lower serum levels with aging, BPH increases with age in men and dogs, while age-dependent prostatic hyperplasia develops in the dorsal and lateral lobes of the rat prostate. These observations have proven difficult to explain. One possibility is age-related altered responsiveness of prostatic cells to androgen. For example, it has been reported that in response to castration, there are lower rates of cell death in the dorsal and lateral lobes of aged as compared to young rats, suggesting that prostatic cells may develop some measure of androgen independence with aging [57]. Isaacs and Coffey [58] reported that aging results in an increase in intra-prostatic DHT levels associated with BPH in both animal and human studies. However, there are studies in which this is disputed [59, 60]. Given that testosterone functions via androgen receptors, the proliferative response of cells within the prostate to androgens presumably is dependent in some way upon the expression of androgen receptors. In a study that we conducted some years ago, we hypothesized that age-dependent hyperplasia in the dorsal and lateral lobes of Brown Norway rats might occur in relation to age-dependent and lobe-specific differ-

ences in androgen receptor expression [36, 55]. Lobe-specific increased androgen receptor protein expression was seen that correlated with age-dependent hyperplasia in the dorsal and lateral lobes of the Brown Norway rat prostate. A series of studies by Prins et al. [61-63] of young adult Sprague Dawley rats also showed lobe-specific autoregulation of the androgen receptor. Evidence for increased androgen sensitivity of prostatic cells in the dorsal and lateral lobes of aging rats was also demonstrated by the observation of increases in cell proliferation and cell cycle markers in response to low levels of testosterone correlated with lobe-specific increases in androgen receptor levels of aged rats. These findings, taken together, provide evidence that the imbalance in cell death and cell proliferation that leads to age-dependent prostatic hyperplasia may be related to an altered sensitivity of the prostate to androgen, and suggest that this may be a function of nuclear androgen receptor expression changes with age.

The studies of the rat ventral, dorsal, and lateral prostatic lobes in response to altered hormonal environment, including differences in epithelial cell apoptosis [57] and androgen receptor expression after castration, suggest a role for factors in addition to androgen in the regulation of lobe-specific androgen receptor expression. In this regard, it has been suggested [55, 64-66] that androgen receptor-dependent transcription of target genes may result in the production and secretion of peptide growth factors, including IGF-I, keratinocyte growth factor, fibroblast growth factor-related proteins such as keratinocyte growth factor, interleukin 6, and others. Suffice to say at this juncture that the exact etiology and pathophysiology of BPH remain unknown. The contributions of androgens and estrogens, growth factors, and chronic inflammation remain under investi-

gation. Additionally, stromal-epithelial interactions are known to be very important for BPH pathogenesis and in the regulation of normal cell growth and differentiation [55].

In sum, androgens are essential for development and differentiated function, as well as proliferation and survival of cells within the prostate gland. In man, dog and rodent, there are age-related decreases in serum testosterone. Despite the lower serum testosterone levels, BPH and CaP increase with age in men while age-dependent prostatic hyperplasia develops in the dorsal and lateral lobes of the rat prostate. Clearly, it is not only the serum testosterone concentration that determines whether or not there is abnormal prostate growth.

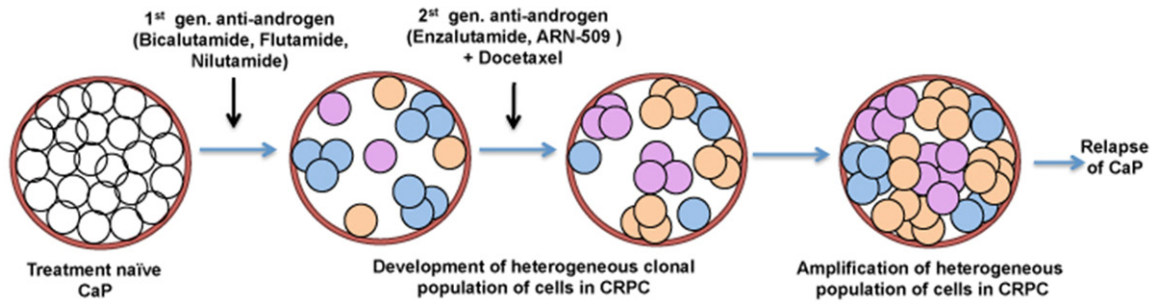### Androgen/androgen receptor signaling and prostate cancer

