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Moving Beyond the Androgen Receptor (AR): Targeting AR-Interacting Proteins to Treat Prostate Cancer

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Abstract

Medical or surgical castration serve as the backbone of systemic therapy for advanced and metastatic prostate cancer, taking advantage of the importance of androgen signaling in this disease. Unfortunately, resistance to castration emerges almost universally. Despite the development and approval of new and more potent androgen synthesis inhibitors and androgen receptor (AR) antagonists, prostate cancers continue to develop resistance to these therapeutics, while often maintaining their dependence on the AR signaling axis. This highlights the need for innovative therapeutic approaches that aim to continue disrupting AR downstream signaling, but are orthogonal to directly targeting the AR itself. In this review, we discuss the preclinical research that has been done, as well as clinical trials for prostate cancer, on inhibiting several important families of AR interacting proteins, including chaperones (such as HSP90 and FKBP52), pioneer factors (including FOXA1 and GATA-2), and AR transcriptional coregulators such as the p160 steroid receptor coactivators (SRCs) SRC-1, SRC-2, SRC-3, as well as lysine deacetylases (KDACs) and lysine acetyltransferases (KATs). Researching the effect of, and developing new therapeutic agents that target, the AR signaling axis is critical to advancing our understanding of prostate cancer biology, and to continuing to improve treatments for prostate cancer and for overcoming castration-resistance.

Keywords

Prostate cancer; androgen receptor; steroid receptor coactivators; castration resistance; androgen receptor signaling

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Introduction

Despite recent advances in treatment, prostate cancer – the second most commonly diagnosed cancer in men worldwide – remains a leading cause of cancer-related death in men, with over 220,800 new cases and 27,540 deaths reported in the United States annually (1-3). Prostate adenocarcinoma (PCa), as a primarily endocrine disease, is frequently dependent (at least initially) on androgens to maintain growth and viability of the tumor. This dependence on androgens provides a therapeutic opportunity. Blocking androgen production or action in PCa has served as the basis for PCa therapeutics for over 70 years (4). In the current standard of treatment, PCa that has recurred following surgical resection or radiation therapy is initially treated via reduction of gonadal androgen secretion, which has been well-documented to have clinical activity (4). This is accomplished either by surgical castration (orchiectomy) or by chemical castration through the use of gonadotropin-releasing hormone (GnRH) analogs. Despite an initial response to gonadal hormone suppression, PCa cells almost inevitably overcome this approach, recurring as what had been previously called a “hormone-refractory” or “androgen-independent” disease. These terms have largely fallen out of use in favor of “castration-resistant prostate cancer” (CRPC), as extensive studies have demonstrated that, frequently, these recurring cancers are neither hormone refractory nor androgen-independent. Instead, many cancers have adapted to castrate conditions, but – through a number of possible mechanisms – still largely depend on the androgen receptor (AR) signaling axis and in some cases, can still respond to androgens (5).

Androgens, including androstenedione, but especially testosterone and dihydrotestosterone (DHT), are particularly important for the maintenance of the normal prostate, and by extension, for the proliferation of PCa (especially early PCa). Androgens, similar to other steroid hormones, are initially derived from cholesterol and are gradually converted by enzymes into their chemically distinct structures. Testosterone, which is synthesized primarily in the testes but can also be synthesized from adrenal precursors, circulates in serum until it diffuses into cells where it is converted by 5 α -reductase into its significantly more active derivative, DHT (6). Of the three isoforms, type II 5 α -reductase is the dominant isoform in normal prostate and benign prostate hyperplasia (BPH), while type I 5 α -reductase is increased in PCa (7). Current therapeutic approaches include depriving PCa of testosterone and DHT by blocking 5 α -reductase via finasteride (which inhibits isoform II and III) and dutasteride (which inhibits isoform I and II). Although long-term studies demonstrate that these inhibitors can significantly decrease PCa incidence (8), their preventative effect is limited to low-grade cancers, thus decreasing the value of these agents for averting PCa deaths (9-11).

Structure and Function of AR and Its Role in PCa

The full-length AR protein can be divided into several functional domains (Figure 1): the amino-terminal domain, which contains an AR transcriptional activation function; the DNA binding domain (DBD); a hinge region; and the carboxy-terminal ligand-binding domain (LBD). The AR signaling axis primarily follows the classical mechanism of nuclear receptor signaling (Figure 2), which relies upon a large network of interacting proteins – and thus

offers a number of potential targets beyond AR itself for therapeutic intervention. When not bound to its ligand, the AR protein is primarily localized in the cytosol, sequestered and stabilized by chaperone proteins. Upon binding of ligand, the AR undergoes a conformational change and translocates to the nucleus (likely via a dynein/microtubule-dependent fashion) (12). In the nucleus, pioneer factors and histone modifiers prime the chromatin for transcription factor binding, allowing the AR to bind as a dimer to androgen response elements (AREs) in the promoter region of its target genes such as transmembrane protease serine 2 (TMPRSS2), prostate-specific antigen (PSA), and many others. Once bound to chromatin, the AR protein recruits additional co-regulators and transcription machinery components that ultimately lead to activation of a transcriptional network that promotes growth and survival.

In addition to the full-length protein, AR variants (ARVs) have also been described (Figure 1). One of the first alternative forms of the AR was observed in 1996, and - through antibody mapping - it was established that this form lacked the LBD (13,14). Although this form was proposed to be a proteolytic fragment of full-length AR, studies have established that ARVs can also arise through alternative mRNA - either derived from alternative splicing (15,16) or chromosomal rearrangements (17). Through the characterization of a PCa cell line that harbors one of these variants - the CWR22 PCa xenograft-derived line - it was discovered that ARVs lacking the LBD are constitutively nuclear, and are able to restore part of the AR signaling activity in a ligand-independent manner (18). In 2009, Hu et. al reported four cryptic exons (one of which had been discovered previously by Dehm et. al (16)). Hu et. al further reported that among the four cryptic exons, alternative splicing led to seven AR splice variants, all lacking the LBD. In addition to these seven variants, other groups - utilizing technologies such as 3' and 5' RACE - have identified several alternative splice variants, some containing the LBD (19,20), and others lacking the LBD (21).

In contrast to its reliance on the AR signaling axis, PCa is resistant to many of the standard treatment modalities utilized against other solid malignancies. The notable exception to this is the taxane family of chemotherapeutics, which are active against several cancers, primarily through the inhibition of microtubule function in the cell. Taxane treatment has been observed to suppress nuclear translocation of the AR, and thus, its transcriptional activity (22,23). Importantly, it has been reported that some ARVs may elude this effect of microtubule inhibitors, as they may lack the microtubule-binding domain present in full-length AR (24,25). Historically, docetaxel and cabazitaxel have been used to treat advanced stage PCas (26,27) after hormonal therapies have lost their activity. However, very recent data suggest that early addition of docetaxel with gonadal suppression may significantly increase patient survival compared to later use of the same chemotherapy (28). It remains to be determined whether this synergistic effect is due to a more potent suppression of AR signaling achieved by the combination regimen (29).

