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Molecular processes leading to aberrant androgen receptor signaling and castration resistance in prostate cancer

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Abstract

Hormone therapies targeting androgen receptor signaling are the mainstay of treatment for patients with advanced prostate cancer. The length of clinical remission induced by hormone therapies varies substantially among treated patients. Why some patients progress rapidly after treatment while others benefit with prolonged remission is a question that remains unsolved. The androgen receptor signaling pathway is the key molecular determinant of castration resistance, and a key target for prostate cancer drug design. Recent advances in characterizing molecular processes leading to the development of castration-resistant prostate cancer, including the discovery of multiple androgen receptor splicing variants, offer opportunities for rational development of new clinical tools or approaches to predict, monitor or control/prevent prostate cancer progression in the castrate setting.

Keywords

androgen receptor; AR; AR signaling; AR splicing variants; castration-resistant prostate cancer; CRPC; hormone therapy; prostate cancer

In 1941, Huggins and Hodges demonstrated the clinical efficacy of hormonal manipulation for the treatment of metastatic prostate cancer [1]. Androgen suppression therapies (ASTs) have since become the mainstay of systemic treatment for advanced prostate cancer. The clinical benefits of androgen suppression are well established when AST is applied in the setting of metastatic disease [2]. Most patients respond to AST by entering into clinical remission, although they eventually experience disease relapse and develop more aggressive tumors, commonly termed ‘hormone-refractory prostate cancer’ or ‘castration-resistant prostate cancer’ (CRPC). In addition to its standard use in patients with metastatic disease, AST is increasingly being applied to prostate cancer patients with a wide spectrum of clinical phenotypes, including men experiencing biochemical recurrence after definitive primary treatment (e.g., radiation or surgery) [3], indicated by a re-elevation of serum

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prostate-specific antigen (PSA) but no other evidence of disease. Annually, an estimated 70,000 men in the USA alone develop biochemical recurrence [4], which is a source of patient anxiety that often provokes consideration of AST treatment options [5].

This widespread use of AST in patients with advanced prostate cancer has prompted a debate regarding the clinical benefit associated with early (before the onset of clinical metastasis) versus late AST, a subject of investigation in numerous ongoing clinical trials [6]. Given that the efficacy of AST is limited by time and the severe side effects are additive with longer duration of AST, these clinical trials seek to determine the optimal timing of AST in various clinical settings [6]. Some clinical trials have provided evidence supporting the survival benefit of early ASTs; however, such benefits have been relatively well established in only a subset of patients [3,7,8], suggesting that the clinical benefit may vary among patients in different disease and treatment categories. Even among men with metastatic prostate cancer, for which AST is the established standard of care, the length of clinical remission induced by AST can range from months to more than 10 years [9]. Among those relapsed patients (i.e., CRPC patients), a subset (~30–60%) may continue to benefit from sequential second-line hormone therapies [10–14]. Although clinical parameters [3,8,15] may provide some prognostic utility in these treatment settings, there are currently no definitive clinical methods that can reliably predict responses to first- or second-line therapies. Therefore, although ASTs have been in use for 70 years, why some patients progress rapidly after treatment while others benefit with prolonged remission is still an unsolved question.

Endocrine approaches to the treatment of advanced prostate cancer have evolved over the last 30 years [16], yet the common therapeutic target remains the same. All existing endocrine therapies (Figure 1), including those currently under Phase III trials for the treatment of CRPC (e.g., MDV3100 and abiraterone), target the androgen receptor (AR) signaling pathway [11–14]. More precisely, a specific domain of the AR, the ligand-binding domain (LBD), is the intended target of all hormone therapy regimens (Figure 1). The importance of the AR as the key target for prostate cancer therapeutic development is supported by the prevailing explanation for endocrine therapy failure – sustained AR signaling despite castrate levels of androgens [16].

