

Partners in crime: deregulation of AR activity and androgen synthesis in prostate cancer

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Prostate cancer remains a leading cause of cancer death, as there are no durable means to treat advanced disease. Treatment of non-organ-confined prostate cancer hinges on its androgen dependence. First-line therapeutic strategies suppress androgen receptor (AR) activity, via androgen ablation and direct AR antagonists, whereas initially effective, incurable, ‘castration-resistant’ tumors arise as a result of resurgent AR activity. Alterations of AR and/or associated regulatory networks are known to restore receptor activity and support resultant therapy-resistant tumor progression. However, recent evidence also reveals an unexpected contribution of the AR ligand, indicating that alterations in pathways controlling androgen synthesis support castration-resistant AR activity. In this report, the mechanisms underlying the lethal pairing of AR deregulation and aberrant androgen synthesis in prostate cancer progression will be discussed.

Prostate cancer and AR addiction

To date, prostate cancer remains the second leading cause of cancer death and the most frequently diagnosed malignancy in the male population in the USA [1]. Locally confined prostate cancers can be effectively treated either by surgical resection or radiation therapy [2]. However, non-organ-confined tumors represent a significant clinical challenge, accounting for significant morbidity. Although the underlying mechanisms are not well understood, prostate cancers respond poorly to standard anti-mitotics used for chemotherapy. Thus, the first line of clinical intervention for patients with non-organ-confined disease capitalizes on the established addiction of prostate cancers to androgen receptor (AR) signaling, and consists of a variety of mechanisms to ablate AR function [3–8]. These regimens are initially effective, resulting in AR activity suppression and tumor regression. However, incurable, ‘castration-resistant’ prostate cancers (CRPC) develop in patients with disseminated disease within a median time of 2–3 years, as a result of reactivation of AR activity [4,9]. Based on these clinical observations, there has been an intensive effort in the field to discern the mechanisms by which AR is reactivated in recurrent disease and to develop novel strategies to thwart this process.

AR regulation in prostate cancer

As a member of the steroid receptor subclass of nuclear receptors, AR functions as a ligand-dependent transcription factor (Figure 1) [4,10]. In the absence of ligand (androgen) binding, the receptor is present diffusely throughout the cytoplasm and held in an inactive state in association with chaperones such as heat shock proteins (HSPs) [11]. Although testosterone is the most prevalent androgen present in sera of human males, it is converted into 5 α -dihydrotestosterone (5 α -DHT), a higher affinity ligand for AR, in prostatic epithelia or prostatic adenocarcinoma cells [12]. The K_d of testosterone for AR is 10⁻⁹ M whereas that of 5 α -DHT is 10⁻¹¹ M. Ligand binding releases AR from HSPs, facilitating AR homodimerization, rapid nuclear translocation, post-translational modification and receptor stabilization [11]. Activated AR dimers subsequently bind DNA at specific sequences termed ‘androgen-responsive elements’ (AREs), serve as a platform for recruitment of co-activators and basal transcriptional machinery, and initiate a program of gene-transcription that results in diverse biological outcomes depending on cell context [13–15]. To date, the best characterized AR target gene is *KLK3*, which encodes the PSA (prostate-specific antigen) protein. The ability to monitor PSA as a marker of AR function has had a major effect on diagnosis and management of human disease [16,17]. Detection of serum PSA, a serine protease secreted by the prostate, affords an easy mechanism through which to assess prostate-specific AR activity. Thus, PSA screening is widely used to monitor disease development, tumor progression, and response to therapeutic intervention. Recent advances revealed that AR requires cooperating ‘pioneer’ factors such as FoxA1 to enhance transcription, and that the predominant sites of AR action lie outside the classic promoter regions of androgen-responsive genes; in fact, AR function appears to be largely manifested through sites of action that lie in distal enhancers located in intragenic, intronic and intergenic regions [18,19].

Although challenges exist for matching identified sites of AR binding to transcriptional output or disease relevance, several recent advances have revealed the divergent function of AR in CRPC (recurrent prostate cancer). First, Wang *et al.* elegantly showed that in CRPC cells, AR occupies binding sites that overlap with but are

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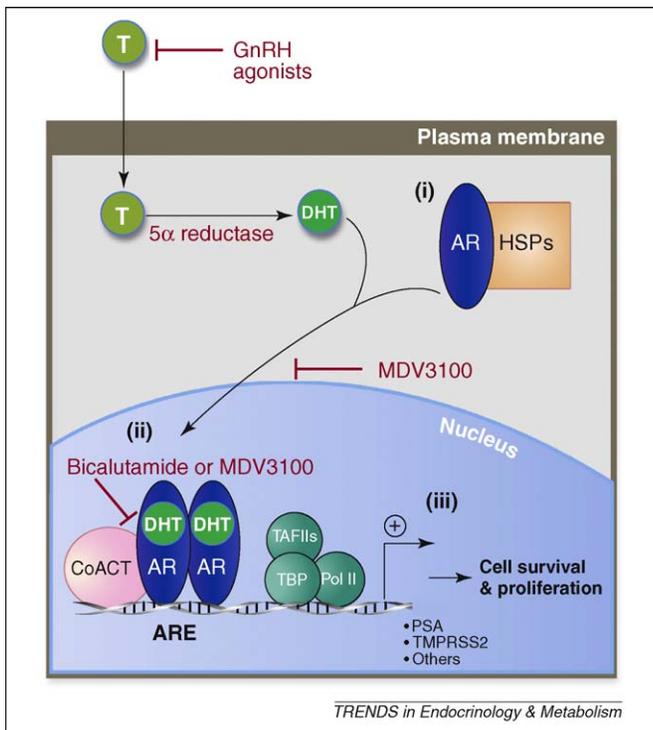