Diagnosis, Treatment, and Current Understanding of Resistance Mechanisms

Patients diagnosed with *localized* PCa will generally undergo surgical resection or radiation therapy, upon which serum levels of prostate-specific antigen (PSA, or gene name KLK3) - a gene product directly induced by active AR - will be monitored as a biochemical marker for

PCa recurrence. Rising level of PSA after prostatectomy or irradiation can serve as an early indicator of recurrence. Once PCa becomes *locally advanced or metastatic*, the next stage in treatment is to start the patient on GnRH analogs to suppress gonadal androgen synthesis. The goal of first-line hormonal treatment is the suppression of gonadal androgens (with a target level of serum testosterone below 50 ng/dL). Despite this suppression of serum testosterone levels, some cancers maintain intra-tumoral androgen levels via local synthesis and metabolism (7,30,31). Alternatively, some PCas have been observed to overexpress AR mRNA (frequently via amplification of the AR gene) and are sensitized to lower levels of androgens. These molecular alterations in the AR axis increase sensitivity to hormone and allow resumption of PCa cell proliferation despite suboptimal levels of androgen in the serum (32,33). Once a patient exhibits resistance to initial androgen suppression therapies, AR antagonists are given to directly inhibit the AR. Flutamide, followed by bicalutamide (Casodex), are amongst the first-generation AR antagonists which inhibit AR by competitively binding to its LBD and disrupting activation (34,35). Sadly, restoration of PCa growth and AR signaling still occur.

In some patients, withdrawing treatment of the first-generation AR antagonist results in a decrease of circulating PSA (36), suggesting that the drug was functioning as an AR agonist. Point mutations in the LBD of the AR have been reported to confer promiscuity or relaxed stringency for ligands (37), for example, in AR-LBD mutant W741L, bicalutamide switches from acting as an AR antagonist to acting as an agonist and driving AR signaling (38,39). In humans, the AR gene is located on the X-chromosome (Xq12), thus, there is only one copy of the AR gene in the male germline. As previously mentioned, AR functions as a homodimer. Consequently, in cells that manage to acquire an AR gene mutation in the single AR gene copy, every molecule of AR will harbor this mutation, resulting in homodimers of mutant AR and greater oncogenic potential. Alternatively and in addition to point mutations, overexpression of AR (frequently via gene amplification) can also result in first-generation AR antagonist-to-agonist conversion (40).

Unfortunately, in patients with metastatic PCa, the reduction in serum PSA after first line hormonal therapy is almost inevitably short-lived. When PSA levels begin to rise, castration-resistant disease has emerged and needs to be treated with second-line hormonal therapies. Currently, these include 1) abiraterone acetate (approved by FDA in 2011)-a selective and irreversible inhibitor of 17- α -hydroxylase/C17-20-lyase (CYP17A1), an enzyme that catalyzes the sequential reactions of the conversion of pregnenolone and progesterone to their 17- α -hydroxy derivatives and the subsequent formation of dehydroepiandrosterone (DHEA) and androstenedione (41-43), and 2) enzalutamide (MDV3100) (approved by FDA in 2012)- a second-generation AR antagonist that can prevent AR from binding its natural ligands: testosterone or 5 α -dihydrotestosterone (44). Although abiraterone is able to suppress circulating androgen to near undetectable levels (45,46), and enzalutamide is probably the most potent FDA approved anti-androgen currently available (as it is clearly active in bicalutamide-refractory patients), there is significant evidence that many PCas remain dependent on the AR signaling axis for growth even after treatment with these agents.

Recently, mechanisms of resistance to second-generation AR antagonists, such as enzalutamide, have also been described. Stemming from the experiences with resistance to bicalutamide, efforts were made to screen for potential mutations that might confer resistance to enzalutamide. In 2013, an *in vitro* screen for potential resistance discovered a point mutation in the LBD of the AR (F876L) that blunts the effect of enzalutamide and allows proliferation of cancer cells (47). Two studies have shown this same acquired somatic point mutation in PCa cells from patients treated with different AR antagonists: one who was treated with enzalutamide (48), and the other with a different second-generation AR antagonist – ARN-509 (49). In addition to point mutations, an AR-independent mechanism for resistance has also been described: a cellular switch by which the AR signaling axis is maintained by an up-regulation of the glucocorticoid receptor – which recognizes and binds the same hormone response element DNA motif as AR (50,51). Resistance to CYP17A1 inhibitors, such as abiraterone, has also been reported in CRPC patient-derived xenograft (PDX) models, through increased expression of the CYP17A1 enzyme, as well as through induction of ARVs (52).

ARVs (Figure 1) are a critical mechanism of resistance observed in PCa (53); they can occur in response to a variety of treatments, and pose a challenge for the management of PCa (54,55). Transgenic mice engineered to express ARV in the prostate exhibit epithelial hyperplasia by 16 weeks and invasive PCa by 1 year of age, as well as expression of genes critical for tumor initiation and progression (56). Regardless of the origin and heterogeneity of these splice variants, they provide a hurdle for our existing therapies: because many of these ARVs lack a LBD, neither treatments targeting hormone production, nor AR antagonists targeted toward the LBD are effective against these ARVs. In fact, several studies have noted increased presence of ARVs in PCa cells resistant to enzalutamide (57) and subsequently was associated with shorter PSA progression-free as well as less overall survival in patient samples with PCa resistant to abiraterone or enzalutamide (58-60).

Due to the transient success of abiraterone and enzalutamide, efforts to develop newer and more potent AR antagonists and androgen synthesis inhibitors are ongoing. Novel AR-directed approaches include ARN-509 (49) and Galeterone (a novel CYP17 inhibitor (61) which is in clinical trials for CRPC, in particular AR-variant-expressing CRPC); their ability to further advance PCa treatment remains to be seen. In addition, AKR1C3, an enzyme that is overexpressed and implicated in driving androgen synthesis in advanced PCa, has been studied for potential therapeutic value (62,63). One attractive feature of inhibition of AKR1C3 over abiraterone is that inhibition of AKR1C3 does not block glucocorticoid synthesis. Small molecule inhibitors for AKR1C3 has already been developed, but their effect remain to be tested in clinical trials to assess for clinical benefit in abiraterone-refractory patients (64).

