New Opportunities for Targeting the Androgen Receptor in Prostate Cancer

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Recent genomic analyses of metastatic prostate cancer have provided important insight into adaptive changes in androgen receptor (AR) signaling that underpin resistance to androgen deprivation therapies. Novel strategies are required to circumvent these AR-mediated resistance mechanisms and thereby improve prostate cancer survival. In this review, we present a summary of AR structure and function and discuss mechanisms of AR-mediated therapy resistance that represent important areas of focus for the development of new therapies.

Prostate cancer is the most common solid tumor in men, accounting for 21% of all cancers in the United States, and is the second-leading cause of male cancer-related death (Siegel et al. 2016). Localized prostate cancer can be cured with surgery and/or radiation therapy. For advanced, metastatic, or recurrent prostate cancer, treatment with androgen deprivation therapy (ADT) is the current standard of care, which exploits the fact that prostate cancer cells are exquisitely dependent on androgens and the androgen receptor (AR) for growth and survival (Huggins et al. 1941). ADT is a chemical form of castration that uses luteinizing hormone releasing hormone (LHRH) analogs to suppress testicular production of androgens by the hypothalamic–pituitary–gonadal axis. ADT can be

used alone or in conjunction with competitive AR antagonists that act peripherally to prevent residual androgens binding the AR (Labrie 2011). Although initially effective, ADT eventually fails, and patients progress to an incurable and lethal stage of disease, known as castration-resistant prostate cancer (CRPC) (Scher et al. 2004; Attard et al. 2016).

For many years, the chemotherapeutic agent docetaxel was the only approved treatment option for CRPC (Tannock et al. 2004). The realization that AR is reactivated in CRPC and remains the primary therapeutic target for this disease led to the development of second-generation AR-targeting agents, abiraterone acetate and enzalutamide, which improve survival of CRPC patients (de Bono et al. 2011; Scher

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et al. 2012). Abiraterone inhibits androgen biosynthesis in the testes and adrenals and within prostate tumor cells by blocking cytochrome P450 (CYP17A1), a critical enzyme for testosterone synthesis (Potter et al. 1995; O'Donnell et al. 2004; Attard et al. 2005). Enzalutamide is a new-generation AR antagonist with greater potency than first-generation agents (Tran et al. 2009). The therapeutic landscape for CRPC broadened even further with recent approvals of cabazitaxel (second-line chemotherapy), radium-223 (bone-targeted radiopharmaceutical), denosumab (anti-RANK ligand monoclonal antibody), and sipuleucel-T (immunotherapy). However, despite the past decade of therapeutic revolution, the fact remains that none of these agents improve survival by more than a few months on average. Moreover, in the majority of cases in which second-generation AR-targeting agents or chemotherapies have failed, AR remains the key driver of disease (Karantanos et al. 2015). Thus, discerning the mechanisms by which AR continues to signal in the face of therapy is critically important.

This review provides an overview of AR structure and function and the key structural alterations in the AR gene and protein associated with resistance to ADT. Areas of research that represent key opportunities for more effective targeting of AR in prostate cancer are discussed.

ANDROGEN RECEPTOR STRUCTURE AND FUNCTION

The AR is an intracellular ligand-activated transcription factor that mediates the biological actions of androgens (Evans 1988). As a member of the steroid receptor subfamily of nuclear receptors (Lu et al. 2006), the AR shares structural and functional homology with the glucocorticoid, mineralocorticoid, progesterone, and estrogen receptors (Germain et al. 2006). The human AR gene is located on the X chromosome at Xq11-12 and is organized into eight exons (Fig. 1A) (Brinkmann et al. 1989). The 90-kb gene encodes a 110-kDa modular protein of 919 amino acids that is organized into four distinct functional domains (Fig. 1A) (Lubahn et al. 1988; Tilley et al. 1989): the ligand-binding domain (LBD), hinge

domain, DNA-binding domain (DBD), and amino-terminal domain (NTD).