Androgen/androgen receptor signaling also is considered to drive prostate malignancy [67], the second leading cause of cancer deaths in the US [68] and worldwide [69]. Given its critical role in the normal prostate, it is perhaps not surprising that the AR signaling axis is crucial for prostate carcinogenesis and subsequent phases of disease progression. For that reason, androgen-deprivation therapy has been the mainstay for CaP treatment for over seven decades, after Dr. Charles Huggins demonstrated that orchiectomy or treatment with high doses of estrogen led to regression of metastatic CaP [70]. Androgen deprivation therapy in the form of LHRH agonists/antagonists has typically been used as treatment for metastatic CaP. In addition, various anti-androgens, including flutamide, bicalutamide, and nilutamide, bind to the androgen receptor and inhibit its activity, and thus have been used along with/without chemotherapy [71-73]. However, many patients receiving androgen-deprivation therapy progress to metastatic castration-resistant CaP, characterized by cancer progression despite low serum testosterone levels (**Figure 2**). Although androgen-deprivation therapy is initially successful in most men, development of resistance is inevitable, normally occurring within a period of 18-24 months [74]. The resultant form of this disease is referred to as castration-resistant prostate cancer (CRPC), and is incurable and most often lethal [75, 76]. To overcome this shortfall, taxane (docetaxel) has been used as the standard of care for meta-

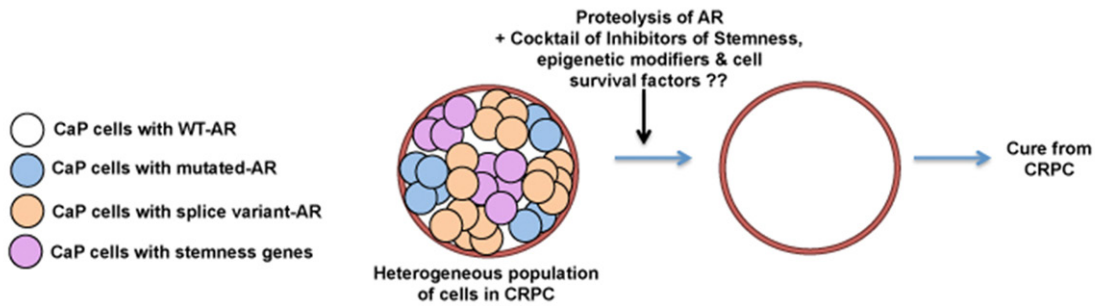static CRPC. However, survival is not long-lasting [77, 78].

Given the importance of androgen receptor signaling during prostate carcinogenesis, and the vast body of work over the past 2-3 decades showing that most CRPC remain dependent on the AR signaling pathway, androgen receptor signaling has remained the major therapeutic target in CRPC even in the face of a very low androgenic environment. The dependence on androgen receptor signaling may be due to the increased AR gene copy number and expression of androgen receptors in the CRPC. Eighty percent of patients exhibit elevated androgen receptor gene copy number, and evidence for mRNA amplification has been observed in 30% of patients with CRPC [79-82]. Moreover, elevated levels of AR have been shown to hypersensitize cancer cells even to castrate levels of androgens [83-85], switch AR antagonists to agonists [86], and promote resistance to variety of AR-targeting agents [87].