Future Direction of PCa Molecular Target Discovery and Drug Design

AR is an excellent molecule to target therapeutically, as its inhibition in PCa patients has limited risk of morbidity (although there are some serious considerations with regard to quality of life) and the presence of a defined LBD has allowed the development and FDA approval of first- and now second-generation AR antagonists. Unfortunately, as outlined

above, mechanisms of resistance to therapies targeting the AR LBD are commonly observed, suggesting that new strategies are needed. Several newer studies aim to design inhibitors capable of blocking required conformational changes of the AR, or protein-protein interactions between the AR and co-regulators (65).

It is worth contemplating what might constitute an “ideal” candidate molecule to target therapeutically. A good candidate for development of a small molecule inhibitor should exhibit favorable biological activity, and favorable technical feasibility (66). In Oncology, biological activity should be assessed through assays that clearly indicate that disruption or degradation of the target slows or kills cancer cells, and that disruption or degradation of the target will yield favorable results in patients. In addition to promising data for the biological importance of the target, the technical feasibility of drugging the molecule (“druggability” of the target) must be considered. Traditionally, this has focused on taking advantage of ligand binding pockets (like the LBD in the AR), especially if structural data – such as X-ray crystals or NMR – is available to help identify targetable regions and guide development (66). Recently, however, much focus has been placed on the development of inhibitors of protein-protein interactions. With increased understanding of macromolecular binding energetics, and advances in assessing structural data, protein surfaces that were previously considered ill-fit for small molecule binding have been reassessed (67,68). Furthermore, research into targetable delivery systems increases the feasibility of using small molecule inhibitors against candidate targets that pose challenges such as toxicity in other tissues, or less favorable bioavailability. One such approach in prostate cancer takes advantage of the prostate-specific expression of prostate-specific membrane antigen (PSMA). PSMA has been successfully utilized to image PCa in mice (69), and has been coupled with nanoparticles to deliver targeted therapeutics in animal models (70), potentially providing a mechanism to limit off-target effects of small molecule inhibitors targeting AR-interacting proteins.

Disrupting or inhibiting the AR signaling axis – either mediated by full-length AR, or ARVs - by targeting the AR-interacting proteins may prove to be a viable strategy to supplement traditional therapeutics in order to enhance their activity, delay the emergence of resistance, or even treat resistant disease. The numerous escape and reactivation mechanisms observed in PCa cells allow them to maintain the benefit of a functioning AR signaling axis, and utilize it for cell growth. It follows then, that new treatments will continue to target this signaling axis, while aiming to overcome the currently known routes to *de novo* or acquired resistance. Although efforts are being made to target other, previously considered “less druggable” domains of the AR, including the N-terminal domain antagonist EPI-001 (65,71), the shifting views on what constitutes druggability of proteins, technological advances in viewing and using structural data for inhibitor development, along with added specificity and delivery mechanisms from targeted therapies such as PSMA-nanoparticles provides the opportunity to expand and capitalize on the dependence of AR for other proteins, in addition to directly targeting androgens and the AR itself.

Chaperones

In theory, targeting chaperones or heat-shock proteins (including HSP90, HSP70, FKBP52, and p23) seems to have high therapeutic potential, as successful inhibition would result in mis-folding or degradation of their client proteins. Although early biochemical studies in yeast suggested that inhibiting chaperone proteins as a way to target the AR axis might hold promise (72), so far this approach has been unsuccessful in PCa either due to unacceptable toxicity of these inhibitors, or emergence of novel ARVs.

For over 30 years, chaperone proteins have been known to regulate maturation, activation, and stability of ligand-free steroid receptors such as AR (73). Although HSP90 was the first identified chaperone protein for the AR, additional chaperone or co-chaperone proteins have since been reported, including: HSP70, FKBP52 (HSP56) and p23 (74,75). Stabilization by chaperones is vital to the ability of the AR to respond to ligand, as evidenced by observations in yeast that mutant forms of the HSP90 yeast-homolog do not decrease overall levels of the AR, but inhibit hormone-dependent transactivation, even in the presence of the potent synthetic androgen metribolome (R1881) (72). This basic mechanism of action presents two general strategies for inhibition of chaperone activity: (1) inhibit the protein-protein interacting domains of the client protein with the chaperone, and (2) inhibit the ATP binding pocket to prevent release of the client protein. Of these approaches, most work with chaperone inhibitors has followed the latter approach.

Given the evidence of Hsp90 mediated transactivation of AR transactivation, the feasibility of targeting HSP90 with small molecules has been examined extensively. And at least *in vitro*, some success has been observed. Geldanamycin, a well-established HSP90 inhibitor (via competitively interacting with its ATP binding pocket) has been observed to decrease AR protein levels (Figure 4A), as well as mRNA for the AR target gene PSA, even in the presence of R1881 in LNCaP PCa cells (76). Despite this, geldanamycin and another major HSP90 inhibitor, 17-allylamino-17-demethoxygeldanamycin (17-AAG), have been investigated *in vivo*, and disappointingly, studies have so far failed to establish any clinically meaningful benefit. Geldanamycin was shown to result in unacceptable liver toxicity in animal models (77), while 17-AAG was discontinued in Phase II clinical trials due to a failure to reduce serum PSA levels (78).

Despite these setbacks, interest in HSP90 inhibition remains, and several new inhibitors are being tested. One of these, ganetespib (STA-9090), was shown to have therapeutic activity against a number of PCa cell lines, while exhibiting significantly better safety and pharmacological profiles (79). In clinical trials, single-agent use of ganetespib was unable to prolong progression-free survival in metastatic CRPC patients – however combination therapy remains an option for future studies (80). As highlighted earlier, ARVs has been established as a major mechanism for CRPC. Two inhibitors, geldanamycin and MJC13 (which block HSP90 and its co-chaperone FKBP52, respectively), were demonstrated to be effective at blocking ligand-dependent AR activity, but were unable to modulate ARv7 protein stability or transcriptional activity (81), suggesting that ARVs may contribute to resistance to HSP90 inhibitors in clinical settings.

Although inhibition of chaperone proteins with regards to their classical function may not prove to be an effective strategy, interest in the field remains as new functions of chaperones have emerged. Chaperones have been shown to have a potentially more direct role in the AR transcriptional program. Live cell imaging experiments have illustrated that chaperones, including HSP90, may help mediate the nuclear translocation and sub-nuclear localization of nuclear receptors (82). Furthermore, it has been suggested that chaperones of the AR might also participate in the regulation of transcriptional complexes on chromatin (83), and that co-chaperone p23 may actually help stabilize AR on its hormone response elements, potentiating AR-mediated transcription (84).