Multiple molecular processes contributing to sustained AR signaling in a castrate setting have been proposed and reviewed extensively [17–19]. Somatic androgen receptor mutations [20], AR gene amplification [21] and AR overexpression [22] may sensitize the AR to reduced levels of androgens or agonizing anti-androgens in CRPC. AR signaling is also enhanced by alterations in AR coactivators and corepressors [23], AR post-translational modifications [24] or AR cross-talk with other signaling molecules [25]. Under these mechanisms, AR signaling still requires, or is enhanced by, ligand binding to the AR LBD. With regard to the requirement for ligand binding, it is also known that existing androgen-suppression regimens do not lead to complete ablation of androgens, and that prostate cancer cells may synthesize androgens *de novo* or from adrenal androgen precursors [26]. Recently, we and others reported the discovery of multiple novel ARs that lack the LBD [27–29]. These AR Δ LBD variants are constitutively active (i.e., active in the absence of androgens) and mediate AR signaling, which is not targeted by any of the existing hormone therapy regimens (Figure 1). In addition, we detected elevated levels of the AR variants (by ~20 fold) in CRPC tissues, suggesting an alternative and potentially more direct explanation for hormone therapy resistance [27–29].

In this article, we will evaluate the evidence supporting each of the above mechanisms, with a focus on mechanisms evolving to activate the AR signaling pathway in the castrate setting. We will also evaluate other mechanisms that bypass the requirement of AR signaling, as

well as a complex immune-mediated model of castration resistance. Many of the key mechanisms are currently driving the development of novel therapies to treat CRPC. However, a lack of therapeutic response indicators is a clear limiting factor in the development of novel endocrine therapies for advanced prostate cancer. We discuss the implications of these recent research advances in addressing this unmet need in the management of advanced prostate cancer.

Molecular components of AR signaling

The biological effect of androgens is mediated through the AR, a member of the nuclear steroid hormone receptor super family. The human AR coding sequence was cloned in 1988, in three independent laboratories [30–32]. The cloning of human AR made it possible to study AR structure and function. The *AR* gene is located on the X chromosome (Xq11–12), spanning approximately 180 kb of DNA with eight canonical exons (exon 1–8) [33]. Like other nuclear steroid hormone receptors (e.g., estrogen receptor and progesterone receptor), the AR protein is organized into four discrete functional domains, an N-terminal domain (NTD), a highly conserved DNA-binding domain (DBD), a short hinge region and a moderately conserved C-terminal LBD [32,33]. Figure 2 illustrates the chromosomal organization and structure of the *AR* gene, the *AR* mRNA transcript and the AR protein domains. The AR NTD, encoded by exon 1, constitutes nearly 60% of the AR protein sequence and harbors a majority of the AR transcriptional activities and AR coactivator interaction interface [34]. The cysteine-rich AR DBD is composed of two zinc finger motifs, which are encoded by exon 2 and exon 3, respectively. The first zinc finger motif mediates the recognition of the specific nucleotide in the AR response element and facilitates DNA binding. The second zinc finger stabilizes the DNA binding. The hinge region, encoded by part of exon 4, contains a bipartite nuclear localization signal as well as important sites for phosphorylation, acetylation and degradation. The LBD, encoded by part of exon 4 and exons 5–8, mediates ligand binding [35–38]. Crystal structure of the AR LBD demonstrated that the C-terminus amino acids of AR assume a ligand-binding pocket by the ordered arrangement of 12 conserved α -helices [33,39].

Upon ligand binding, the AR undergoes a conformational change to a more active form, allowing subsequent nuclear translocation, homodimer formation and DNA binding [39]. Subsequent to AR binding to the AR response elements located on the target gene, essential AR cofactors (coactivators and/or corepressors) are recruited to the transcriptional machinery, which regulates gene transcription [40]. To date, more than 170 AR cofactors have been identified. Coactivators enhance and corepressors reduce the AR transactivation function by direct regulation of transcriptional control through physical interaction with general transcription factors and RNA polymerase, facilitating AR DNA binding and chromatin remodeling, and changing AR folding and AR subcellular localization [39,41].