In some patients with CRPC, AR mutations have been detected in primary CaP prior to androgen deprivation therapy, and it is generally believed that androgen deprivation therapy-mediated selection of such mutations can underlie resistance in patients with CaP [87-92]. However, much higher frequencies of AR mutations (5-30%) have been reported in CRPC tissue, circulating tumor cells, and circulating cell-free DNA samples compared to pre-treated tumor samples [80-82, 89, 93-98]. These AR mutations are clustered in domains responsible for ligand-binding (at the AR C-terminal domain) or trans-activation activity (the AR N-terminal domain). Such alterations by mutation facilitate AR signaling in CRPC by conferring ligand promiscuity or ligand-independent transcriptional activity, thereby allowing AR to be activated even in the presence of low/absent levels of androgens. The best known AR mutation is the T878A mutant, first identified in the LNCaP cell line [99]. This is the archetypal promiscuous receptor, activated by estrogen, progesterone, and glucocorticoids [92, 97, 99-104]. AR mutations H875Y and L702H broaden ligand specificity by enabling AR activation by glucocorticoids [103-106]. AR mutations also confer agonistic properties to antiandrogens. For example, cancer cells with the T878A mutation are activated by flutamide and nilutamide, and those with the H875Y or W742C/L mutations are activated by

A Development of heterogeneous cells in CRPC

1st gen. anti-androgen (Bicalutamide, Flutamide, Nilutamide)

2st gen. anti-androgen (Enzalutamide, ARN-509) + Docetaxel

Treatment naïve CaP

Development of heterogeneous clonal population of cells in CRPC

Amplification of heterogeneous population of cells in CRPC

Relapse of CaP

B Hypothetical treatment option and cure for heterogeneous CRPC

Proteolysis of AR + Cocktail of Inhibitors of Stemness, epigenetic modifiers & cell survival factors ??

CaP cells with WT-AR
CaP cells with mutated-AR
CaP cells with splice variant-AR
CaP cells with stemness genes

Heterogeneous population of cells in CRPC

Cure from CRPC

Figure 2. Model by which the treatment naïve prostate cancer (CaP) changes from complete androgen dependence to castration-resistant prostate cancer (CRPC). Treatment with 1st generation of anti-androgens kills a majority of wild-type, androgen receptor (AR)-expressing androgen sensitive cells. However, some of these cells acquire AR mutations, some express AR splice variants, and some trans-differentiate to cancer stem-like cells by expressing stemness genes. Second generation anti-androgens attacks only some of the mutated AR expressing cells; because of the plasticity of the CaP cells, some survive, repopulate heterogeneous cancer cells throughout the prostate, and there is cancer relapse. To target all mutated and splice variant AR positive-cells and cancer stem-like cells, total AR degradation together with inhibitors of stem-like cells will be necessary. Only this way will a lethal environment be created that will eradicate all CaP cells irrespective of their heterogeneity.

nilutamide or bicalutamide, respectively [96, 97, 99, 101, 107-109].

Furthermore, increased expressions of constitutively active AR splice variants (AR-Vs) represent another layer of complexity that pertains to the molecular mechanism for disease progression after castration-resistance or androgen-deprivation [110]. Many alternatively spliced AR-Vs lack the C-terminal ligand-binding domain but retain the transactivating N-terminal domain, leading to constitutively active AR in the absence of ligands [111, 112]. AR-Vs are either truncated versions of full-length AR (AR-V1 to AR-V11) or have missing/skipped exons (AR-V12 to AR-V14 and AR-V567es) [110]. Of the different AR isoforms identified in CaP, AR-V7 and ARv567es are the most common [110, 113, 114]. Both are upregulated in metastatic CRPC compared with hormone-naïve metastatic disease [114-116], although only

V7 has been consistently described in human samples.

In addition to AR gene amplification, AR mutations and AR splice variants, AR activation in CRPC can occur in response to increased intratumoral synthesis of testosterone and dihydrotestosterone (DHT) from weak androgens produced by the adrenal glands as well as from de novo androgen synthesis from cholesterol [117, 118]. A number of studies demonstrated that intratumoral levels of androgens in metastatic CRPC are elevated compared with untreated primary prostate cancers [119, 120]. Increased levels of AKR1C3, HSD3B2 and CYP17A1, enzymes that are involved in androgen synthesis, have been detected in the intratumoral tissue from CRPC patients [119, 121]. Therefore, abiraterone acetate, a steroidal anti-androgen that inhibits intratumoral androgen biosynthesis by blocking the hydroxylase and

lyase activities of CYP17A, has been used to treat CRPC. However, the benefit of this treatment is also short-lived.

Why has targeting androgen receptor signaling not dramatically improved overall survival in individuals with metastatic castration-resistant prostate cancer?