When taking into consideration some of the other key cancer-associated proteins and pathways (such as AKT (85)) that are stabilized by HSP90 and other chaperones, it is even more remarkable that HSP inhibitors failed to have effect in PCa clinical trials. However, the functions of chaperones for AR stabilization as well as sub-cellular trafficking, and their potential roles as part of the transcriptional machinery highlight their importance and potential as therapeutic targets. New efforts at targeting chaperones may enhance specificity for the cancer, and ameliorate toxicity by focusing on inhibition of the protein-protein interaction between AR and specific chaperones, or by focusing on inhibiting the AR axis by attacking the transcriptional or trafficking roles of chaperones. It remains to be seen if these strategies prove to be more selective or less amenable to ARV-mediated resistance.

Pioneer factors

Pioneer factors are critical for priming the chromatin to allow AR to bind and become transcriptionally active and thus targeting pioneer factors could have high therapeutic potential in PCa. Two classes of proteins have been identified as key pioneer factors for AR recruitment to chromatin: FOXAs (specifically FOXA1), and GATAs (especially GATA-2). These factors are the earliest chromatin binding factors and, unlike other transcription factors, they are able to bind *condensed* chromatin (86). FOXA1 contains a winged-helix structure (similar to the DNA binding domain of linker histone, H1) that allow it to bind the major groove of DNA (87) and make the chromatin more accessible to other transcription factors to initiate productive transcription (86). Several mechanisms for inhibition can be envisioned to target their role in the AR signaling axis: inhibitors could degrade FOXA1 and/or GATA-2, prevent interaction of FOXA1 and/or GATA-2 with chromatin, or interfere with the ability of FOXA1/GATA-2 to interact with the AR. Few small molecule inhibitors for these proteins have been investigated, therefore all of these avenues continue to have potential.

The importance of FOXA1 in allowing the AR to bind chromatin and initiate transcription was first described in 2003, when experiments showed that FOXA1 binding to the core KLK3 enhancer preceded AR binding, and that overexpressing mutant forms of FOXA1 significantly ablated KLK3 transcription (88). Evidence for the relationship and dependence of the AR on FOXA1 was further supported by experiments demonstrating the co-localization of the two proteins on chromatin (89), including in castration-resistant PCa cells that exhibit a novel AR transcriptional program (90). Elevated FOXA1 expression levels were associated with poor prognostic features (higher Gleason scores) and clinical outcomes

(early biochemical recurrence) in three studies (91-93). In one study, overexpression of FOXA1 resulted in broad increases in AR binding on chromatin, and that these new AR binding sites are primarily unresponsive to DHT stimulation (93). These data raise the hypothesis that patients with CRPC may also benefit from inhibiting FOXA1 levels and/or activity.

However, the pioneering effect of FOXA1 for AR is far more complex and important questions about the clinical value of inhibiting FOXA1 to treat PCa have been raised by other studies reporting somewhat contradicting results. Specifically, it has been proposed that FOXA1 is not required for AR-chromatin interaction at canonical AREs, but is instrumental in recruiting AR to low-affinity half-AREs by opening local chromatin (94). In the presence of high levels of FOXA1, these open chromatin regions serve as reservoirs that retain AR via abundant half-AREs, and decrease availability of AR for full-ARE sites. Thus, suppressing FOXA1 levels can release AR to bind new full-AREs across the genome, resulting in substantial reprogramming of the AR cistrome and AR-dependent gene expression, even in the absence of androgen (94,95). As a result of the above AR reprogramming and alternative hormonal response, these studies have also reported that FOXA1 downregulation activates a castration-resistant AR transcriptional program (94), while lower FoxA1 levels are significantly associated with increased metastatic potential and poor prognosis (95), and that silencing FOXA1, surprisingly, results in increased cell progression to S phase (95). These data contradict, to some degree, the other studies discussed in the previous paragraph, and raise some concern about the potential usefulness of FOXA1 inhibition for targeting the AR signaling axis and inhibiting PCa growth. To further complicate matters, the AR and the ARv7 exhibit, at least partially, different propensities for FOXA1 dependency, suggesting that targeting FOXA1 may not overcome resistance mediated by AR splice variants (96).

Another pioneer factor that can serve as a candidate target for the inhibition of the AR signaling axis is GATA-2. Similar to FOXA1, GATA-2 has also been shown to co-localize with the AR on chromatin, with enhanced binding of GATA-2 at AREs in response to androgen (89). Increased GATA-2 expression has also been correlated with poor prognosis and more aggressive cancers (97,98), and has been implicated as a potential driving gene in metastatic PCa, as evidenced in patient expression data as well as cell phenotypic assessments, which demonstrated that silencing GATA-2 decreases cell migration, tissue invasion and focal adhesion (99). Recently, GATA-2 has been garnering more attention as a key player in AR-mediated transcription.

GATA-2 is another pioneer factor that regulates AR function and, unlike FOXA1, also promotes AR expression. GATA-2 silencing resulted in decrease in both AR mRNA and protein levels in androgen-free and R1881-treated cells (100), diminishing the expression of not only full length AR, but also ARV (98). This effect on full-length AR and ARVs was recapitulated by the small molecule GATA-2 inhibitor K-7174 (98). Simultaneously, GATA2 is necessary for optimal transcriptional activity of both full-length AR and ARV, as demonstrated by the inability of exogenously transfected ARs (both full-length AR and ARV) to drive transcription in the absence of GATA-2 (98). As ARVs have emerged as a mechanism of CRPC resistance to androgen synthesis inhibitors, AR antagonists and even,

potentially, chaperone inhibitors, the finding that inhibition of GATA-2 may not only target the full-length AR signaling axis, but also decreases the expression and function of ARVs is critical. Interestingly, in studies looking at ETS factor reprogramming of the AR after deletion of PTEN (which is common in PCa), there is an even greater number of new AR binding sites on chromatin that contain the GATA-2 DNA binding motif (*101*). GATA-2 has been reported to regulate IGF-2 expression in PCa cells, resulting in the downstream activation of AR-independent genes vital for chemoresistance (*102*). Taken together, these findings suggest a potential critical role for GATA-2 in both AR-dependent and AR-independent signaling and thus making it an attractive and promising candidate for PCa therapeutics.

Several mechanisms for inhibition can be envisioned to target the role of pioneer factors in the AR signaling axis: a) inhibitors could degrade FOXA1 and/or GATA-2, b) prevent interaction of FOXA1 and/or GATA-2 with chromatin, or c) interfere with the ability of FOXA1/GATA-2 to interact with the AR. Few small molecule inhibitors for these proteins have been investigated, therefore all of these avenues continue to have potential.

AR transcriptional partners

It is important to note that AR itself has no intrinsic enzymatic activity; instead, it serves as a scaffold – recruiting various protein machinery that can modify chromatin and result in active transcription. AR-interacting partners on the chromatin represent an exciting pool of targets for innovative therapeutics either through inhibiting their activity, or by preventing their recruitment to the AR. Although numerous proteins are involved in the initiation of transcription, three categories of proteins stand out as potentially offering some specificity for PCa over the ubiquitous, and required transcription machinery in normal tissues and cells: the p160 family of steroid receptor coactivators (SRCs), lysine acetyltransferases (KATs), and lysine deacetylases (KDACs).