Figure 1. AR regulation in prostate cancer. Once activated by ligand (testosterone or DHT) binding, (i) the AR is released by chaperones (including HSPs), translocates to the nucleus, (ii) binds to DNA at androgen response elements (AREs) and recruits a series of co-activators (Co-act) that facilitate formation of active transcription complexes. (iii) The resultant gene expression program outcomes are dependent on cell context and include secretion, differentiation, survival, migration and proliferation. AR activity is targeted in invasive disease through depletion of testicular ligand synthesis (e.g. GnRH agonists), often used in combination with direct AR antagonists (e.g. bicalutamide). A novel AR antagonist, MDV3100, both competes with AR for androgen binding and reduces nuclear accumulation of AR.

distinct from those observed in tumor cells that are responsive to hormone ablation. Remarkably, AR appears to be enriched in castration-resistant cells to regulate genes whose products control transitions into and through mitosis. One target gene of relevance, *UBE2C*, is required for castration-resistant cell growth, and is overexpressed in clinical CRPC specimens [18,19]. Second, resurgent AR activity in VCaP xenograft models after castration promotes recurrent ETS transcription factor expression from the transmembrane protease, serine 2 (TMPRSS2):ERG chromosomal translocation present within this model system [20]. Given the high frequency of TMPRSS2:ERG translocations in prostate cancer and the importance of ERG signaling for tumor phenotypes, these findings are of clinical significance. Most recently, two independent groups demonstrated that AR activation induces a close proximity of the TMPRSS2 and ERG chromosomal loci, and in the presence of ionizing radiation, actually promotes formation of the TMPRSS2-ERG chromosomal translocation [21,22]. These striking findings present a model in which AR activation binds chromatin to alter gene expression, but by doing so, actively supports chromosomal aberrations. Combined, it is clear that the ability of AR to bind AREs and elicit a resultant gene expression program is crucial for both early stage and castration-resistant disease, and that these gene-expression profiles might be different.

AR as the primary therapeutic target for non-organ-confined disease

The biochemical goal of first-line intervention for non-confined tumors is to effectively suppress the trans-activation potential of AR, regimens collectively referred to as androgen depletion or endocrine therapy [4,5,7]. GnRH agonists (e.g. leuprolide) represent the most frequently used means to deprive AR of ligand [7]. These drugs suppress the release of luteinizing hormone (LH) from the anterior pituitary, and prevent Leydig cell testosterone biosynthesis in the non-castrated male. Although these agents result in an initial spike of testosterone release [23], testicular androgen synthesis is subsequently suppressed, resulting in serum testosterone levels similar to those seen in surgically castrated men (<0.2 ng/mL) [24]. These strategies are sometimes used in combination with direct AR antagonists such as bicalutamide, which compete for agonist binding [4,7]. In addition, the most commonly administered AR antagonists are thought to elicit conformational changes in the receptor that recruit co-repressors (rather than co-activators) to sites of AR binding, thus assisting in active transcriptional repression [25]. That regimens are initially effective is incontrovertible; the vast majority of patients with disseminated disease show marked PSA declines (thus providing biochemical evidence of AR inactivation) and tumor remission [4,7]. At the cellular level, hormone therapy results in both cell cycle arrest and cell death [15,26]. However, recurrent tumor formation is common, and there remains no effective, durable means to treat this latter stage of disease [4,7]. For CRPC, docetaxel shows modest efficacy in prolonging life but is not curative [27]. Development of detectable, recurrent tumors is almost invariably heralded by a preceding rise in serum PSA, thus indicating that AR is reactivated for disease progression [17]. Based on these observations and interrogation of AR function in models of human disease, it is widely accepted that AR is the key driver of prostate cancer progression and is required at all stages of disease for tumor maintenance [4,7]. As such, it is of the utmost importance to fully delineate the mechanism(s) by which AR becomes reactivated in recurrent disease, and to discern the underlying pathways that impinge on this process.

AR reactivation in castration-resistant disease

Over the past decade, it has become clear that there are several mechanisms through which AR can be inappropriately reactivated in the presence of GnRH agonists (chemical castration) and direct AR antagonists. Means of AR reactivation in such castration-resistant tumors can be loosely classified into mechanisms that: (i) impinge directly on AR modulation; (ii) involve alterations in AR cofactors or (iii) promote intratumor ligand synthesis (Figure 2). Each is considered below.

