Review

Androgen Receptor as a Driver of Therapeutic Resistance in Advanced Prostate Cancer

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Abstract

The role of the androgen receptor (AR) signaling axis in the progression of prostate cancer is a cornerstone to our understanding of the molecular mechanisms causing castration-resistant prostate cancer (CRPC). Resistance of advanced prostate cancer to available treatment options makes it a clinical challenge that results in approximately 30,000 deaths of American men every year. Since the historic discovery by Dr. Huggins more than 70 years ago, androgen deprivation therapy (ADT) has been the principal treatment for advanced prostate cancer. Initially, ADT induces apoptosis of androgen-dependent prostate cancer epithelial cells and regression of androgen-dependent tumors. However, the majority of patients with advanced prostate cancer progress and become refractory to ADT due to emergence of androgen-independent prostate cancer cells driven by aberrant AR activation. Microtubule-targeting agents such as taxanes, docetaxel and paclitaxel, have enjoyed success in the treatment of metastatic prostate cancer; although new, recently designed mitosis-specific agents, such as the polo-kinase and kinesin-inhibitors, have yielded clinically disappointing results. Docetaxel, as a first-line chemotherapy, improves prostate cancer patient survival by months, but tumor resistance to these therapeutic agents inevitably develops. On a molecular level, progression to CRPC is characterized by aberrant AR expression, *de novo* intraprostatic androgen production, and cross talk with other oncogenic pathways. Emerging evidence suggests that reactivation of epithelial-mesenchymal-transition (EMT) processes may facilitate the development of not only prostate cancer but also prostate cancer metastases. EMT is characterized by gain of mesenchymal characteristics and invasiveness accompanied by loss of cell polarity, with an increasing number of studies focusing on the direct involvement of androgen-AR signaling axis in EMT, tumor progression, and therapeutic resistance. In this article, we discuss the current knowledge of mechanisms via which the AR signaling drives therapeutic resistance in prostate cancer metastatic progression and the novel therapeutic interventions targeting AR in CRPC.

Key words: Androgen receptor, taxanes, prostate cancer, therapeutic resistance, tumor progression, castration resistance, epithelial-mesenchymal transition.

Prostate Cancer Progression to Metastatic Castration-Resistant Disease

Prostate cancer is the most common malignancy diagnosed in the United States, and the second most common cause of cancer-related deaths. In 2013, a total of 238,590 new cases of prostate cancer were diagnosed and 29,720 died of prostate cancer. Prostate cancer accounts for 28% of the cancer diagnoses in men and 10% of cancer deaths in men [1]. Although local prostate cancer is curable with radical prostatectomy or radiation therapy, advanced prostate cancer can only be palliated with chemical or surgical castration. Ninety percent of men with castration-resistant prostate cancer (CRPC) will develop bone metastases [2]. Upon progression to CRPC, median survival has historically been less than 2 years [3-5]. Advanced prostate cancer is initially treated

with androgen deprivation therapy (ADT)[6]. Chemical or surgical castration, defined as a serum testosterone (T) <50 ng/mL, causes temporary disease regression by initiating apoptosis of malignant prostate cells and indirectly impacting the tumor microenvironment [7, 8]. Progression to CRPC is usually identified by a rising prostate specific-antigen (PSA) despite castrate levels of T, indicating aberrant androgen receptor (AR) reactivation [7, 9, 10] and inhibition of apoptotic pathways [11, 12]. A complex series of molecular events such as oncogene activation, tumor suppressor gene inactivation, apoptosis evasion, intratumoral androgen production, and aberrant AR activation lead to the development of castration resistance [13]. This article will focus on the role of androgen deprivation and AR in the emergence of therapeutic resistance.

The AR Axis in the Prostate Gland

The AR is a nuclear steroid receptor transcribed from the *AR* gene located on Xq11-12[14, 15]. Eight exons encode four functional motifs: an amino-terminal domain, a DNA-binding domain (DBD), a hinge region, and a ligand-binding domain (LBD)[16-18]. The amino-terminal domain contains a transactivation domain, AF1, which is the primary transcriptional regulatory region, and the LBD contains the secondary transcriptional regulatory region, AF2. The DBD is composed of two zinc fingers that are critical to DNA recognition and binding. The hinge domain contains the nuclear localization signal that regulates translocation of the AR into the nucleus, which indirectly effects transcriptional activity [19-21].

Once synthesized AR settles in an inactive form in the cytoplasm bound to chaperone proteins, such as heat shock protein 90 (hsp90). Circulating T levels, of testicular or adrenal origin, are sequestered by sex hormone binding protein (SHBP). Dissociation from SHBP and diffusion across the prostatic plasma membrane brings T into proximity of the cytochrome p450 enzyme 5α-reductase (SRD5A1, SRD5A2), producing the cognate ligand of AR, dihydrotestosterone (DHT). The presence of SRD5A1 generates a DHT rich environment in the prostate, where DHT is more potent than T and is four to five times more concentrated than T[22, 23]. Thus inactive AR binds DHT, causing a conformation change that frees it from its cytoplasmic chaperone proteins. The androgen-AR complex homodimerizes, translocates to the nucleus to bind androgen response elements, and recruits co-activators and co-repressors, which then stimulate transcription of androgen-dependent proteins [5, 24, 25].