The LBD folds into 12 α -helices to form a ligand-binding pocket that mediates androgen binding (Matias et al. 2000; Sack et al. 2001) and is the target site of all clinically used AR antagonists. Upon ligand binding, the AR adopts an active conformation that induces formation of an AF-2 activation domain (He et al. 2004). Unlike the other steroid receptors, whose AF-2 surface is the primary site of interaction with coregulator proteins (Danielian et al. 1992; Heery et al. 1997), the AR AF-2 preferentially interacts with FXXLF motifs in the amino terminus of AR (Ikonen et al. 1997; Berrevoets et al. 1998; Langley et al. 1998; Schaufele et al. 2005). This so-called N/C interaction facilitates cross talk between receptor domains that is critical for receptor stabilization, reduced ligand dissociation, and enhancing DNA-binding affinity (He et al. 2000; He and Wilson 2002). The AF-2 domain can recruit specific AR coregulatory proteins containing FXXLF and LXXLL motifs that contribute to receptor function (He et al. 2002). Linking the LBD to the DBD is a small, flexible hinge region, which contains a bipartite nuclear localization signal (NLS) that is exposed on ligand binding and mediates interaction with importin- α to translocate AR from the cytoplasm into the nucleus (Cutress et al. 2008). The hinge is an important site for posttranslational modification of the receptor, including acetylation, ubiquitination, and methylation (Clinkemalie et al. 2012). The DBD is α -helical in structure and contains two cysteine-rich zinc finger motifs that mediate receptor homodimerization and direct interaction with the major groove of DNA (Luisi et al. 1991; Schoenmakers et al. 1999; Shaffer et al. 2004) at specific recognition sequences known as androgen response elements (AREs), which are located in the enhancer and promoter regions of AR target genes (Claessens et al. 2001). The consensus ARE is comprised of two hexameric half-sites of the sequence 5'-TGTTCT-3' separated by a 3-base-pair spacer (Claessens et al. 1996; Schoenmakers et al. 1999; Claessens and Gewirth 2004). The AR also binds DNA at ARE half-sites in collaboration with transcription factors such as FoxA1,

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Figure 1. Chromosome, gene, and protein organization of the androgen receptor (AR) and AR variants. (A) The human AR is encoded by a single gene located at Xq11-12 and is normally organized into eight exons, encoding a protein of ~919 amino acids. The full-length AR protein is divided into structural and functional domains: a large amino-terminal transactivation domain (NTD) containing activation function-1 (AF-1) and -5 (AF-5) and two LxxLL-like motifs ²³FQNL²⁷ and ⁴³³WHTLF⁴³⁷, a DNA-binding domain (DBD), a small hinge region (H) containing a nuclear localization signal (NLS), and a ligand-binding domain (LBD) containing activation function 2 (AF-2). Gain-of-function mutations in the AR gene as a result of therapy-mediated selection pressure colocalize to the LBD. Alternative splicing of cryptic exons (CEs) or exon skipping can give rise to carboxy-terminally truncated AR isoforms. (B) Structure of two clinically relevant AR-Vs, AR-V7 and ARv567es.

GATA2, and Oct1 that facilitate access of AR to the chromatin (Bolton et al. 2007; Massie et al. 2007; Wang et al. 2007; Lupien and Brown 2009). The AR amino-terminal domain (NTD) mediates the majority of AR transcriptional activity through two activation domains (AF-1 and AF-5) that recruit complexes of coregulators (Chmelar et al. 2007; DePriest et al. 2016) and the basal transcriptional machinery (Jenster et al. 1995; McEwan and Gustafsson 1997; Shang et al. 2002) to regulate the expression of AR target genes (Glass and Rosenfeld 2000; Ra-

chez and Freedman 2001). Whereas the LBD and DBD are highly conserved and structured regions of the AR, the NTD has limited stable secondary or tertiary structure and is considered an intrinsically disordered protein domain, which presents challenges for therapeutic targeting of this domain. Despite its general lack of structure, the NTD does fold on interaction with coregulators or DNA, the conformation of which is dictated by the particular binding partner or DNA sequence (Kumar et al. 2004; Brodie and McEwan 2005).