Direct AR modulation

AR deregulation Aberrant AR expression, alterations in upstream regulatory factors and/or upregulation of required cofactors each significantly contributes to resurgent AR activity in CRPC. First, the great majority of CRPCs show marked induction of AR mRNA and protein

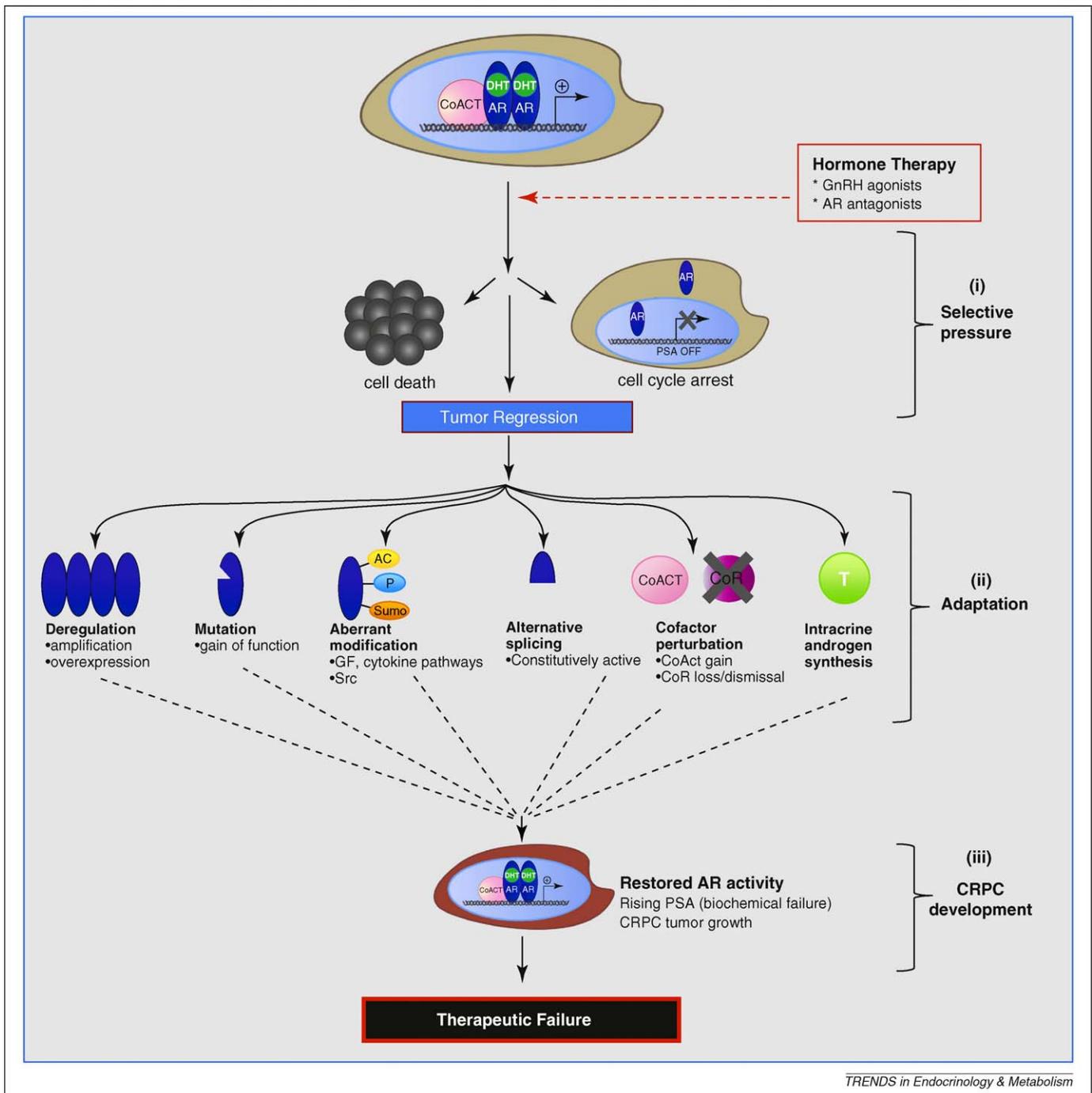


Figure 2. AR deregulation and CRPC development. (i) Successful targeting of AR activity by testicular androgen depletion and AR antagonists results in tumor cell death or cell cycle arrest. (ii) Subsequent adaptation events occur that restore AR signaling, including amplification or overexpression of AR, gain-of-function somatic mutation of AR, aberrant AR post-translational modification (frequently driven by growth factor (GF) or cytokine signaling), alternative splicing events that result in hyperactive receptors, cofactor dysregulation and/or intracrine androgen synthesis. (iii) Resurgent AR activity induces incurable CRPC tumor development and is most often heralded by a preceding rise in PSA (therapeutic failure).

expression [28,29]. A fraction can be accounted for by amplification of the AR locus itself [30–32], thus implicating genomic instability in CRPC. Little is understood about the additional mechanisms that promote enhanced AR expression. Loss of Pur-alpha, which can modulate the AR transcript through the 5' untranslated region, has been implicated in this process in model systems, but its clinical relevance remains uncertain [33]. Regardless of mechanism, it has been clearly demonstrated that deregulation of AR alone can

have a major effect on prostate biology and therapeutic response. Transgenic animals in which AR was modestly overexpressed in a prostate-specific manner showed evidence of both hyperplasia and carcinoma *in situ*, providing *in vivo* evidence for the pro-tumorigenic functions of AR overexpression [34]. Oncogenic transformation and progression to metastatic disease was observed in a transgenic model of prostate-specific AR-E231G (a mutant AR) expression, thus validating the concept that AR gain-of-function mutations are sufficient

to drive tumor development and progression [35]. In addition, analyses of xenograft models in which hormone therapy-sensitive tumors progressed to recurrent tumors after castration revealed that the major molecular change associated with CRPC was elevation of AR itself, reinforcing the hypothesis that disease progression is reliant on sustained AR signaling [36]. These same studies effectively demonstrated that AR induction alone is sufficient to bypass androgen depletion therapy and weaken the antagonist capabilities of bicalutamide. Collectively, these studies point to AR deregulation as a major mechanism of recurrent AR activity and CRPC formation.