The p160 Steroid Receptor Coactivators—The p160 family of steroid receptor coactivators, including steroid receptor coactivator-1 (SRC-1, or NCOA1, RIP160), steroid receptor coactivator-2 (SRC-2, or NCOA2, GRIP1, TIF2) and steroid receptor coactivator-3 (SRC-3, or NCOA3, AIB1, RAC3), have been well established to play an important role in endocrine-related cancers, including ovarian (*103*), breast (*104*), endometrium (*105*), and prostate (*98,106-109*). The p160 SRCs have also been implicated in other diseases, as well as in lipid, carbohydrate, amino acid, and drug metabolism (reviewed in (*110*) and (*111*)). As the SRCs are involved in a number of diseases and cancers, targeting these proteins may have high therapeutic potential. One of the key challenges for successful inhibition, however, will be finding a therapeutic window such that the SRCs levels are low enough to benefit the patient (Figure 4C), but does not impact the important transcriptional roles played by the SRCs in normal tissues and cells.

The p160 SRCs are best known for their eponymous functions as coactivators for steroid receptors (including AR, estrogen receptor, progesterone receptor, mineralocorticoid receptor, glucocorticoid receptor (reviewed in (*112*))), but have also been shown to coactivate a variety of other transcription factors including NF- κ B, TAT, and AP-1 among others

(113-116). Among the conserved features in the p160 family of SRCs are a basic Helix-loop-Helix Per/ARNT/Sim (bHLH-PAS) domain through which it can mediate various protein-protein interactions. They also contain three LxxLL motifs, which serve to recognize and bind activated steroid hormone receptors. The C-terminus of the SRCs contains two activation domains which can recruit CBP and p300, as well as other coactivators and histone modifiers, such as CARM-1 and PRMT1 (117-119). These features provide the backbone for the many interactions and activities attributed to the SRCs.

The role of the SRCs in PCa has been investigated for well over a decade, with one of the first studies implicating the SRCs arising in 2001. Patients with PCa that recurred after androgen deprivation therapy were shown to have elevated expression of SRC-1 and SRC-2, which was sufficient to potentiate AR signaling despite suboptimal levels of androgen (38). Since then, overexpression of SRC-1, SRC-2 and SRC-3 mRNA has been observed in patients with primary PCa as well as patients treated with androgen deprivation therapy, although there is some heterogeneity among the findings (120). This has been recapitulated recently in an expression dataset of patients with primary and metastatic PCas, which show increased levels of overexpression of the SRCs as disease progresses (121). This is emphasized through the observation that the p160 SRCs have been shown to coactivate the AR not only with androgens, but even in the presence of partial agonists (122).

SRC-1 has been shown to be important in both ligand-dependent PCas and CRPCs that retain expression of the AR (123). SRC-1 has also been proposed to play a role in mediating resistance after androgen deprivation therapy (38). Perhaps the most well studied p160 SRC in PCa is SRC-2. SRC-2 has been shown to have a strong relationship with androgens and the AR itself; a study that profiled SRC-2 expression in cell lines and patient samples in normal and androgen-deprivation conditions showed that SRC-2 mRNA and protein are increased in PCa cells in the absence of androgens, and that SRC-2 overexpression is correlated with biochemical recurrence in patients (124). Recent studies have shown that increased mRNA levels of SRC-2 (frequently associated with NCOA2 gene amplification) may occur in up to 20% of primary, and 63% of metastatic tumors (121). In mouse models, SRC-2 has been shown to be induced upon castration, and in this model, has been demonstrated to be critical for the development of CRPC (108).

SRC-3 has also been shown to play an important role in the progression of PCa. Although SRC-3 has been shown to be upregulated at the mRNA level in patients with PCa (121), the number of patients with increased mRNA is unimpressive when compared with SRC-1 or SRC-2. However, immunohistochemistry for SRC-3 in patient tissue samples has demonstrated frequent overexpression at the protein level and has associated it with higher grade prostate tumors as well as worse overall survival (125). This finding suggests that SRC-3 may be extensively regulated at the post-translational level in PCa. For example, the E3 ubiquitin ligase adaptor SPOP (speckle-type poxvirus and zinc finger (POZ) domain protein) binds SRC-3 and promotes its ubiquitination and proteolysis. Mutations in the SPOP substrate-binding cleft are frequent in human PCas and interfere with SRC-3 ubiquitination and degradation, resulting in higher protein levels (126,127). *In vitro*, SRC-3 has been shown to be required for PCa cell proliferation and survival (107). SRC-3 has also been implicated as positively regulating genes in the insulin-like growth factor/AKT

signaling pathway in PCa cells, resulting in increased cell proliferation (*128*). In addition to playing a role in cell proliferation, SRC-3 has also been shown to participate in driving a more metastatic-like program in PCa cells. In this study, SRC-3 was associated with increased cell migration and invasion by regulating matrix metalloproteinases (*129*). The importance of SRC-3 (and SRCs in general) in PCa was highlighted in a study of the impact of SRC-1 knockout in a prostate tumorigenesis model in mice. The authors observed an increase in SRC-3 expression during prostate tumorigenesis, which they explained as a possible compensatory effect that allows cancer formation despite the loss of SRC-1 (*130*). This is an important concept, as the SRCs (as well as other proteins that have structurally similar family members) may be able to compensate for each other, and thus efforts to inhibit SRCs may need to be directed towards inhibiting all three rather than specifically targeting only one member.

Evidence for targeting the SRCs to inhibit the AR signaling axis does exist: peptide-mediated targeted inhibition of the p160 coactivator interface on the AR itself results in a loss of AR activity in both androgen-sensitive, as well as castration-resistant PCa cells (*115*). Most nuclear receptors, through their AF-2 domain (which is typically located on the carboxy-terminus), interact with the LxxLL motifs on the p160 SRCs. One study, investigating the relationship between SRC-1 and the AR, mutated the LxxLL domain on SRC-1, rendering that domain nonfunctional. Surprisingly, the mutant SRC-1 was still able to activate the AR and potentiate AR-signaling. The authors discovered that SRC-1 can interact with the glutamate-rich region located in the amino-terminal AF-1 domain on the AR (*131*). Importantly, targeting the SRCs may also serve to interfere with ARV signaling, as evidenced through the observation that overexpressing FOXO1 – which binds to the Tau-5 domain on the AR (conserved on both full-length and most characterized ARVs) – can compete for SRC-1 binding, and abrogate ARV transcriptional activity (*132*).