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ANDROGEN RECEPTOR MATURATION AND FOLDING

Newly synthesized AR undergoes a highly ordered maturation process facilitated by the molecular chaperones' heat shock protein (HSP)70 and HSP90, and their cochaperones, to be correctly folded into its high-affinity ligand-binding form (Pratt and Toft 2003). Following synthesis, AR is rapidly bound by the cochaperone HSP40 to prevent aggregation of the unfolded receptor (Fan et al. 2003). HSP40 recruits HSP70 and stimulates its ATPase activity, allowing HSP70 to bind and deliver AR to HSP90 via the HSP organizing protein (HOP) (Chen and Smith 1998; Hernandez et al. 2002). HOP is one of many cochaperones that contain a tetratricopeptide repeat (TPR) domain; a protein-protein interaction surface characterized by three or more TPR motifs of a degenerate 34 amino acid sequence (Scheufler et al. 2000). Through its TPR domain, HOP binds to a highly conserved MEEVD sequence in both HSP70 and HSP90 to transfer AR from one chaperone to the other through a process that is still relatively uncharacterized (Chen et al. 1998). HSP90 binds as a homodimer directly to AR through the AR-LBD and assembly of this receptor-chaperone heterocomplex induces the ATP-bound "closed" form of HSP90 (Pratt and Toft 1997), which is stabilized by the cochaperone p23 (Dittmar et al. 1997; Morishima et al. 2003). The presence of ATP weakens the affinity of HSP90 for HOP, and the HOP-HSP40-HSP70 complex is displaced by any one of several TPR cochaperones, known as immunophilins (Carrello et al. 1999). Members of the immunophilin family, including TPR-containing proteins FKBP51, FKBP52, CYP40, or PP5, display peptidyl-prolyl-isomerase (PPIase) activity that promotes AR-HSP90 heterocomplex assembly (Ni et al. 2010). The intrinsic ATPase activity of HSP90 together with the PPIase activity of the immunophilins fold AR into its functionally mature state by opening the AR ligand-binding pocket to make it accessible to androgens (Fang et al. 1996; Grenert et al. 1999; Ni et al. 2010). The mature AR is maintained in the cytoplasm in a heterocomplex that includes cochaperone SGT α , which

anchors the receptor to cytoskeletal microtubules and actin filaments (Buchanan et al. 2007; Trotta et al. 2012); immunophilins, which link AR to the dynein motor protein (Galigniana et al. 2002; Harrell et al. 2002); and HSP90 and its cochaperones, which protect AR from degradation and hold it in a ligand-inducible state (Pratt and Toft 1997). In the absence of ligand, FKBP51 is the major immunophilin in the AR-HSP90 complex. Binding of cognate ligand to the AR results in displacement of FKBP51 by FKBP52, which facilitates trafficking of AR along microtubules into the nucleus via retrograde movement of the dynein motor complex (Buchanan et al. 2007; Galigniana et al. 2010).

STRUCTURAL CHANGES TO THE AR AS CASTRATION RESISTANCE MECHANISMS

The failure of ADT to achieve durable inhibition of prostate tumor growth was previously attributed to an outgrowth of tumor cells that were androgen-independent and no longer required AR signaling for survival. However, it is now recognized that prostate cancer cell "addiction" to the AR is manifested in adaptive changes that ensure persistent AR signaling in the face of ADT (Knudsen and Scher 2009). This concept formed the basis of a drug screen that identified the now widely used AR antagonist enzalutamide (xtandi), which was undertaken in prostate cancer cell lines engineered to express high levels of AR to reflect this common clinical scenario (Tran et al. 2009). Similar research has led to the identification of newer AR antagonists including ARN-509 (Clegg et al. 2012) and ODM-201, both of which are currently in phase III clinical testing (see Clinicaltrials.gov identifiers NCT01946204, NCT02489318, NCT02531516, NCT02200614, and NCT02799602).

The most recent, large-scale genomic analysis of CRPC suggests that >70% of CRPC cases harbor AR pathway aberrations, consistent with adaptive changes to maintain AR activity (Robinson et al. 2015). Understanding how these aberrations drive AR signaling in the castrate environment remains a major research focus.

AR Gene Amplification

The first studies of AR pathway aberrations showed genomic amplification of the AR locus in up to 30% of recurrent primary or castration-resistant prostate tumors, with little or no amplification detected in untreated prostate cancer (Visakorpi et al. 1995; Koivisto et al. 1997; Bubendorf et al. 1999; Linja et al. 2001). More recent studies, using whole-exome sequencing or targeted sequencing of cell-free DNA in metastatic CRPC, indicated that AR gene amplification emerges more frequently than previously estimated, occurring in 45%–60% of cases (Taylor et al. 2010; Grasso et al. 2012; Carreira et al. 2014; Robinson et al. 2015; Wyatt et al. 2016), and is associated with therapeutic resistance and poorer overall and progression-free survival (Romanel et al. 2015; Salvi et al. 2015; Wyatt et al. 2016). The increase in AR mRNA and protein levels that result from AR gene amplification has been shown to sensitize prostate cancer cells to castrate levels of androgens (Visakorpi et al. 1995). Moreover, using isogenic prostate cancer xenograft models, Chen and colleagues showed that increased AR mRNA was the primary change in the progression to castration resistance and was sufficient for resistance to castration or the AR antagonist bicalutamide (Chen et al. 2004).