AR mutation or alternative splicing Alterations of AR also occur that can either significantly alter the spectrum of ligands that act as agonists or bypass the need for ligand altogether. A large number of somatic, tumor-derived mutations of AR have been identified, of which the majority result in 'promiscuous' ligand binding, facilitating activation of the receptor by non-androgen steroid hormones (e.g. progesterone, estrogens, cortisol and weak androgens [6,9,37]). A subset of these somatic mutations also converts known AR antagonists into agonists. Clinical situations referred to as 'the anti-androgen withdrawal effect', in which cessation of therapies based on direct AR antagonism resulted in lower PSA levels, suggest that alterations in AR might change the cellular response to these therapeutically used receptor antagonists [38]. Indeed, a somatic mutation of AR, T877A, identified in human disease, results in flutamide-mediated receptor activation [39]. More recent studies identified mutations that result in receptor activation by bicalutamide [40], and analyses of CRPC specimens support the current hypothesis that specific AR antagonists select for development of specific AR somatic mutations [41]. Because the overall frequency and effect of somatic AR mutation in prostate cancer remains uncertain (and ranges from 8% to 25% in tumors analyzed), additional studies using relevant tissue (CRPC) are needed. In addition, it will be important to define the ligands that act as agonists for clinically relevant somatic mutants of AR. At present, it is clear that specific mutations are selected for during disease progression in a subset of tumors, resulting in increased ligand promiscuity and responsiveness, and active promotion of CRPC via non-androgens.

In addition, AR can be alternatively spliced in CRPC, resulting in receptors that decisively bypass the need for ligand [42–44]. These 'constitutively active' splice variants were first identified in prostate cancer cell lines, and were shown to result in both cryptic exon usage and exon exclusion [42–44]. Although the precise number and frequency of the alternatively spliced variants remains to be rigorously determined, those identified to date retain the most crucial transactivation domain of the receptor (AF1, located in the N-terminus) and the DNA binding domain, but are devoid of the ligand binding domain (LBD). It has been long appreciated that deletion of the LBD results in constitutively active AR [45], and revolutionary among these findings was the observation that LBD-deficient

splice variants are enhanced in CRPC. Because these mutants would not be amenable to inhibition by LBD-directed AR antagonists (such as bicalutamide, TOK-001 or MDV3100), upregulation of such AR splice variants presents a significant clinical challenge. Combined, it is apparent that alterations in the AR coding region, through either somatic mutation or alternative splicing of the resultant transcript, play significant roles in human disease progression.

AR post-translational modifications Recurrent AR activity can be achieved in the presence of hormone therapy through post-translational modification(s) that do not require alterations in the AR locus or mRNA processing. Not surprisingly, AR activity is modulated by disparate mechanisms that include serine/threonine phosphorylation, tyrosine phosphorylation, acetylation, ubiquitylation and sumoylation [9,46]. Some uncertainty remains with regard to the overall effect of individual modification events on subsequent modifications and total AR activity, but several key findings point to evidence for aberrant AR modification playing a role in human disease. For example, phosphorylated AR is associated with reduced survival in patients unresponsive to hormone therapy, thus implicating phosphorylation-derived AR modifications with disease progression [47]. Underlying mechanisms of aberrant phosphorylation events are emerging, and in many cases are attributed to growth factor receptor activation. Deregulated epidermal growth factor (EGF) activity can induce AR phosphorylation at Ser-578, resulting in castration-resistant receptor activity and tumor cell proliferation [48]. Other growth factors including insulin-like growth factor (IGF)1 (recently reviewed in [49]) bolster AR activity in a low-ligand environment, supporting the contention that under certain conditions, peptide growth factors support overall AR activity. These findings are of potential disease relevance, as IGF1 is locally induced in human disease [50]. Conversely, tyrosine phosphorylation of AR appears to be predominantly driven by oncogene activation, especially via Src. This phosphorylation event is found with higher frequency in castration-resistant tumors, and modeling of aberrant tyrosine phosphorylation supports the contention that deregulated tyrosine phosphorylation promotes ligand-independent AR activity and concomitant cellular proliferation [51]. Notably, EGF function is also partially dependent on Src-mediated AR tyrosine phosphorylation, supporting a role for multiple phosphorylation events in mediating growth factor-induced AR activation [51]. Intriguing new studies suggest that the tumor microenvironment might promote both events, as a neuroendocrine cell-derived protein (parathyroid hormone-related protein, PTHrP) appears to promote EGF and Src-mediated AR modification and resultant adaptation to a low androgen environment [52]. Neuropeptides released by this cell type appear to serve similar functions [53]. Alternatively, AR phosphorylation can be enhanced through altered phosphatase activity. Recent reports indicate that protein phosphatase (PP)1 associates with AR and regulates both receptor subcellular localization and stability [54]. These collective

observations underscore the importance of external signals in modulating nuclear receptor function through phosphorylation cascades.