AR gene amplification and AR overexpression in CRPC

AR gene amplification was first reported in the early 1990s [21]. Since then, the reported incidence of *AR* gene amplification has varied between 20 and 33% in CRPC patients, but very few cases have been reported in untreated primary prostate cancer [21,42]. Very recently, *AR* amplification was examined in circulating tumor cells derived from patients undergoing second-line hormone therapies [43,44]. These studies revealed a much higher rate (50–85%) of *AR* copy number gain in CRPC patients. The variation of the assessed *AR* amplification rate in the recurrent prostate cancer specimens could be explained by cohort differences, tumor heterogeneity and technical limitations in the early 1990s. The fact that *AR* gene amplification was rarely detected in the primary prostate tumor suggests that *AR* gene amplification contributes to the development of CRPC. Amplification of the wild-type

AR (i.e., no mutations) was associated with a substantially increased level of AR mRNA and protein [42,45], although increased AR protein cannot be completely explained by AR gene amplification alone [45–47]. Interestingly, AR amplification was more frequently observed in patients who initially responded to hormone therapies and later gradually developed resistance, but was rarely seen in those who displayed no initial response to androgen deprivation or those who developed resistance in less than 12 months [42].

Since existing hormone therapy regimens do not completely deplete androgens, an increased level of wild-type AR protein as a result of AR gene amplification or other mechanisms may confer an elevated response to a diminished level of testosterone or dihydrotestosterone (DHT), therefore, causing a survival and growth advantage upon castration. Based on the finding that the AR gene is the only gene consistently upregulated during progression to CRPC in multiple xenograft models, Chen *et al.* presented evidence supporting increased AR expression being both required and necessary for the progression of prostate cancer to a castration-resistant state [22]. In addition, an increased AR level can convert an AR antagonist (the anti-androgen bicalutamide) to an agonist [22], suggesting an alternative explanation (other than AR mutation, see later) for the well-known paradox termed ‘anti-androgen withdrawal response’ [48], in which tumor regression was observed in approximately 50% of patients undergoing complete androgen blockade (luteinizing hormone-releasing hormone analog plus anti-androgen) following discontinuation of anti-androgens. This pivotal study by Chen *et al.* has motivated the search for second-generation anti-androgens with greater affinity, using cells that overexpress AR. Examples of second-generation anti-androgens include RD162 and MDV3100 [11]. Both compounds inhibit AR nuclear localization, AR DNA binding and AR coactivator recruitment [11]. MDV3100 is currently under Phase III clinical trials for the treatment of CRPC [11,14,49,50]. Another example of a second-generation anti-androgen that is currently in Phase I clinical trials is TOK-001 (VN/124-1) [51,52]. TOK-001 possesses several anticancer properties that target the AR: direct AR antagonism, androgen suppression via CYP17 inhibition (see later) and AR protein downregulation [51,52]. Interestingly, TOK-001 also inhibits growth of AR-negative prostate cancer cells.

A general consensus that further supports the importance of AR expression in CRPC is that increased AR protein expression is characteristic of aggressive prostate cancer in various disease settings. However, study results that rely on immunohistochemical (IHC) assessment of AR expression need to be interpreted with caution, as differences in the performance of the anti-AR antibody as well as tissue processing methods related to the specific treatment setting can dramatically influence the results. Higher AR expression was observed in more aggressive prostate tumors when compared with less aggressive tumors, and thus may serve as a prognostic IHC marker in patients treated with surgery [53,54]. Using tissue IHC data, Donovan *et al.* demonstrated that AR levels at baseline (i.e., detected at the time of surgical treatment but prior to hormone therapy) were positively associated with shorter time to PSA progression after AST [53]. However, IHC studies in CRPC specimens did not show significant correlation between AR protein expression level and patient survival [46,55,56]. A recent study found significantly lower AR protein expression in the metastatic CRPC tumors compared with the primary prostate tumor [57]. It is worth noting that in this study, an AR antibody specific to the AR LBD was used, therefore detecting only full-length AR proteins with an intact LBD. In light of the presence of multiple AR splicing variants that lack the AR LBD (see later) [27–29], future studies comparing results derived from the use of multiple AR antibodies may reveal findings suggestive of the relative importance of the full-length AR and the AR variants.