AR Gene Mutations

The first indication that AR mutations may contribute to castration-resistance came from studies of the AR-dependent prostate cancer cell line, LNCaP (Wilding et al. 1989; Veldscholte et al. 1990; Culig et al. 1999). A single point mutation, T878A (originally reported as T877A), in the AR-LBD of LNCaP cells confers AR activation not only by androgens but also progestins, estrogens, glucocorticoids, and certain AR antagonists (hydroxyflutamide, nilutamide, bicalutamide). This phenotype, known as ligand promiscuity, facilitates AR activation in a low-androgen environment. Efforts to define the frequency of somatic AR gene mutations in prostate cancer have revealed that they are rare in primary disease but emerge in tumors pro-

gressing on ADT or following second-line hormonal manipulations, supporting the notion of clonal selection during treatment (Gundem et al. 2015). Early studies using PCR-based methods identified more than 85 mutations in clinical prostate cancer that occur within distinct regions of the AR NTD, hinge, and LBD (McPhaul et al. 1991; Suzuki et al. 1993; Taplin et al. 1995; Tilley et al. 1996; Buchanan et al. 2001; Gottlieb et al. 2004; Steinkamp et al. 2009). The biological consequence of these mutations was attributed to their location, with AR-LBD mutations conferring ligand promiscuity and AR-NTD mutations enhancing AR transactivation capacity through altered coregulatory interactions (Buchanan et al. 2001; Gottlieb et al. 2004). In contrast to these early findings, recent next-generation sequencing efforts have identified few mutations within the AR-NTD and instead ascribe the LBD as the primary mutational hotspot in CRPC, with a reported incidence of up to 20% (Taylor et al. 2010; Barbieri et al. 2012; Grasso et al. 2012; Robinson et al. 2015). The T878A mutation present in LNCaP cells is one of the most frequent clinically observed AR mutations. In addition to conferring agonist activity to first-generation AR antagonists, the T878A mutation also converts second-generation antagonists enzalutamide and ARN-509 into partial agonists (Lallous et al. 2016) and has been identified in patients progressing on abiraterone (Azad et al. 2015a; Romanel et al. 2015). Other AR mutations frequently observed across clinical studies include L702H, W742C/L, and H875Y, all of which confer resistance to AR antagonists, abiraterone, and/or hydrocortisone to facilitate castration resistance (Fig. 1A) (Lallous et al. 2016).

AR Splice Variants

AR splice variants (ARVs) derived from mRNA species that lack part or all of the exonic sequence encoding the LBD can be grouped into two broad classes: (1) truncated owing to the incorporation of a cryptic exon (CE); or (2) exon skipping events (Fig. 1B). ARV mRNAs were first identified in cell lines but have since been detected in patient specimens, with recent RNA-seq data indicating the presence of at least

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16 distinct ARV mRNA species in primary prostate cancer (The Cancer Genome Atlas Research Network 2015) and 23 in metastatic CRPC (Robinson et al. 2015). However, it must be noted that only two variants, AR-V7 and ARv567es, have been definitively detected at the protein level (Efsthathiou et al. 2015; Qu et al. 2015; Welte et al. 2016; Henzler et al. 2016). This is partly caused by the dearth of ARV-specific antibodies but also the fact that abundance of ARVs is likely much lower than the full-length receptor, with an early study suggesting that AR-V7 and ARv567es mRNAs are present at ~0.03%–7% of the full-length receptor in CRPC bone metastases (Hornberg et al. 2011). A subsequent study validated this finding for AR-V7, suggesting that it is expressed at ~5% of the levels of the canonical AR transcript in CRPC (Robinson et al. 2015).

The combination of constitutive receptor activity with the absence of a functional LBD renders ARVs resistant to conventional ADT (Chan and Dehm 2014). This has led to the obvious hypothesis that these variants play a critical role in driving CRPC. Whereas ARVs have been shown to be required for castrate-resistant growth in various models (Chan et al. 2015), their clinical relevance has been harder to decipher (for a recent summary of this topic, see Luo et al. 2017). This is, at least, in part attributed to the aforementioned difficulty in detecting ARV proteins. Recent progress in this area, such as the analytical and clinical validation of an AR-V7 IHC method (Welte et al. 2016), suggests that this particular issue will ultimately be overcome. Nevertheless, the clinical significance of ARVs as a resistance mechanism in CRPC will remain equivocal until ARV-specific drugs are developed (Sharp et al. 2016).