In addition to phosphorylation, AR is regulated by ubiquitylation, sumoylation and acetylation events that might influence disease progression. The ubiquitin E3 ligase ring finger protein (RNF)6 promotes AR activity through selective modulation of cofactor recruitment (such as androgen receptor-associated protein (ARA) [54] and this function is enhanced in castration-resistant tumors [55]. Although similar observations were reported with TRIM68 [56], the Mdm2-mediated ubiquitylation of AR results in receptor destabilization and loss of activity [57]. Thus, different ubiquitylation events appear to result in disparate effects on AR activity, and the underlying events that control these processes are incompletely understood. By contrast, conjugation of SUMO-1 to AR occurs rapidly after androgen binding, and cleavage of this process by SENP1 (SUMO-specific protease 1) and SENP2 promotes gene-specific AR activation [58]. It has been suggested that this post-translational modification helps 'fine tune' receptor activity. It will be of interest to determine how this process is regulated in human disease.

Alterations in AR cofactors

AR cofactors are *cis*-acting transcriptional modulatory proteins that substantially influence AR function. Given the prevailing posit that agonists induce recruitment of co-activators and antagonists promote conformational changes that promote recruitment of co-repressors, two hypotheses emerge. First, it is predicted that deregulation of co-activators or loss of co-repressors promote unchecked AR activity and disease progression. Second, it is predicted that changes in overall AR levels alter the stoichiometry of assembled complexes. Both predictions appear to be correct and have disease relevance.

Co-activators

To date, several hundred putative AR co-activators have been identified that enhance ligand-dependent AR activity in model systems. These co-activators serve pleiotropic functions at the chromatin level, including recruitment of basal transcriptional machinery, modulation of function or recruitment of chromatin remodeling enzymes (e.g. histone acetylase), and/or altered AR conformational changes. A subset of AR co-activators, including SRC1, SRC2, SRC3 and ARA70, appears to be enhanced in human disease [13,59–61]. The importance of deregulated co-activator expression might be significant, as excessive co-activator expression might not only sensitize cells to a low-hormone environment but also convert nuclear receptor antagonists into partial or full agonists. As AR is known to regulate a distinct transcriptional program in hormone-sensitive versus castration-resistant models of disease [19], an attractive hypothesis is that altered cofactor expression and/or regulation assist in eliciting the CRPC-specific transcriptional program.

Co-repressors

Loss of AR co-repressor function can convert therapeutic antagonists into agonists or promote agonist sensitization

(reviewed in [62]). Such events can occur through down-regulation of the co-repressor itself (such as occurs with prohibitin) [63,64], through dismissal of the co-repressor from the AR complex (as seen with nuclear co-repressor (NCoR) in the presence of macrophage-induced TAB2 signaling) [65], and/or through aberrant co-repressor mislocalization (such as observed with Hey1) [66]. In addition to AR modulation, co-repressors perturbed in prostate cancer might crosstalk with pathways directly associated with prostate cancer growth. For example, reduction of the AR co-repressor enhancer binding protein (Ebp)1 is not only associated with resistance to hormone therapy, but also alters the proliferative response to heregulin [67]. Similarly, crosstalk between the AR and cell cycle machinery is mediated by cyclin D1, which acts through cyclin-dependent kinase-independent functions to suppress AR activity [15,68]; this function of cyclin D1 is abrogated in human disease through downregulation, mislocalization or alternative splicing events [15,69]. As a result of such growth factor and cell cycle crosstalk functions embedded within selected AR cofactors, alterations within these cofactors might impinge both on AR signaling and connected pathways to yield a powerful pro-tumorigenic signal. Challenges remain with regard to discerning which of the many coregulators identified to date play crucial roles in recurrent AR activity and prioritizing those that could be developed as viable therapeutic targets.

Intratumor ligand synthesis

Most recently, it has become apparent that resurgent AR activity in CRPC can be accounted for in part through intratumoral androgen synthesis mediated by intracrine and paracrine mechanisms. As mentioned above, prostate cancer is a disease whose growth is dependent on the male sex hormone testosterone, which is converted in the prostate by steroid 5 α -reductase type 2 (*SRD5A2*) to yield the more potent androgen 5 α -DHT [70] (Figure 3). Importantly, prostate cancer is a disease of the aging man and thus grows under the influence of androgens even as testicular output of testosterone wanes. An alternative source of androgens in the aging man is the adrenal gland, in which circulating dehydroepiandrosterone (DHEA) is converted in the prostate via the sequential actions of 3 β -hydroxysteroid dehydrogenase (3 β -HSD/ketosteroid isomerase type 1 and type 2 (*HSD3B1*, *HSD3B2*), type 5 17 β -HSD (*AKR1C3*)); and 5 α -reductase type 2 to yield 5 α -DHT [71–73]. In CRPC, this intratumoral synthesis of androgens provides a mechanism by which the effects of a gonadotropin-releasing hormone (GnRH) agonist on Leydig cell testosterone synthesis can be surmounted. Indeed, increases in the androgenic synthetic pathway occur in CRPC as part of an adaptive response to androgen ablative therapy [74–76].