AR gene mutation

Androgen suppression therapy also seems to confer a selection pressure for somatic *AR* gene mutations because *AR* gene mutations were found in a higher frequency in patients with more advanced disease [58–64]. Many *AR* gene mutations have been reported in prostate cancer tissues and are summarized together with *AR* gene mutations associated with other endocrine diseases in the McGill Androgen Receptor Gene Mutation Database [65]. The *AR* gene mutations in the LBD may alter ligand binding, leading to AR activation by AR antagonists and other ligands that do not activate wild-type AR. AR LBD mutations may explain the paradoxical anti-androgen withdrawal response, manifested as PSA decline and tumor regression in a substantial proportion of CRPC patients receiving combined androgen blockade (luteinizing hormone-releasing hormone and anti-androgen therapies) after the agonist activities of the anti-androgens were removed [48]. Therefore, this gain-of-function mutation may lead to a growth advantage in the absence of sufficient AR natural ligands, or the presence of anti-androgens. For example, the first characterized *AR* gene mutation T877A was found in both LNCaP prostate cancer cells and in clinical tissues. AR-T877A can be activated by androgens, the AR antagonist flutamide, estrogens and progesterone [18,66,67]. This broadened ligand-binding capability of AR-T877A to progesterone has been confirmed by crystallographic studies [68,69]. However, subsequent investigations found no association between AR mutations detected in bone marrow specimens from CRPC patients with either anti-androgen withdrawal response or survival [60].

It is a general consensus that the incidence of AR mutation is relatively low (~10%) in CRPC specimens. As such, the role of the AR mutations in CRPC progression is not clear [61,63]. Technical limitations may explain some of the discrepancies in previously reported AR mutation rates [20,70]. It remains possible that a higher mutation rate might be revealed using more comprehensive approaches such as next-generation sequencing technologies, although such studies will almost certainly reveal the diversity and intratumor heterogeneity of various AR mutations, among which only a subset have been functionally characterized [20,58,59,71].

All naturally occurring AR mutants characterized so far contain a LBD (thus, they still require the presence of ligands for AR signaling) with one exception. Ceraline *et al.* isolated an AR mutant – Q640X. AR-Q640X does not contain a LBD owing to a nonsense mutation immediately after the coding sequences for the DBD. This mutant mediates AR signaling independent of any ligands [72]. The prevalence of this constitutively active AR mutant in CRPC, however, has yet to be fully characterized.

AR splicing variants

Shortly following the cloning of human AR in 1988, a number of laboratories generated AR deletion mutants with intact NTDs and DBDs but lacking LBDs [37,73]. These artificially generated AR Δ LBD variants were demonstrated to be constitutively active. Although findings from Ceraline *et al.* provided one example that such variants could occur naturally through a simple mutation [72], similar mutations have not been verified. As a result, it is impossible to reliably detect the AR Δ LBD variants in clinical specimens. Recent discoveries of naturally occurring AR variants derived from alternative splicing made it possible to detect and study AR Δ LBD variants [27–29]. These studies, including one from our group, suggest that AR signaling could occur in the complete absence of ligand binding, owing to the expression of constitutively active AR Δ LBD variants. These AR variants are encoded by newly discovered AR transcripts with ‘intronic’ insertions (i.e., cryptic exons) downstream of the coding sequences for the AR DBD. Owing to the premature stop codons present in those cryptic exons, the translated AR proteins are truncated, retaining the NTD, DBD and a

short variant-specific peptide, but lacking the LBD. These AR Δ LBD variants mediate genomic functions despite the absence of androgens or the presence of anti-androgens. The functional differences between the wild-type full-length AR and the AR variants are illustrated in Figure 3.

The clinical relevance of these truncated AR variants has also been characterized. Such studies were made possible by variant-specific mRNA probes that detect the AR variants but not the full-length AR, or *vice versa*, as well as antibodies developed against variant-specific peptides. Significantly elevated expression of the AR variants, measured at both the mRNA and protein levels, was detected in CRPC specimens, as well as in a subset of prostate specimens from untreated (i.e., hormone naive) patients who experienced biochemical recurrence after radical prostatectomy [28,29]. Thus, progressive acquisition of ligand-independent AR signaling, through AR splicing, may occur during prostate cancer progression in both castrate and noncastrate conditions. Collectively, these studies provide strong evidence supporting an alternative and potentially more direct explanation for castration resistance in prostate cancer.