Although the role of ARVs as a key driver of CRPC remains to be definitively resolved, recent findings have shown the utility of AR-V7 as a marker that predicts lack of response to AR-targeted therapies in CRPC. Antonarakis and colleagues (2014) first showed that men with detectable expression of AR-V7 mRNA in circulating tumor cells (CTCs) had lower response rates to enzalutamide and abiraterone. Since that time, numerous studies have verified the capacity of AR-V7 mRNA to predict response to these

AR-targeted therapies (for review, see Li et al. 2017); moreover, an AR-V7 protein immunofluorescent assay on the Epic Sciences CTC detection platform developed by Scher and colleagues (2016) had similar predictive capacity. Importantly, the efficacy of chemotherapy is independent of AR-V7 expression in CTCs and other patient material (Antonarakis et al. 2015; Onstenk et al. 2015; Scher et al. 2016). Although AR-V7 clearly has great promise to guide treatment decision-making, key questions remain. First, the optimal method for measuring AR-V7 (i.e., qRT-PCR, in situ hybridization, immuno-detection) and the most suitable source of patient material (i.e., CTCs, whole blood) remain to be determined (Luo et al. 2017). Second, results from a randomized controlled trial (NCT02269982) to prospectively evaluate the predictive utility of AR-V7 are not yet available. Finally, the presence of AR mutations and/or copy number alterations in plasma cell-free DNA also provides predictive and prognostic information (Azad et al. 2015a; Wyatt et al. 2016); whether AR-V7 has greater and/or independent clinical utility compared with these genomic alterations remains to be determined. In short, the widespread application of AR-V7 as a tool to predict treatment response is far from assured. Nevertheless, given the estimate that AR-V7 testing in CRPC patients could result in savings of \$150M per annum in the United States alone by reducing ineffective use of abiraterone and enzalutamide (Markowski et al. 2016), the ongoing interest in this potential biomarker application is clearly warranted.

FUTURE PERSPECTIVES

The recent evolution in understanding AR structure and function is facilitating new strategies to target this essential factor in prostate cancer. Below, we highlight fields of biology and therapy that we believe represent key areas of focus in the near future.

Coregulators

AR is regulated by a diverse and large suite of more than 300 protein coregulators that activate

(coactivators) or repress (corepressors) its activity (Chmelar et al. 2007; Heemers and Tindall 2007; DePriest et al. 2016). The complexity of this network is immense and is indicative of the extent and diversity of the coregulators with potential impact on AR function (Fig. 2); moreover, new coregulators continue to be identified (Paltoglou et al. 2017). As coregulators are essential for the transcriptional activity of AR, it is not surprising that coregulator expression and activity is altered throughout the course of prostate cancer progression and in response to AR targeting therapies, with enhanced activity of coactivators and reduced activity of corepressors being a hallmark of CRPC (Chmelar et al. 2007; Heemers and Tindall 2007). We anticipate that the AR coregulator field will experience rapid progress in the next decade, facilitated by new methodologies and a better appreciation of their functional role in CRPC. For example, with the advent of more sensitive mass spectrometric methods for isolating protein:protein interactions (Mohammed et al. 2016), identification of additional AR coregulators associated with chromatin in specific disease states will be possible. Such methods will also allow robust comparisons of the protein “interactomes” of wild-type AR, and specific AR mutants and variants. Additionally, the power of integrative “omics”—incorporating cistromic, transcriptomic, genomic, and proteomic data, among others—will expedite identification of the coactivators that are critical for AR activity in castrate-resistant settings (Paltoglou et al. 2017; Stelloo et al. 2017).

As our understanding of AR coregulator biology continues to evolve, we believe that these factors will become a major class of therapeutic target in CRPC (reviewed in Biron and Bedard 2015; Foley and Mitsiades 2016). Considerable progress toward the development of small molecules that inhibit coactivator binding to the AR-NTD, AF-2 in the AR-LBD or binding function 3 in the AR-LBD has already been made (Lack et al. 2011; Munuganti et al. 2013; Myung et al. 2013; Ravindranathan et al. 2013). In many of these cases, the precise receptor binding surface for coregulators interacting with AR are unknown, limiting current efforts to develop drugs

that modulate their function. We propose that integration of genome-wide technologies to define coregulator activity in critical disease-specific contexts combined with rational drug design will accelerate this field. As an example of this workflow, an integrated transcriptomic/kinomic approach identified the first kinase “chaperone” of the AR, choline kinase A (CHKA) (Asim et al. 2016). CHKA expression is associated with poor outcome, potentially because it stabilizes the AR to enhance the AR signaling axis. This approach has high potential for rapid translation to the clinic because kinases are highly “druggable” (Fleuren et al. 2016).