We have enormous understanding about androgen receptor signaling, AR gene amplification, AR mutation, AR splice variants and intratumoral androgen synthesis in CaP tumor tissue before and after androgen deprivation therapy. However, overall survival has not improved beyond few months. Although clinical data indicate the benefits of second generation antiandrogens such as Enzalutamide (MDV3100) and ARN509, recent evidence also indicates the emergence of resistance. Enzalutamide is ineffective in preventing the growth of CRPC cell lines such as 22Rv1 unless the AR splice variant, AR-V7, is specifically knocked down [122]. Two independent groups have demonstrated the appearance of a specific missense mutation (F876L) in the AR ligand binding domain in cell lines, xenograft tumors, and clinical samples that have undergone prolonged treatment with enzalutamide and ARN509 [123, 124]. Additionally, patients who are poor responders to enzalutamide treatment show increased expression of the glucocorticoid receptor in bone marrow biopsies [125]. Elevated glucocorticoid receptor expression has been implicated as a compensatory mechanism to overcome AR antagonism in vitro and in vivo. Therefore, it appears that the benefits of anti-androgen therapy are short-lived, and alternative approaches to combat emerging resistance are needed for the effective management of CRPC. One flaw of Enzalutamide and ARN509 is that these second generation antiandrogens, as well as earlier antiandrogens, target the AR-ligand binding domain. Now we know that CRPC also expresses AR splice variants that lack the ligand-binding domain, and therefore neither Enzalutamide or ARN509 alone will be effective in that scenario. More recent therapeutic modalities have attempted to target the AR-transactivation domain rather than the ligand-binding domain.

EPI-001, a small-molecule antagonist of AR N-terminal transactivation domain that inhibits protein-protein interactions necessary for AR transcriptional activity, has recently been developed. EPI-001 inhibited transcriptional activity of AR and its splice variants and reduced the growth of CRPC in a xenograft model [126-128]. These findings suggest that the development of small-molecule inhibitors that target the AR transactivation domain could be a promising strategy for CRPC. Currently, a stereoisomer of EPI-001, EPI-506, is under phase I/II clinical testing (NCT02606123) in post-abiraterone and post-enzalutamide settings. However, the outcomes of these clinical trials are not published yet.

Another alternative therapeutic approach is one that directly targets the degradation of the androgen receptor protein itself rather than its ligand binding and/or transcriptional activity. A plant-derived carbazole alkaloid, mahanine, showed inhibition of both ligand-dependent and ligand-independent AR transactivation, as well as AR protein degradation, leading to a decline in AR target gene expression [129]. Niclosamide, an FDA-approved antihelminthic drug, was identified as a potent inhibitor of the AR-V7 variant in prostate cancer cells. Niclosamide significantly down-regulated AR-V7 protein expression by enhancing protein degradation through a proteasome-dependent pathway [130]. It also inhibited AR-V7 transcription activity and reduced the recruitment of AR-V7 to the PSA promoter [130]. More importantly, this drug potentiates the effects of enzalutamide in vitro and in vivo, and resensitizes enzalutamide-resistant CaP cells [130]. This finding has elicited a phase I trial to assess the utility of niclosamide in combination with enzalutamide for treating AR-V7-positive CRPC (NCT02532114). The clinical outcome has not been published yet. There are additional selective agents that target AR degradation (SARDs; UT-69, UT-155, and (R)-UT-155) and markedly reduce the activity of wild-type and splice variant isoforms of AR at submicromolar doses [131]. However, the efficacy of these agents in clinical settings has yet to be determined.

It is important to emphasize that prostatic epithelial cells demonstrate enormous plasticity in response to androgen ablation. This innate characteristic of prostatic epithelial cells may give rise to different populations of cells, some of which are not dependent on androgen. Consequently, androgen receptor positive and negative cells might co-exist as important examples of cellular heterogeneity within CRPC.