Attempts at targeting the SRCs for therapeutic benefit have begun with a number of compounds proposed as inhibitors for the p160 family. Gossypol, Verrucaric acid, and the cardiac glycoside Bufalin have all been shown to inhibit SRC activity and to have activity against breast cancer cells expressing high levels of SRCs (*133-135*). Bufalin was shown to decrease SRC-3 protein levels, although it may also have additional mechanisms for SRC inhibition (*134*). Bufalin inhibits all three SRCs, which may help avoid compensatory effects. In addition, it has been shown that the microRNA miR137 is able to target SRC-1, SRC-2 and SRC-3, providing an alternative option for combined inhibition of the entire class of p160 SRCs (*136*). Although there is concern that the SRCs are required for normal cell function and that their inhibition may thus prove too toxic, they remain – at least in theory – a potentially valuable therapeutic target. Many of the mechanisms of resistance in PCa arise through reestablishing at least part of the AR signaling axis: increased expression of the AR, mutations in the LBD, functional ARVs, or the use of other steroid receptors. All of these mechanisms of resistance signal through the p160 SRCs; therefore effectively inhibiting the SRCs would provide a relatively comprehensive blockade of most of the currently known resistance mechanisms in PCa.

KATs—Another group of AR transcriptional partners are the lysine acetyltransferases (KATs, or HATs (histone acetyltransferases)). KATs, such as p300 and CREB-binding

protein (CBP) are, in general, proteins that acetylate lysine residues on histones or other proteins by utilizing acetyl-CoA as a donor. KATs, and the acetylation they catalyze, are generally considered to play an activating role in transcription. As a result, the therapeutic potential from their inhibition in cancer – if specificity can be achieved – could be high. For PCa, research into inhibition of KATs has mixed results: PCa-specific inhibition of p300 and CBP – which play key roles in the regulation of transcription – has been difficult to achieve due to the ubiquitous expression of these proteins. However another KAT, Tat-interacting protein 60 (Tip60), seems to have high potential as a target for novel therapies.

The association between CBP or p300 and nuclear receptors, and the importance of these KATs for efficient nuclear receptor signaling and transcription was first demonstrated in 1996. In this study, endogenous CBP and p300 was required for signaling of the retinoic acid and glucocorticoid receptors (137). The role of KATs in PCa became even more prominent when it was shown that, in the absence of androgen, interleukin-6 (IL-6) was able to transactivate AR. In this setting, p300 mediated the transcriptional activity of un-liganded AR, and silencing p300 by RNAi was sufficient to mitigate this IL-6-activated AR (138). The role of CBP was also established when it was shown that CBP enhances AR transcriptional activity in the presence of androgen, as well as in the presence of first-generation anti-androgens hydroxyflutamide and bicalutamide (139).

While inhibitors of KDACs (the deacetylating counterparts to KATs) have been widely studied, less work has been done on developing small molecule inhibitors for KATs. This may be, in part, due to the complexity in teasing apart differences among several closely related KATs. Although CBP and p300 are commonly grouped together (and are often written collectively as CBP/p300), differences between their activities have been documented: one study noted that inhibition of p300, but not CBP, resulted in increased cell death, better inhibition of AR function, and a decrease in cell invasion in PCa cells (Figure 4D). This study used C646, an inhibitor for both CBP and p300, but refined the specificity of their results through RNAi (140). Interestingly, this same study noted that the ability of AR to bind to chromatin was not affected by the use of C646. Another well-studied agent for KAT inhibition is curcumin – which is notable as a component of the spice turmeric, and has been implicated as an anticancer agent for decades (141). *In vitro* use of curcumin in prostate lines has resulted in decreased AR activity in both androgen-dependent as well as castration-resistant PCa cells (142). Despite this, the ubiquitous expression of KATs such as CBP and p300, and their importance in normal tissue as well as cancer cells severely limit the therapeutic window in which to treat patients.

Tip60, a less well-known (compared to CBP and p300) acetyltransferase, has also been studied for its role in PCa and AR signaling. Tip60 was first shown to interact with the AR in 1999. In this screen, Tip60 – originally known as an interacting protein with the human immunodeficiency virus protein TAT – was discovered to interact with full length AR, as well as estrogen and progesterone receptors, and to coactivate transcription of liganded AR to similar degrees as CBP, p300, and SRC-1 (143). After the initial report, Tip60 was discovered to acetylate lysines in the AR hinge region, which was determined to be required for its transactivating capacity for the AR (144). Interestingly, Tip60 has also been shown to play key roles in maintenance of DNA integrity, as deletion of its acetyltransferase activity

has been shown to result in DNA damage, halting of cell cycle progression, and induction of apoptosis (145,146). Analysis of the cellular localization of Tip60 has revealed that Tip60 has a relatively diffuse expression pattern throughout the cell in benign prostate tissue and in localized PCa. However, there is an accumulation of Tip60 protein after androgen withdrawal, and significant nuclear localization has been observed in CRPC disease – potentially contributing to the castration-resistance phenotype (147).

Tip60, therefore, remains a potential target. Recently, several publications have investigated the use of Tip60 inhibitors for antagonizing AR signaling and PCa cell growth. NU9056 was identified through a screen for acetyltransferase inhibitors, and was determined to have specificity for Tip60 over other KATs (with over 29-fold selectivity over p300). The same study showed that NU9056 decreased cell viability in a number of PCa cell lines with IC₅₀s in the low micromolar range, suggesting that Tip60 may be a promising candidate target for novel therapeutics (148). More recently, another group has developed and tested another small molecule inhibitor for Tip60 – TH1834 – reporting similar inhibition of cell viability when used in breast cancer (149). The dual roles of Tip60 – coactivating the AR, and maintaining the integrity of DNA – peg Tip60 as a very attractive candidate for therapeutic intervention in PCa.

KDACs—The counteracting enzymes to the KATs are the lysine deacetylases (KDACs or HDACs (histone deacetylases)). The KDACs consist of four classes of proteins, termed Class I (including HDAC1, HDAC2, and HDAC3), II (including HDAC7), III (including the sirtuins) and IV. KDACs are primarily responsible for removing the acetylation modification from lysines on histones or other target proteins. As the counterpart to KATs, KDACs and deacetylation are generally considered to be more repressive with regard to transcription (150,151), which may make them a less obvious target for inhibition in PCa therapy. However, KDACs have also been shown to play an important role in regulating post-translational modifications on a wide array of transcription factors and proteins, including the AR (152), GATA-2 (153), and many more general cancer-important proteins including c-Myc, E2F, Rb, p53, among others (reviewed in (154)). Numerous studies have looked into KDAC inhibition, often with promising *in vitro* or preclinical data, although as will be explored in this section, little success so far has been observed for PCa in clinical trials.