Chaperones

The critical role of chaperones in AR maturation and signaling makes these proteins attractive therapeutic targets (reviewed in Azad et al. 2015b). This notion is supported by the significant focus to date on developing molecular inhibitors of HSP90 as novel agents for prostate cancer therapy (reviewed in Butler et al. 2015). Through binding the amino-terminal ATP-binding pocket of HSP90, inhibitors such as 17-allylamino-17-desmethoxygeldanamycin (17-AAG) and AUY922 prevent HSP90 from achieving its ATP-bound state and thus formation of the substrate-HSP90 complex (Prodromou et al. 1997; Janin 2010). Consequently, rather than being folded, AR and other HSP90 substrates are degraded by the ubiquitin proteasome pathway, a process that is fatal to prostate cancer cells (Solit et al. 2002; Williams et al. 2007; Centenera et al. 2012). Although HSP90 inhibitors have been in clinical development for prostate cancer for many years, one of the underlying issues is that agents currently in clinical trial, all of which inhibit the ATP-binding pocket, induce a resistance mechanism known as the heat shock response (Bagatell et al. 2000; Butler et al. 2015). The heat shock response, mediated by heat shock factor 1 (HSF1), is a highly conserved mechanism that induces expression of cytoprotective proteins HSP70, HSP40, and HSP27 to protect organisms against heat and other environmental stresses (Richter et al. 2010). Inhibition of HSP90 releases HSF1, resulting in in-

creased expression of heat shock proteins that protect the cell and mediate resistance against the cytotoxicity associated with HSP90 inhibition (Bagatell et al. 2000; McCollum et al. 2006). We and others have endeavored to circumvent this issue by investigating inhibitors that target sites on HSP90 other than the amino-terminal ATP-binding pocket, such as the carboxy-terminal nucleotide binding pocket (Shelton et al. 2009; Matthews et al. 2010; Eskew et al. 2011) or the middle-linker domain (Armstrong et al. 2016). These agents show excellent antitumor activity in prostate cancer cell lines and one has shown efficacy in vivo (Eskew et al. 2011), which warrants further investigation of this class of compound to enhance their potency, solubility, and pharmacokinetic properties.

Alternative chaperones that have been investigated as potential targets for prostate cancer include HSP70, HSP27, and CLU. In addition to its role in AR maturation, HSP70 is a potent antiapoptotic factor in both normal and cancerous cells (Mosser et al. 1997). Targeting HSP70 expression with short-interfering RNA (siRNA) or antisense oligonucleotides sensitizes prostate cancer cells to apoptosis induced by a variety of cancer treatments, including ionizing radiation and taxol (Gabai et al. 2005). Despite these findings, further clinical evaluation of targeting HSP70 has been hampered by a lack of tolerable HSP70 inhibitors (Britten et al. 2000). Whereas HSP27 and CLU are not as amenable as HSP90 or HSP70 to small molecule inhibitors because they lack an ATP-binding site, antisense oligonucleotide strategies have been successfully used to achieve knockdown of these chaperones clinically. The second-generation HSP27-targeting antisense oligonucleotide OGX-427 induces proteasomal degradation of the AR, inhibits AR transcriptional activity, and results in induction of apoptosis and suppression of PSA levels and metastasis in cell line and xenograft models of prostate cancer (Kumano et al. 2012; Shiota et al. 2013; Lamoureux et al. 2014). OGX-427 has been evaluated alone and in combination with docetaxel in metastatic CRPC patients, demonstrating an acceptable safety profile and declines in PSA, measurable disease

and CTC count (Hotte et al. 2012; Chi et al. 2016), and further clinical evaluation of OGX-427 is ongoing. Modulation of CLU expression with siRNA or antisense oligonucleotides has also been efficacious preclinically, inhibiting both tumor growth and metastasis in xenograft models, including synergy with ionizing radiation, docetaxel and enzalutamide (Miyake et al. 2000; Gleave et al. 2001; Zellweger et al. 2002; Lamoureux et al. 2011; Matsumoto et al. 2013). On the basis of these data, the CLU antisense oligonucleotide OGX-011 entered clinical evaluation and has progressed to phase III testing (Chi et al. 2005, 2010; Saad et al. 2011) (Clinicaltrials.gov identifiers: NCT01188187; NCT01578655).

Targeting chaperone proteins for the treatment of CRPC holds significant promise but the therapeutic potential of many HSP inhibitors has been limited by their poor efficacy and/or toxicity profiles in the clinic. An alternative approach to using these agents may be in combination therapy strategies, particularly as most of the chaperone inhibitors mentioned above have shown the capacity for synergy with existing prostate cancer treatments including radiation therapy, taxanes and AR antagonists. The potential for enhanced therapeutic benefit with combinatorial treatment strategies for CRPC is discussed in greater detail later in this review.