The relative importance of the seven AR Δ LBD variants we reported in our study [29] has been further characterized in more detail. The seven AR variants are named numerically, from AR-V1 to AR-V7, according to the genomic positions of the variant-specific intronic sequences. The two AR transcript variants identified by Dehm *et al.*, named AR1/2/2b and AR1/2/3/2b [27], contain transcribed 'intronic' sequences identical to those in AR-V3 and AR-V4, respectively. The three AR variant transcripts identified by Guo *et al.*, named AR3, AR4 and AR5 in that study [28], contain transcribed 'intronic' sequences identical to those in AR-V7, AR-V1 and AR-V4, respectively. Therefore, the seven AR variants identified in our study match all those reported in other studies. Preliminary data presented in Table 1 summarize the diverse characteristics among the seven AR variants in terms of their abundance in clinical specimens, their suitability for antibody development and their functional activities.

While the discovery of the AR variants made it possible to detect them in clinical specimens, whether these discoveries can be translated into the clinical setting as molecular indicators of therapeutic response awaits further investigation. Carefully designed studies addressing whether AR variant expression levels can be used to predict or indicate hormone therapy response have not yet been performed. While the detection methods are being developed and can be optimized for these purposes, such studies entail the application of the detection methods in a clinical cohort that is uniformly managed and followed up after endocrine therapy regimens. Existing clinical trials assessing the second-line hormone therapies may provide the platform for such studies.

Role of AR cofactors

Alterations in AR coregulator levels and function may contribute to the development of CRPC. Studies regarding the AR cofactors in prostate cancer have been reviewed previously [23]. Upregulation of coactivators was proposed as a mechanism to sensitize the AR to low-level androgens [49]. For example, several AR coactivators, including TIF2, SRC1 and TIP60, are overexpressed in CRPC [74,75]. In the presence of overexpressed TIF2, AR signaling was activated by physiological concentrations of adrenal androgens [74]. Depletion of TIF2 reduced the AR target gene expression and also slowed down the proliferation of both AR-dependent and AR-independent prostate cancer cells [76]. Clinicopathological studies also showed that aberrant expression of several AR cofactors was associated with poor prognosis [23]. Heemers *et al.* showed that androgen controls

approximately over 30% of cofactor genes in prostate cancer cells [77], suggesting that a coordinated AR and AR cofactor network regulates AR signaling.

Cross-talk with growth factor signaling pathways & AR phosphorylation

The cross-talk between growth factor and AR signaling pathways in prostate cancer cells has been well documented. Considerable evidence supports the ability of growth hormones and cytokines to activate AR. For example, IL-6, KGF, EGF and IGF-1 can each stimulate AR target gene expression in the absence of the AR ligand [78–80]. Evidence from these studies also suggests that the MAPK and PI3K/AKT pathways may be the mediating pathways [78,79]. Importantly, expression levels of IGF-1, IL-6 and EGF are elevated during progression to CRPC [81–83]. Activation of the MAPK and PI3K/AKT pathway may result in phosphorylation of AR coactivators, such as SRC-1 and TIF2 [78,79], or the AR protein itself (see later).

Androgen receptor agonists induced AR phosphorylation at the seven serine residues (serines-16, -94, -81, -256, -308, -424 and -650) [84]. R1881 treatment leads to phosphorylation of all these serine sites except serine-94, which is constitutively phosphorylated. Evidence of direct phosphorylation of AR by growth factors has been presented. The expression of HER2/neu, a member of the EGF receptor family, is increased in a subset of hormone-refractory LAPC4 xenografts and overexpression of HER2 in androgen-dependent prostate cancer cells promotes androgen-independent cell growth [85]. Yeh *et al.* have shown that AR can be phosphorylated by MAPK, which mediates AR transactivation by HER2/neu [86]. *PTEN* tumor-suppressor gene is frequently mutated and functionally inactivated in metastatic CRPC [87]. With loss of *PTEN* function, AKT activity is enhanced and promotes prostate tumor growth in castrated mice [88]. AKT binds to AR and phosphorylates serine-210 and serine-790 [89,90]. AKT activation provides additional pathways that mediate activation of AR by HER2/neu, as blocking of the AKT pathway abrogates the HER2/neu-induced AR signaling in the absence of androgens [90]. AR serine phosphorylation by MAPK or AKT may sensitize AR to low levels of DHT [91]. Increased levels of phosphorylated AKT (pAKT⁴⁷³) and phosphorylated AR (pAR²¹⁰) during the transition from hormone-naïve to castration-resistant prostate cancer has been associated with shortened disease-specific survival [92].