The role of intratumoral synthesis in CRPC has been controversial because it does not adequately explain why AR receptor antagonists (e.g. flutamide and bicalutamide) failed and why early chemopreventive trials of finasteride (a selective 5 α -reductase type 2 inhibitor) decreased prostate cancer incidence but resulted in the appearance of a more aggressive disease in some patients [77]. Proponents of the intratumoral synthesis of androgens point out that

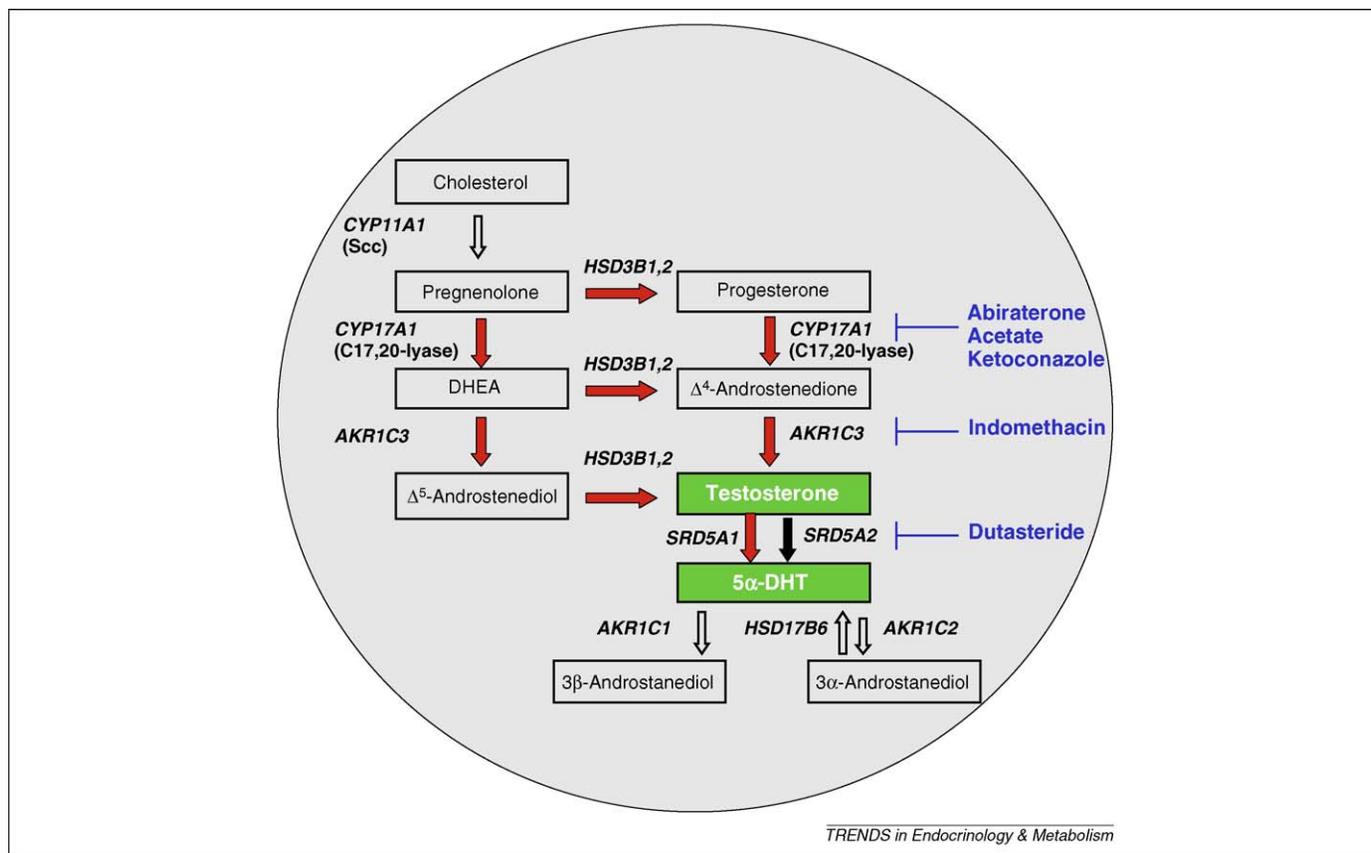


Figure 3. Altered androgen biosynthesis and CRPC. High-affinity ligands for the AR are shown in green boxes. Gene names are in italics. Steps in the classic pathway that show consistent elevated transcript expression in metastatic or recurrent tumors are marked with a red arrow and those with decreased transcript level are shown with a black arrow. Changes in steps indicated by the open arrows need to be further studied. Drugs that are available for clinical use that can block each of the major steps in androgen biosynthesis are indicated in blue. Dehydroepiandrosterone (DHEA) can also be of adrenal origin and 3 α -androstanoediol might be produced by the backdoor pathway on route to 5 α -DHT. Only the final step in the backdoor pathway to 5 α -DHT is shown for clarity.

testosterone and 5 α -DHT are very potent hormones, and low concentrations might be sufficient to outcompete the effects of low affinity AR antagonists and thus activate AR [78]. In addition, the more aggressive tumors observed in the original finasteride prostate cancer chemopreventive trial are now widely accepted as being the result of a sampling artifact due to the increased sensitivity of biopsies to detect cancer in the drug arm [79]. Studies on the use of finasteride to reduce intraprostatic 5 α -DHT show that hormone levels are suppressed by only 68–86%, suggesting that other routes to this hormone exist [80]. Two routes to the synthesis of 5 α -DHT that are independent of *SRD5A2* are steroid 5 α -reductase type 1 (*SRD5A1*) and retinol dehydrogenase (RoDH)-like 3 α -hydroxysteroid dehydrogenase (HSD) (*HSD17B6*) [80,81] (Figure 3). The latter enzyme catalyzes the back (oxidative) conversion of 3 α -androstanoediol to 5 α -DHT. The recognition that two 5 α -reductase isoforms are involved in intraprostatic synthesis of 5 α -DHT has led to the development of dutasteride, which inhibits both isoforms. Dutasteride is currently in clinical trials for the treatment and prevention of prostate cancer [80]. Earlier studies with dutasteride to treat benign prostatic hyperplasia indicated that it failed to reduce serum DHT levels altogether [82], and intraprostatic levels of DHT fell from 3.23 ng/g to 0.21 ng/g [83]. Alternatively, 5 α -DHT can be formed by the ‘backdoor pathway’ in which 3 α -androstanoediol is oxidized to 5 α -DHT via RoDH-like 3 α -HSD. In this pathway, Δ^4 -andros-