Non-LBD Targeting of the AR

The AR-NTD is the predominant mediator of AR transcriptional activity, making it an extremely attractive therapeutic target for prostate cancer, but its intrinsic disorder and flexible structure has hindered efforts to achieve this goal. Two different types of NTD-targeting agents are currently in development. The first is the small molecule, EPI-001, which directly binds the NTD at the AF-5 activation domain (De Mol et al. 2016), thereby blocking AR/coactivator interaction and nuclear localization to inhibit AR and ARV activity (Andersen et al. 2010; Myung et al. 2013). EPI-001 has shown promising preclinical efficacy (Andersen et al. 2010; Myung et al. 2013), although it has been

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reported to have off-target activity (Brand et al. 2015). A derivative of EPI-001, EPI-506, is currently being assessed in a phase I/II trial (Clinicaltrials.gov identifier: NCT02606123). The second type of AR-NTD targeting agent includes peptide decoys comprised of NTD sequences that competitively bind AR coactivators. A decoy peptide comprised of the entire NTD (decoy AR₁₋₅₅₈) inhibits transcriptional activity of the AR under both androgen-dependent and -independent conditions in vitro and in vivo, implying that the AR-LBD is not the target of these agents (Quayle et al. 2007).

Significant homology between the DBD of the steroid receptors (Brinkmann et al. 1989) has made this region a challenge to target therapeutically. DNA-binding polyamides that bind ARE sequences to prevent AR from interacting with DNA have been developed and show inhibition of subsets of androgen-regulated genes depending on the specific sequence of the polyamide (Nickols and Dervan 2007; Chenoweth et al. 2009). For example, the sequence 5'-AGAACA-3' inhibits a similar number of DHT-induced transcripts as bicalutamide in prostate cancer cell lines, whereas the sequence 5'-WGWCGW-3' inhibits a significantly lower number of transcripts (Nickols and Dervan 2007). Given the diversity in sequences of AREs, mixtures of polyamides will likely be required to achieve sufficient AR inhibition with these agents for the treatment of prostate cancer. More recently, an in silico drug screen approach identified a targetable site within the AR-DBD that led to development of the morpholiny thiazole VPC-1449, which displays potency against the AR in LNCaP cells and LNCaP xenografts similar to that of enzalutamide (Chenoweth et al. 2009; Li et al. 2014). VPC-1449 did not reduce AR levels or prevent AR nuclear localization but reduced interaction of AR with the *PSA* and *TMPRSS2* gene promoters, indicating blockade of DNA binding (Dalal et al. 2014). VPC-1449 specifically inhibited AR and ARV transcriptional activity at low micromolar concentrations without cross-reactivity with GR, PR, or ER α (Dalal et al. 2014), and therefore represents a potential new avenue of treatment for ARV-mediated CRPC.

Genomic Interplay between Hormone Receptors

Recent studies in breast cancer have revealed extensive interplay at the cistromic level between estrogen receptor- α (ER α), the key driver of tumor growth, and other hormone and orphan receptors (Mohammed et al. 2015; Singhal et al. 2016; Swinstead et al. 2016). Such cross talk shapes and reprograms the transcriptional activity of ER α to influence disease progression. For example, we recently showed that progesterone activation of the PR results in a physical association with ER α , which consequently redirects ER α to specific sites on chromatin to induce a transcriptome that is associated with good clinical outcome. Conversely, retinoic acid receptors (Hua et al. 2009), ER- β (Charn et al. 2010), and AR (Hu et al. 2016) can antagonize the transcriptional activity of ER α by competing for specific chromatin binding sites: indeed, we have proposed that this is one key mechanism by which androgens suppress breast cancer growth (Hickey et al. 2012).

We predict that steroid receptor cross talk has a similar level of influence on AR in the prostate. Although the functions of certain receptors, including GR, PR, ER α , and ER β , have been investigated in prostate cancer (Arora et al. 2013), the vast majority of this work has been performed in isolation from AR. Nevertheless, two studies have shown considerable overlap (~50%) between AR and GR chromatin binding sites in prostate cancer (Arora et al. 2013; Sahu et al. 2013), with current evidence suggesting that GR has a dual role depending on AR status: in AR-positive and androgen-responsive cells, GR can antagonize AR activity, whereas in AR-negative CRPC settings, GR could act as a “surrogate” AR to regulate an androgen-related transcriptional program and drive tumor growth (Kach et al. 2015). This finding has high clinical relevance, because corticosteroids (ligands of GR) are often used to treat patients with CRPC (Heidenreich et al. 2014). Despite these recent findings, very little is known at a molecular level about the specific mechanisms and outcomes of AR/GR cross talk and the same is true for interplay between AR and other steroid

receptors. The challenge now is to elucidate how the chromatin interactions of these steroid receptors dictate the AR cistrome and transcriptional activity and thus prostate cancer growth and progression.