In addition to serine and threonine AR phosphorylation, AR tyrosine phosphorylation has been reported [93–95]. AR can be phosphorylated at tyrosine-534 by Src tyrosine kinase in response to several growth factors, including EGF, heregulin and IL-6 [93,94], or at tyrosine-267 and tyrosine-363 by Ack1 [95]. AR phosphorylation at tyrosine-267 regulates androgen-independent recruitment of AR to the androgen-responsive enhancers and transcription of AR target genes in the absence of androgens [95,96]. In clinical specimens, an increase of Src family kinase activity during the development of CRPC is associated with worse treatment outcomes [97].

Studies of AR signaling cross-talk in CRPC have led to clinical studies targeting IL-6, IGF receptors, EGF receptor and Src kinase in the treatment of CRPC [98–100].

Intracrine androgen production

Castration blocks gonadal testosterone synthesis, but androgens can also be synthesized from adrenal androgens in prostate tissues using steroidogenic enzymes expressed in target cells [101,102]. Initial studies carried out in animal models demonstrated that by increasing the serum dehydroepiandrosterone concentrations in castrated animals, prostate weight and intraprostatic DHT levels were elevated compared with the control castrated animals [101]. It was assessed that approximately 40% of androgens in men are produced by peripheral

target tissue from adrenal steroid precursors [102]. In addition to adrenal androgens, prostate cancer cells may produce androgens *de novo*. Dillard *et al.* demonstrated for the first time that high-passage LNCaP cells are able to synthesize testosterone from cholesterol without depending on testicular and/or adrenal androgens [103]. Locke *et al.* showed that tumor explants isolated from CRPC tumors in LNCaP xenografts were capable of *de novo* conversion of [¹⁴C]acetic acid to DHT, using an *ex vivo* radiotracing assay coupled to HPLC radiometric/mass spectrometry detection [104]. In clinically relevant settings, Geller *et al.* reported for the first time that castration did not completely eliminate prostate cancer tissue DHT levels [105]. Studies from Mohler *et al.* confirmed these findings by measuring intraprostatic tissue testosterone and DHT levels in CRPC patients [106,107]. In these studies, DHT levels in the prostate tissues collected after androgen ablation therapy were decreased substantially (by 91% in one study and 82% in another study carried out by the same group, compared with hormone-naïve benign prostate), yet at a level sufficient to activate AR. Interestingly, intraprostatic testosterone levels remained at similar levels after ASTs [106,107]. Studies from Nishiyam *et al.* also demonstrated that after androgen deprivation therapy, prostatic DHT levels remained at approximately 75% of the pretreatment levels [108,109]. Other evidence supporting the role of intracrine tumor androgen synthesis is the upregulation of enzymes involved in androgen synthesis in the CRPC specimens [104,110,111]. However, simultaneous expression of the enzymes CYP17A1 and HSD3B1, both of which are essential for the synthesis of androgens from pregnenolone, can only be detected in a small portion of the castration prostate cancer tissues [112].

Based on the overall evidence presented above, it seems plausible to inhibit testicular, adrenal and intracrine androgen sources by targeting the metabolic enzymes in CRPC patient management [113]. CYP17 is one of the key enzymes in androgen synthesis in both adrenal gland and tumor tissues [114,115]. CYP17 consists of two enzymatic components, 17- α hydroxylase and C17,20-lyase. The former enzyme converts pregnenolone to 17 α -hydroxypregnenolone and progesterone to 17- α hydroxyprogesterone. The latter converts 17 α -hydroxypregnenolone to dehydroepiandrosterone, and 17- α hydroxyprogesterone to androstenedione [114,116]. Abiraterone is a potent CYP17 inhibitor. A Phase III trial evaluating abiraterone in the treatment of CRPC is ongoing [49,115,117]. Another example of a CYP17/20-lyase inhibitor that is under clinical trial (Phase I/II) is TAK-700 [201].