tene-3,17-dione and testosterone are not precursors of 5 α -DHT [84,85]. This pathway starts with the conversion of pregnenolone to progesterone catalyzed by 3 β -HSD2 (*HSD3B2*), formation of 17 α -hydroxyprogesterone catalyzed by 17 α -hydroxylase (*CYP17A1*), 5 α -reduction to yield 5 α -pregnane-17 α -ol-3,20-dione (catalyzed by 5 α -reductase isoforms), and 3-ketone reduction to yield 5 α -pregnane-3 α ,17 α -diol-20-one (catalyzed by *AKR1C2*) followed by *CYP-17,20-lyase* (*CYP17A1*) to yield androsterone. Androsterone is then reduced to 3 α -androstanoediol by the action of *AKR1C3*. This ‘backdoor pathway’ is thought to be important in the aging man when adrenal output of these steroids contributes more to prostate steroidogenesis. Thus far, ‘RoDH-like 3 α -HSD’ (*HSD17B6*) has not been targeted for androgen ablative therapy in prostate cancer because its role has only been recently elucidated.

The importance of intratumoral androgen synthesis after chemical or surgical castration has gained credence based on several observations. First, crucial genes involved in androgen synthesis in the prostate are upregulated at the transcript level in CRPC. These include *HSD3B2* (1.8-fold increase); *AKR1C3* (5.2-fold increase), *SRD5A1* (2.1-fold increase); *AKR1C2* (3.4-fold increase); and *AKR1C1* (3.1-fold increase), where the latter two enzymes produce 3 α -androstanoediol and 3 β -androstanoediol, respectively, from 5 α -DHT [76] (Figure 3). These findings were observed in Affymetrix expression microarrays and validated by quantitative reverse transcription PCR [76]. In a separate

study, the relative expression of the following transcripts changed in castration-resistant metastases compared with primary prostate tumors; *CYP17A1* (16.9-fold increase), *HSDD3B2* (7.5-fold increase), *AKR1C3* (8.0-fold increase), *SRD5A1* (2.6-fold increase), and *SRD5A2* (9.4-fold decrease) [75]. These studies show that the ratio of AKR1C3:SRD5A2 transcripts increases, and this might result in a decrease in the ratio of 5 α -DHT:testosterone within tumor samples. It was found that primary prostate tumors from eugonadal patients had a 5 α -DHT:testosterone ratio of 10:1, whereas this ratio was reversed to 0.25:1 in metastatic tumors [75]. Importantly, testosterone levels measured by liquid chromatography–mass spectrometry in metastatic tumors are well within the range to stimulate AR. These studies suggest that in CRPC, the disease might become more dependent on testosterone than on 5 α -DHT.

The second piece of evidence showing the importance of intratumoral androgen synthesis after chemical or surgical castration comes from xenograft studies. Using a LNCaP (an AR-dependent prostate cancer cell line) mouse xenograft model for CRPC, increases in transcripts for androgen-synthesizing enzymes were observed after extended castration and were coincident with increased PSA [86]. These studies suggest that during tumor reoccurrence, not only is there an increase in local androgen synthesis but this is sufficient to cause the induction of androgen-sensitive genes. Importantly, there were additional changes in proteins responsible for the build-up of free cholesterol and cholesterol synthesis (low density lipoprotein receptor (LDLr), scavenger receptor (SR)B1, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, steroidogenic acute regulatory protein (StAR), acyl-coenzyme A:cholesterol acyltransferase-(ACAT)1 and 2, and ATP-binding cassette (ABC)A1) [87,88] and changes in the expression of side-chain cleavage enzyme (*CYP11A1*) [86], suggesting that *de novo* steroidogenesis from cholesterol might take place in CRPC. Metabolism studies in castration-resistant tumors provided evidence for *de novo* synthesis of 5 α -DHT from [¹⁴C]-acetate [86]. In addition, metabolism studies with [³H]-progesterone provided evidence that intermediates in the backdoor pathway to 5 α -DHT accumulate [74]. One caveat with these xenograft experiments is that in mice, *CYP17A1* is not expressed in the adrenal, placing additional selection pressure on these tumors to synthesize their own androgens. However, the importance of this work is that even under conditions in which the mice are castrated and the adrenal is not producing DHEA, the tumors adapt to make their own androgens. These data indicate that after chemical or surgical castration, CRPC can be promoted by intratumoral androgen synthesis and that *de novo* synthesis from cholesterol might also occur.