Combination Strategies to Target the AR

An improved understanding of the adaptive mechanisms used by prostate cancer cells to maintain AR signaling has underpinned the emerging paradigm that combination therapy incorporating novel and/or existing therapeutic agents may be a more effective approach to treat advanced prostate cancer. This concept is supported by mathematical modeling approaches indicating that tumor recurrences are inevitable when patients are treated sequentially with single agents, and that simultaneous administration of at least two agents is essential to achieve durable response or cure (Bozic et al. 2013). Rational combinatorial strategies with classes of agents that target different aspects of AR signaling have been the subject of many preclinical and clinical investigations. Combining existing clinical agents is particularly attractive, as extensive pharmacokinetic and pharmacodynamic data are readily available and it circumvents the need to acquire new drug licensing approvals. One successful example of this approach is the addition of the microtubule targeting chemotherapeutic docetaxel to standard ADT, which is based, in part, on the observation that docetaxel is capable of inhibiting AR expression and subcellular localization (Zhu et al. 2010; Darshan et al. 2011) and may therefore target ADT-resistant subclones of prostate tumor cells. Meta-analysis of six clinical trials evaluating chemotherapy plus ADT (termed “chemohormonal therapy”) revealed improved progression-free and overall survival over ADT alone in patients with hormone-naïve metastatic prostate cancer (Ramos-Esquivel et al. 2016). This treatment regimen is now considered a valid first-line therapy for patients with metastatic, hormone-naïve prostate cancer by the National Comprehensive Cancer Network (see nccn.org/professionals/physician_gls/pdf/prostate.pdf) and the European Society of Medical Oncology

(Parker et al. 2015). Current clinical trials investigating combinations of existing AR-targeting prostate cancer treatments are extensive and include ADT plus enzalutamide (Clinicaltrials.gov identifiers: NCT00268476, NCT02058706, NCT02677896) or abiraterone (NCT00268476, NCT01715285, NCT01786265, NCT01751451); docetaxel plus enzalutamide (NCT02288247, NCT01565928, NCT02685267, NCT02453009) or abiraterone (NCT02036060, NCT01400555); and enzalutamide plus abiraterone (NCT01650194, NCT01949337, NCT02268175).

Strategies to combine prostate cancer treatments generally aim to maximize therapeutic efficacy while preventing cross-resistance between the drugs, thereby improving survival outcomes (van Soest et al. 2013). With this in mind, it is generally accepted that these combinations will be insufficient to prevent resistance and control progression to CRPC if they ultimately only target ligand activation of the AR. The development of combination strategies incorporating novel molecular drugs that target distinctly different aspects of the AR pathway will be critical to achieve more complete and durable suppression of AR signaling. For example, the HSP90 inhibitor AUY922, which induces AR protein degradation (Centenera et al. 2015) and inhibits AR signaling independent of AR mutations and ARVs (Gillis et al. 2013), markedly enhanced inhibition of AR signaling when used in combination with the AR antagonist bicalutamide in preclinical studies (Centenera et al. 2015). Likewise, agents that interfere with AR transcriptional activity, such as histone deacetylase inhibitors or DNA topoisomerase II inhibitors, act synergistically with AR antagonists to enhance prostate tumor cell killing in vitro and in vivo (Marrocco et al. 2007; Li et al. 2015; Carter et al. 2016).

These preclinical studies provide support for further clinical investigation, but trial design for combination therapies requires more careful consideration of patient selection, dose range and schedule, drug-drug interactions, and the potential for overlapping or unpredictable toxicities to normal tissues. To address the complexities of administering drugs simultaneously, phase I combination studies are beginning to

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use flexible and innovative designs that allow dose adjustments for maximizing target engagement, tolerability, and efficacy (Yap et al. 2013). Another important consideration is the timing of treatment, as both mathematical evidence and neoadjuvant combination trial results indicate that treating patients early in their disease course, before a sufficient number of resistant cells conferring CRPC have developed, will be key to the success of combination therapy (Bozic et al. 2013; Taplin et al. 2014).

CONCLUDING REMARKS

The importance of AR signaling through all stages of prostate cancer progression is underscored by the adaptive changes to this pathway that occur in response to androgen targeting therapy and which directly drive treatment resistance. The diversity of mechanisms prostate cancer cells deploy to maintain AR activity creates a major clinical challenge. Improving our understanding of these AR resistance mechanisms, and translating them into the next generation of AR targeting agents, will be key to improving the health and well-being of men with prostate cancer.

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New Opportunities for Targeting the Androgen Receptor in Prostate Cancer

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