For PCa, much of the focus on KDACs has been on Class I KDACs, including HDAC1, HDAC2 and HDAC3. The dysregulation of Class I KDACs have been implicated in cancer, as they have been established to play key roles in the regulation of cell cycle (155,156). For PCa, Class I KDAC dysregulation has been observed even in early stages (including tumor initiation) (157). Through IHC analysis of patient samples, it has been observed that HDACs –1, –2 and –3 are all strongly expressed in tumor sections, and each has been associated with earlier biochemical recurrence, and enhanced cellular proliferation, while HDAC1 and –2 have also been associated with dedifferentiation of tumors (157).

Despite the correlation between increased HDAC1 expression levels and PCa cell growth, the relationship between HDAC1 and AR is more complex. It has been demonstrated that acetylation of AR enhances its ability to recruit coactivators and to drive transcription (158), and that HDAC1 may play a role in deacetylating and repressing AR transcription (144). In

contrast, another study has demonstrated that HDAC1 and HDAC3 are important for AR function in androgen-sensitive as well as CRPC cells, and that inhibition can decrease not only AR target genes, but xenograft tumor size (159). In this study, KDAC inhibition did not directly repress the AR itself – instead, KDAC inhibition blocked the assembly of the RNA polymerase II complex required for transcription. Importantly, the authors draw attention to this biphasic response to KDAC inhibition, and noted that the level of KDAC inhibition needed to achieve an AR-inhibitory effect was unlikely to be achievable in patients (159). In addition to HDAC1, Sirtuin 1 – a member of the Class III KDACs – has also been shown to play an important role acting as a corepressor for ligand-dependent AR transcriptional activity. Perhaps more important for therapeutic approaches, the same study also identified Sirtuin 1 as being required for the AR-inhibitory activity of the first-generation AR antagonist bicalutamide (160).

Despite the evidence suggesting that KDACs – by deacetylating the AR – impair coactivator recruitment to AR, and thus may repress AR-mediated transcription, the importance of KDACs in driving cell cycle and cell proliferation have led to numerous studies investigating the use of KDAC inhibitors for inhibiting cancers (including PCa). KDAC inhibitors have been documented to result in cell death in a number of cancer types with a variety of proposed mechanisms (161). With the large number of KDAC inhibitors available for the specific classes, or individual KDAC members, the focus here will remain on those used in clinical trials for PCa (descriptions of the KDAC classes and examples of inhibitors reviewed in (162)).

Although several KDAC inhibitors have obtained FDA approval for treatment of hematologic malignancies (both vorinostat in 2006 (163) and romidepsin in 2009 (164) were approved for cutaneous T-Cell lymphoma, and early in 2015, panobinostat was approved for patients progressing with multiple myeloma (165)), KDAC inhibitors have yet to be proven to be effective in human trials for PCa. Vorinostat – a KDAC inhibitor which blocks activity of both Class I and Class II KDACs (166), failed to decrease circulating PSA levels in a clinical trial for PCa patients who had progressed on one previous chemotherapy, while also resulting in unacceptably toxicity for patients (167). And earlier this year, a phase II study using SB939 (pracinostat) in patients with recurrent or metastatic PCa was completed. SB939 is an orally active, pan-KDAC inhibitor (168), but although SB939 was tolerated at the given dose, the study concluded that SB939 did not meet primary endpoints for PSA decline, and should not be studied further for single agent use in CRPC (169).

Although the need for novel therapeutics in PCa is great, the complexities of KDAC inhibition – especially in PCa - raise concern about the use of these inhibitors in patients. With the above-mentioned evidence that KDACs deacetylate and thus repress AR activity (144) (and thus that inhibition of KDACs may increase or stabilize the AR signaling axis), and the antithetical evidence that inhibition of KDACs may decrease the ability of AR to recruit functional transcriptional complexes (albeit at very high concentrations) (159) (and thus that potent inhibition of KDACs may decrease the AR signaling axis), it may not be easily feasible to utilize inhibitors that have activity across the different classes (or maybe even for multiple members) of KDAC enzymes. More research needs to be done to achieve inhibitors with higher specificity, and to know which KDACs to inhibit and at what stage.

Although in general KDAC inhibitors appear to have a low toxicity profile in patients (170), it is worth considering that several KDAC inhibitors have had difficulties with toxicity in clinical trials. Vorinostat – which has been demonstrated to have a fairly mild toxicity profile, and is currently FDA approved for some hematologic cancers – failed in a CRPC trial, in part due to unacceptable toxicity (171). Another KDAC inhibitor, romidepsin, resulted in withdrawal of 11/35 CRPC patients from a phase II trial due to toxicity issues (172). This may suggest that patients with CRPC may be more susceptible than other oncologic patients to the broader effects of KDAC inhibition. Such a difference may be related to the systemic androgen deprivation instituted in PCa patients, as a phase II study using vorinostat combined with tamoxifen for patients with hormone therapy-resistant breast cancer showed that vorinostat was well tolerated (173). Still, interest in KDAC inhibitors as a potential route for PCa therapeutics remains, and it is likely that more inhibitors will be brought to clinical trials.

Other AR interacting proteins and miRNAs

Although chaperones, pioneer factors, steroid receptor coactivators, and chromatin remodelers (including KDACs and KATs), make up perhaps the more well-studied AR-interacting molecules, there are numerous other factors and molecules to consider targeting.

One of the earlier proteins shown to directly interact with the AR is AR-associated protein 70 (ARA70), which was identified in 1996, and shown to potentiate the transcriptional activity of the AR (174). More work on ARA70 has indicated that there are actually two splice variants, ARA70- α and ARA70- β , and that these have distinct and opposing functions. ARA70- β has been shown to promote cell growth and invasion in Matrigel, and overexpression of ARA70- β was demonstrated to drive transcription of genes important for cell division and adhesion. In contrast, ARA70- α may act as an inhibitor for the AR (175).

Homeobox protein B-13 (HOXB13) is a member of the homeobox family, which contains a number of transcription factors which share the homeobox DNA-binding domain and are often associated with regulating development. HOXB13 was reported to have anti-proliferative effects on PCa cells *in vitro* (176), and to play a role in the localization of AR to chromatin (177). Interestingly, a germline mutation in HOXB13, G84E, is associated with a significantly increased risk for hereditary PCa (178). HOXB13 had been shown to be important in the posterior development of mice, and that HOXB13 expression persisted in some adult organs, including the prostate (179). This work was followed up with a HOXB13 knockout study that demonstrated that HOXB13 is required for normal development of the prostate (180). In PCa, the role of HOXB13 is controversial. Overexpression of HOXB13 in LNCaP PCa cells has been shown to inhibit cell proliferation, and this effect was reversed upon androgen stimulation, suggesting that HOXB13 may be a repressor (176). Other studies have revealed a more complex role. HOXB13 has been shown to shift the localization of AR away from AREs on chromatin (through interacting with the AR DNA binding domain), and instead result in the recruitment of androgen responsive complexes to promoters with a HOXB13-response element [177]. A more recent study also demonstrated reprogramming of the AR cistrome during PCa tumorigenesis, and has implicated FoxA1 and HOXB13 as key mediators of this AR shifting to new sites on chromatin (181).