Third, abiraterone acetate (a CYP17 α -hydroxylase/CYP17,20 lyase inhibitor) has shown important clinical response in individuals with CRPC, leading to a reduction in bone metastases [89]. This response suggests that non-localized disease is still dependent on androgens, because this inhibitor blocks the conversion of either pregnenolone to DHEA or progesterone to Δ^4 -androstene-3,17-dione (Figure 3). This blockade could occur either in the adrenal or the prostate to prevent DHEA formation. Irrespective of where the blockade occurs, subsequent conversion of

DHEA to testosterone in the prostate is prevented. An important clinical outcome of abiraterone acetate was a further decline of one log unit in plasma testosterone levels in CRPC. Use of abiraterone acetate to treat CRPC can have the unintended consequence of inhibiting the conversion of pregnenolone to DHEA in the adrenal and lead to the diversion of pregnenolone into desoxycorticosterone, which is a potent mineralocorticoid with glucocorticoid activity. To prevent the overproduction of desoxycorticosterone, abiraterone acetate is usually co-administered with dexamethasone to suppress the adrenal–pituitary axis and block adrenocorticotrophic hormone formation [90]. Clearly, the effectiveness of abiraterone acetate has stimulated a re-emergence of therapeutic approaches to block adaptive androgen synthesis in CRPC.

Fourth, in a recent small clinical study involving 10 patients, 80% showed slower progression of CRPC when they were given ketoconazole (a less selective CYP17 α -hydroxylase/CYP17,20 lyase inhibitor than abiraterone acetate) in combination with dutasteride (Figure 3) [90]. A combination of agents that block androgen synthesis at several steps in CRPC could be a useful treatment strategy.

AKR1C3 is a prime therapeutic target downstream of CYP17 α -hydroxylase/CYP17,20 lyase. This enzyme catalyzes the penultimate step in testosterone biosynthesis in the prostate. Moreover, in metastatic disease we have seen that the ratio of 5 α -DHT:testosterone clearly favors testosterone accumulation, suggesting that as the disease progresses, testosterone might be the more dependent hormone [75]. These findings also raised the question of whether AKR1C3 is the only reductive 17 β -HSD expressed in prostate cancer. Type 3 17 β -HSD (*HSD17B3*), also known as androgenic 17 β -HSD, catalyzes the conversion of Δ^4 -androstene-3,17-dione to testosterone in the Leydig cells and was thought to be Leydig cell specific [91,92]. Recently, evidence has emerged that this enzyme is also expressed in prostate cancer, but based on transcript levels, aldo-keto reductase family 1 (AKR1)C3 appears to be the dominant player [75]. Interestingly, AKR1C3 is potently and selectively inhibited by indomethacin suggesting that NSAID analogs that do not inhibit cyclooxygenase (COX)-1 or COX-2 might be effective agents for CRPC [93]. Taken together, these findings make it apparent that alterations in androgen synthesis and androgen metabolism pathways are frequently observed in CRPC, presenting new opportunities for means to target AR activity and resultant tumor progression.

Conclusions and future directions

The studies described in this paper illustrate that deregulation of the mechanisms that control both AR activity and androgen levels promote disease progression and lethal tumor phenotypes. With this knowledge in hand, some crucial next steps and questions should be considered. First, how can the information be clinically translated? For example, if it is known that a patient harbors tumors with somatic mutations of AR, AR splice variants or altered cofactor expression, is this information useful for directing treatment options? Second, are the mechanisms that underlie recurrent AR activity mutually exclusive? It is likely that different tumors display a repertoire of these mechanisms. Thus, molecular profiling of the tumor might

ultimately have diagnostic and therapeutic value. In the future, a component of this molecular profiling must include sensitive and specific methods for measuring intratumoral androgen levels so that changes in the expression of androgen-synthesizing genes can be validated at the functional level and enzymes can be targeted with specific inhibitors. Third, can more potent ligands be produced that suppress receptor function given the known mechanisms of androgen and AR deregulation? Recently, the darylthiohydantoin RD162 and MDV3100 were developed as more potent ligands for AR than bicalutamide. These compounds also reduce the efficiency of nuclear accumulation of AR, and impair binding to androgen response elements and coactivator recruitment. Of the first 30 patients with CRPC treated with MDV3100, 13/30 (43%) showed sustained declines in PSA [78]. There is also promise in designing single agents that could block androgen biosynthesis and AR function simultaneously. Analogs of abiraterone acetate (e.g. 3-hydroxy-17-(1H-benzimidazole-1-yl)androst-5,16-diene) not only block CYP17 α -hydroxylase/CYP17,20-lyase but are also potent anti-androgens and cause marked downregulation of AR protein expression [94]. These agents are now entering clinical trial (TOK-001) and could be potentially important if CRPC is characterized by adaptive androgen synthesis and concurrent AR mutation to make the receptor more promiscuous to other ligands. Fourth, what mechanisms underlie the observed induction of enzymes that drive intratumor androgen synthesis? It will be important to determine the mechanisms by which these genes are induced and how their expression levels become permanently elevated. The promoters of the AKR1C1–AKR1C3 genes contain several half-sites for steroid hormone response elements. In addition, they all contain an anti-oxidant response element [95]. Whether inflammatory responses leading to generation of reactive oxygen species to activate the anti-oxidant response element occurs remains an open question. Fifth, can AR cofactors or other crucial effectors of ligand-bound receptor be developed as therapeutic targets? Advances in this area could provide new means to suppress AR activity, even in the presence of deregulated ligand synthesis.

In summary, it is clear that current means of therapeutic intervention for disseminated prostate cancer are circumvented in CRPC by both AR deregulation and aberrant androgen synthesis. This lethal pairing represents a major mechanism of tumor progression, and future efforts for development of new means to treat CRPC will need to consider both partners in crime.

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