Understanding CaP phenotypic and functional cellular heterogeneity is critical for the optimal treatment of CRPC [132-138]. To better understand this cellular heterogeneity, it is essential to identify the individual cell types within the tumor. The predominant histological subtypes found in prostatic adenocarcinoma [139] are luminal secretory cells, rare neuroendocrine cells, and some basal cells, with luminal epithelial cells accounting for the highest percentage within the gland. However, all these luminal epithelial cells are not the same. In fact, using multiple xenograft models and over 70 patient tumor samples, a comprehensive study was conducted recently to dissect the phenotypic, functional, and tumorigenic heterogeneities in human CaP. Four subtypes of prostate cancer cells, AR+PSA+, AR-PSA+, AR+PSA-, and AR-PSA, were seen in untreated human CaP tissue, reconfirming that Cap tissue is comprised of a heterogeneous pool of cells [140]. In another elegant study, the same group demonstrated that the PSA-/lo CaP cells possess unlimited tumor-propagating activity, whereas PSA+ CaP cells have limited activity [141]. Results from this study reinforce the intrinsic stem cell nature and castration-resistant properties of the PSA-/lo cancer cells. The heterogeneous properties of CaP cells present the ultimate challenge to effective therapy in this disease.

The revelations regarding heterogeneity of CaP cells bring us full circle to our earlier studies in which we observed a diversity of responses among cells in the aging Brown Norway rat prostate. We noted cellular heterogeneity in the normal rodent prostate gland, both during its development in various lobes (ventral, dorsal, lateral and anterior) and in response to androgen ablation by castration. To our surprise, we observed that although cell proliferation occurs in all lobes, the presence of physiological or exogenously administered androgen resulted in significant differences in cell death and androgen responsiveness in the ventral compared to the dorsal and lateral lobes [54, 55, 57, 142-145]. While luminal epithelial cells in the ventral lobe are very sensitive to androgen ablation, indicated by significant loss of total DNA content and cell death, the dorsal and lateral lobes showed almost no change in total cell numbers or cell death. Sensitivity to androgen withdrawal also was found to depend on the age of the rat and the presence and absence of various survival factors (telomerase activity,

TGF-alpha, Bcl2) [146-148]. These results suggest that although prostatic epithelial cells are dependent on androgen for their growth and survival, epithelial cells exhibit plasticity such that their function can be modified over time so as to form a heterogeneous pool of cells within the prostate gland, even before androgen withdrawal. These properties become even more apparent following androgen withdrawal (**Figure 1**).

It is interesting to note that approximately a quarter of CRPC patients who develop an aggressive phenotype have low to no AR expression, acquire neuroendocrine signatures, loss of function of tumor suppressor genes (PTEN, RB1, and TP53), overexpression of stemness markers (SOX2, Nanog, Oct4), and epigenetic reprogramming factors (EZH2, BMI) [149-161]. Therefore, unidirectional targeting of androgen receptor signaling will not result in a major benefit or cure from CRPC. A clear understanding about the cellular heterogeneity and plasticity of prostate epithelial cells is necessary to develop an optimal combinatorial strategy to treat or prevent CRPC. A hypothetical model is proposed in **Figure 2**.

Conclusions and future directions

It is clear that androgens and androgen receptor signaling are crucial for prostate growth and homeostasis, and for the development of BPH and CRPC. However, it is also evident that in the face of decreasing serum androgen, as during aging, prostatic cells adapt to survive in low concentrations of androgen. Thus, although androgens are essential, prostatic hyperplasia can occur in men, dogs and rats despite decreased androgen. Clearly, it is not only serum androgen concentration that determines whether or not there is abnormal prostate growth. This also is true of CRPC; heterogeneous cells evolve with various mutations and splice variants in AR after androgen deprivation. Therefore, traditional treatment with anti-androgen targeting to the ligand-binding domain of AR typically is not effective or curative for CRPC. Beside AR modifications, CRPC cells acquire various epigenetic changes and overexpression of stemness genes that make these cells extremely heterogeneous. Therefore, to treat CRPC effectively, anti-androgens are needed that target the AR transactivation domain or completely degrade ARs (wild-

type, mutated and splice variants). The hope is that these, used in combination with other chemotherapy, will be able to create a lethal environment that kills all CaP cells irrespective of their heterogeneity. Until we understand the cellular heterogeneity in CRPC, it will remain difficult to cure this deadly disease.

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