Other pathways that bypass the AR signaling

Proposed molecular mechanisms leading to CRPC can be summarized into two broad categories: those that still require signaling through the AR, and those that bypass the dependence on AR signaling. While multiple lines of compelling evidence have established that, in most cases, CRPC does not lose dependence on AR signaling but instead is characterized by sustained AR signaling despite castrate levels of serum testosterone (<0.2 ng/ml) [118], it is also known that prostate cancer cells may evolve mechanisms to bypass the AR signaling pathway. Pathways that bypass AR signaling mainly operate in rare tumors with distinctive morphologies, such as neuroendocrine tumors and small cell carcinoma [119]. Approximately 10% of prostate cancer tumors show significant neuroendocrine (NE) differentiation, which can be induced by androgen ablation [120]. NE cells produce a number of growth factors, including serotonin and bombesin, that promote the growth of near-by exocrine tumor cells via paracrine mechanisms [121,122]. Although NE differentiation often escapes clinical detection because NEs do not make PSA, it may contribute significantly to the development of CRPC due to its multiple drug-resistance features [120]. The NE pathway can be targeted by somatostatin analogs and has been explored in clinical trials showing objective clinical responses [123–125].

Overexpression of *BCL-2*, an anti-apoptotic gene, is another proposed AR-bypassing pathway mediating the development of CRPC. *BCL-2* is not normally expressed in the secretory prostate epithelial cells, but is expressed in CRPC [126–128]. Inhibition of *BCL-2* with antisense oligonucleotide prevented the emergence of CRPC in xenograft models [129]. IHC studies have demonstrated increased *BCL-2* expression in CRPC specimens compared with hormone-naïve prostate cancer tissues [127,128]. Docetaxel, the first US FDA-approved chemotherapy for CRPC, phosphorylates and inactivates *BCL-2* [100].

Role of inflammation in CRPC

In addition to the cell-autonomous mechanisms leading to the development of CRPC proposed above, a complex immune-mediated model of CRPC has been investigated. Approximately 20% of human cancers are caused by chronic inflammation [130]. Recent studies provide evidence supporting the role of inflammation in prostate cancer progression in the castrate setting. It was recently shown that inflammatory responses triggered by the death of androgen-deprived primary cancer cells contribute to progression to CRPC by activating I- κ -B kinase- β (IKK- β) in B cells, which leads to the production of cytokines that subsequently activate I- κ -B kinase- α (IKK- α) and STAT3 in the target cancer cells to enhance cell survival in androgen-deprived conditions [131]. In addition, activation of NF- κ B, a key mediator of inflammation, may result in constitutive AR activation [132]. Cyclooxygenase-2 (COX-2) is a proinflammatory enzyme necessary for prostaglandin synthesis. Analysis of data from the Radiation Therapy Oncology Group (RTOG 92–02) trial concluded that increased COX-2 expression was significantly associated with biochemical failure, distant metastasis and any failure in patients treated with androgen deprivation plus radiotherapy [133]. Use of COX-2 inhibitors combined with androgen therapy has been evaluated in clinical trials targeting patients with biochemical recurrence [134].

Expert commentary & five-year view

Multiple mechanisms have been proposed to explain the development of CRPC subsequent to ASTs. Sustained AR signaling despite castrate levels of androgens still remains the key mechanism for CRPC development. Future treatment regimens for CRPC will focus on effective inhibition of AR signaling. The existence of constitutively active AR splicing variants implies that existing regimens will not be sufficient to completely suppress AR signaling. Translational development of novel approaches targeting the AR splicing variants awaits further knowledge regarding the relative contribution of the AR variants and the full-length AR to the development of CRPC, as well as more detailed profiling and characterization of additional AR splicing variants that are yet to be discovered. A more complete understanding of the role of intracrine androgens in CRPC is needed. The complex immune-mediated model of CRPC will stimulate clinical trials combining immune therapy and hormone therapy given the recent FDA approval of sipuleucel-T (Provenge, Dendreon, WA, USA), the first immune therapy for metastatic prostate cancer.