Treatment with siRNA against HOXB13 resulted in loss of cell proliferation in LNCaP cells (177,181). Although no specific inhibitor of HOXB13 has been reported, it is interesting to note that there is a report that a FoxA1 enhancer element regulates HOXB13 in the prostate, potentially linking an important pioneer factor for the AR (FoxA1) with an interesting coregulatory transcription factor (HOXB13) (182).

Other potentially interesting molecules that may impact and disrupt the AR signaling axis are microRNAs (miRNAs). Several miRNAs, including miRNAs that are suppressed in primary and, in particular, metastatic PCa can target and regulate the expression of the AR itself and its p160 SRC coactivators (183,184). One of the most notable examples is miR-31. miR-31 expression levels have been shown to inversely correlate with those of AR, while expression of miR-31 is decreased in PCa patient samples; this decrease has been shown to occur through enhanced promoter methylation (185). In another example, miR-137 has been shown to target and decrease the expression of all three p160 SRCs in PCa cells. Similar to miR-31, miR-137 is epigenetically silenced in many cancers (136). The methyltransferases responsible for promoter methylation or hypermethylation of miRNA genes, or other proteins that may inhibit or regulate the AR or interacting factors may also be considered as potential therapeutic targets to disrupt the AR signaling axis. Several well-studied demethylating agents, including 5-azacytidine and 5-aza-2'-deoxycytidine, have been brought to clinical trials in patients with liquid tumors. Additionally, 5-azacytidine has shown some promising results in a phase II clinical trial for metastatic PCa with demonstrated clinical resistance to docetaxel. In this study, a majority of patients demonstrated a decrease in PSA levels after 12 weeks of therapy (186).

Conclusions and Future Directions—Identifying new targets and developing new therapeutics for these targets is crucial to continue extending lifespan and improving quality of life for men suffering from PCa. Despite the historical benefit of directly targeting the AR, or the synthesis of its ligands, it is becoming increasingly apparent that directly inhibiting the AR is not enough; PCa overcomes these therapies, often remaining reliant upon the AR signaling axis.

Nuclear receptors, including the AR, rely on a vast network of cooperating molecules to enact their signaling effects on the cell. Taking advantage of these interacting proteins for therapeutics may provide an additional avenue to combat the castration-resistant phenotype in PCa. Research into AR-associated proteins and development of inhibitors for these molecules has shown that there are opportunities for clinical benefit, with high potential for next-generation PCa treatment or combination therapies.

Significant advances have been made since the first orchiectomy was shown to have activity against PCa, yet over the past 70 years, the primary focus remains directly targeting the AR LBD or ligand. As technology advances, and our understanding of PCa biology moves forward, it is critical that PCa research moves beyond the “low-hanging fruit” of inhibiting the LBD of the AR. Given the continued resistance to AR-directed therapies, and the potential role that ARVs may play in developing resistance to therapies, the development of targeted therapeutics that disrupt protein-protein interactions presents an attractive direction. By focusing on disrupting interactions with the AR, especially in combination with

inhibition of the AR itself, future therapeutic agents may provide more robust inhibition of PCa cells and, ideally will pose a roadblock to the development of resistance. In addition to developing inhibitors of protein interfaces or of AR interacting proteins, the use of targeting agents, such as PSMA-targeted nanoparticles, will likely be a key component of more effective drug development. Future research efforts should be directed towards moving beyond the AR and disrupting other elements of the AR signaling axis, likely with rational combinations of other targeted therapies, in order to make continued advances in our understanding of PCa and CRPC biology, and to bolster our ability to effectively treat this disease.

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A. Full-length AR

B. ARv7 (AR3)

C. ARv567es

Figure 1.

Comparison of the full-length AR and two AR variants. The full-length AR is comprised of eight exons (A) which make up the amino-terminal domain (NTD), the DNA-binding domain (DBD), a hinge region, and the carboxy-terminal ligand binding domain (LBD). The ARv7 - also known as AR3 - contains exons 1-3 (B), comprising the NTD and the DBD, followed by a short cryptic exon encoding 16 amino acids which are not present in the full-length AR. The LBD is missing in the ARv7, resulting in a constitutively active variant. The ARv567es (C) contains exons 1-4 but is missing exons 5-7. In addition, exon 8 has a translational frame-shift.

Figure 2.

Representative cartoon of the canonical AR signaling axis in a normal prostate epithelial cell. Androgens diffuse through the plasma membrane into the cytosol, where they bind to the chaperone-sequestered AR and induce a conformational change, resulting in its nuclear translocation and dimerization. In the nucleus, pioneer factors such as GATA-2 and FoxA1 make the chromatin more accessible to other transcription factors and allow dimerized AR to bind to its hormone response elements on chromatin. Once bound, AR recruits co-regulator proteins that help assemble the transcriptional machinery and result in the transcription of the AR-target genes.

Figure 3.

Representative cartoon of currently FDA-approved prostate cancer therapies, their mechanisms of action, and proposed mechanisms of resistance. Therapies include: GnRH analogs that suppress gonadal androgen synthesis; small-molecule androgen synthesis inhibitors (e.g. abiraterone) that can block enzymatic steps required for androgen steroidogenesis; androgen receptor antagonists (e.g. enzalutamide) that bind to the AR LBD and prevent activation; microtubule inhibitors can disrupt the cell cycle and also may prevent AR translocation into the nucleus. Mechanisms of resistance include: intratumoral androgen production to locally generate ligand for the AR; overexpression of AR (frequently through amplification of the AR gene) to enhance sensitivity to low levels of ligand; AR LBD mutations to relax stringency for the activating ligand; ARVs which are constitutively active; and other nuclear receptors substituting for the AR.

Figure 4.

(A) Chaperone (e.g., Hsp90) inhibitors can disrupt AR protein stabilization or proper folding, and result in degradation and/or dysfunction of the AR protein. (B) Pioneer factor (e.g., FoxA1, GATA2) inhibitors may prevent the AR from accessing response elements on chromatin, or may interfere with the ability of AR to recruit coregulators needed for transcriptional activity. (C) p160 steroid receptor coactivator (SRC) inhibitors may prevent the recruitment of the transcriptional machinery components required for efficient transcription. (D) KAT and KDAC inhibitors may directly modulate post-translational

modifications on the AR itself, reducing its activity or may interfere with chromatin remodeling to make the chromatin less amenable for active transcription.

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