The current trend in managing patients with advanced prostate cancer emphasizes the importance of the AR signaling pathway and its implications in disease management. In addition to the standard use in patients with metastatic disease, AST is increasingly being used in patients with a wide spectrum of clinical phenotypes. A substantial proportion of patients who failed first-line hormone therapies may continue to benefit from sequential second-line hormone therapies [10–12]. The relatively long treatment history of these patients in a contemporary clinical setting will drive the use of second-line endocrine approaches for the treatment of CRPC. Due to the lack of definitive clinical methods that predict individual responses, objective criteria have not been established to guide if and

when to commence first-line or second-line hormone therapies. The development of molecular indicators of castration resistance may address this unmet need in the clinical management of patients with advanced prostate cancer and in clinical trials assessing novel endocrine therapies. Given that the efficacy of AST is limited by time and the severe side effects are additive with a longer duration of AST, as well as the modest survival benefit with nonhormonal treatments, better characterization of appropriate candidates for the less toxic and potential beneficial effects of secondary hormonal therapies could provide a valuable tool for patients and clinicians.

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Figure 1. Hormone therapy regimens commonly used to treat advanced prostate cancer

The hypothalamus–pituitary axis controls testicular androgen synthesis by regulating release of LH. LHRH analogs disrupt this axis and leads to suppression of LH release. T is converted to DHT in the prostate by 5- α reductases (type I and II), which can be inhibited by finasteride and dutasteride. Adrenal androgens, mainly DHEA and DHEA-S, may be used by the prostate cells as precursors of androgens. Abiraterone and ketoconazole inhibit enzymes involved in steroid biosynthesis.

AR: Androgen receptor; DHEA: Dehydroepiandrosterone; DHEA-S: Dehydroepiandrosterone sulfate; DHT: Dihydrotestosterone; GnRH: Gonadotropin-releasing hormone; LH: Luteinizing hormone; LHRH: Luteinizing hormone-releasing hormone; T: Testosterone.

Figure 2. The human androgen receptor gene, transcript and protein structure

Canonical exons and coding regions (from the ATG translation start site to the TGA stop codon) in AR mRNA are indicated. The peptide sequence positions in relation to the four AR domains are marked.

AR: Androgen receptor; DBD: DNA-binding domain; LBD: Ligand-binding domain; NLS: Nuclear localization signal; NTD: N-terminal domain.

Figure 3. Differences in AR signaling mediated by the full-length AR with intact LBD, and an AR variant that lacks LBD

Activation of the full-length AR requires binding of androgens. Upon androgen binding, cytoplasmic AR undergoes conformational changes, dissociates from the HSP90 chaperone, and enters into the nuclei to initiate gene transcription. The AR splicing variant lacks the ligand-binding domain and is not expected to associate with cytoplasmic chaperones, leading to ligand-independent nuclear localization and activation. While the full-length AR forms homodimers, it is not known whether AR variants also undergo dimerization. AR: Androgen receptor; DHT: Dihydrotestosterone; P: Prostate-specific antigen; T: Testosterone.

Clinical relevance, functional relevance and other characteristics of the seven AR variants.

Table 1

AR variant	Peptide length [†]	Transcript abundance	Activity in PC-3 cells [‡]	Activity in LNCaP cells [‡]	Antibody developed [§]	Endogenous protein detection
AR-V1	19	High	No	Yes	Yes	Not detected
AR-V2	19	Not detected [¶]	Unknown [#]	Unknown	Yes	Not detected
AR-V3	53	Medium	Unknown	Unknown	Yes	Unknown
AR-V4	53	Not detected [¶]	Unknown	Unknown	Yes	Unknown
AR-V5	1	Low	Unknown	Unknown	No	Unknown
AR-V6	6	Low	Unknown	Unknown	No	Unknown
AR-V7	16	High	Yes	Yes	Yes	Yes

[†] Detailed variant-specific C-terminal peptide sequences following AR DNA-binding domain are available in Figure 1 of Hu *et al.* [29].

[‡] Determined by prostate-specific antigen reporter assays using constructs containing the full-length coding sequences of the AR variants.

[§] Antibodies were developed using variant-specific peptide sequences. Note that AR-V1/V2 and AR-V3/V4 have identical C-terminal variant-specific peptide sequences although the transcripts differ by the presence or absence of exon 3 duplication.

[¶] AR-V2 and AR-V4, which contain a duplicated exon 3, are expressed only in CWR22Rv1 cells and have not been detected in clinical specimens.

[#] Unknown: not yet verified.

AR: Androgen receptor.