Prostate glandular epithelial cells depend on androgens to stimulate androgen-dependent cell processes necessary for their growth and survival. ADT as the effective treatment for prostate cancer as it leads to prostate tumor regression [6]. ADT can be achieved surgically with orchiectomy or chemically with luteinizing hormone-releasing hormone (LHRH) agonists, LHRH antagonists, or anti-androgens. Normal expression of gonadotropin-releasing hormone from the hypothalamus stimulates release of luteinizing hormone (LH) from the pituitary, which activates synthesis of androgens from the testes, adrenals, and peripheral tissues. ADT decreases the amount of circulating T present in the serum by 90%[26, 27], which then limits AR nuclear translocation and transcriptional activation. In addition to impairing AR signaling activation, ADT induces dramatic apoptosis in normal, benign and prostate epithelial cells [7, 9, 22]. LHRH agonists and antagonists inhibit the release of LH via negative feedback inhibition of the hypothalamus-pituitary-adrenal/gonadal axis and direct inhibition respectively. When compared to leuprolide, an LHRH agonist, degarelix, an LHRH antagonist, had a statistically significant improvement in progression free survival and overall survival [28, 29]. There were no significant differences in overall survival or disease-specific survival in patients with metastatic prostate cancer treated with bilateral orchiectomy or LHRH agonists or among different LHRH agonists [30-33]. Though LHRH agonists ultimately lead to inhibition of gonadal androgen production, they initially cause a "flare," which should be blocked with the addition of anti-androgens [34, 35]. As competitive inhibitors of AR, anti-androgens compete with T and DHT for the LBD of AR, preventing activation of the downstream pathway [33, 34]. Extragonadal sources of androgens however may allow consistent AR signaling activation [36, 37].

The testes are responsible for the production of 90-95% of circulating androgens, and the adrenal gland contributes the remainder [26]. Adrenal cortical cells and testicular Leydig cells convert cholesterol to pregnenolone, the primary substrate in the enzymatic pathway of androgen synthesis. In the zona reticularis of the adrenal cortex, pregnenolone is converted to dihydroepiandrosterone (DHEA) by CYP17A enzyme. Small amounts of DHEA are used to produce androstenedione and T. In the testicle, pregnenolone is converted to T via a series of enzymatic reactions with CYP17A, HSD3B2, and HSDB vb17B. In the LNCaP xenograft model, prostate cancer cells are capable of *de novo* intratumor production of DHT despite castrate levels of serum T, making it a poor surrogate marker of total body androgen levels [7, 38, 39]. The residual DHT in castration-resistant prostates on ADT is lower than that found in normal prostates but

sufficient to stimulate the ARs [24, 37, 39, 40]. In CRPC androgen synthesis inhibition can be accomplished by inhibition of enzymes involved in the steroidogenesis pathway occurring in the testes, adrenal, and tumor cells. Abiraterone irreversibly inhibits CYP17A1 halting the pathway to androstenedione, DHEA, T, and estradiol in both the testes and adrenal glands by inhibiting conversion of pregnenolone to 17-OH pregnenolone and 17-OH pregnenolone to DHEA[24, 26]. However, a "backdoor pathway" to DHT synthesis exists that bypasses the therapeutic efforts of androgen synthesis inhibitors [18, 24, 26]. With CYP17A1 inhibition, progesterone accumulates, which can then be converted to DHT through a series of reactions catalyzed by steroidogenic enzymes, including steroid 5-α reductase types 1 and 2[18, 24, 38]. Resistance to androgen synthesis inhibition develops in part by intratumoral androgen production through the backdoor pathway as well as adaptive changes to the AR that allow continued transcription of androgen-dependent genes in an androgen-depleted microenvironment[38, 39]

Aberrant AR Reactivation during Prostate Tumorigenesis

The ARs in tumors cells exposed to ADT undergo selective alterations that result in aberrant AR reactivation, which ultimately allows the AR pathway to remain active despite the shortage of androgenic ligands. AR amplification, promiscuity, and splice variant isoforms are not observed in prostate cancer cells not previously treated with ADT indicating that these changes occur as an adaptive response to ADT [7, 14, 18, 41, 42]. Ligand-independent AR activation can resist androgen signaling targeting and microtubule-targeting chemotherapy (taxanes), a phenomenon that is exploited in the context of EMT [43].

AR Amplification: Following ADT, the AR gene is the most commonly upregulated gene in prostate cancer [44]. AR amplification leads to AR overexpression, which is present in approximately 30% of CRPCs but is not seen in treatment-naïve prostate cancers [5, 14, 41]. Several studies have demonstrated CRPC tissue have increased expression of ARs compared to both benign prostate tissue and prostate cancers not previously treated with hormonal ablation[37, 39, 41, 45, 46]. Exposure to ADT with resulting castrate levels of circulating androgens selects for AR amplification to allow continued AR signaling and castrate-resistance [7, 47]. The increased number of AR improves the likelihood that AR will come into binding proximity with the scarce androgenic ligands. AR overexpression enhances the cellular response to androgens promotion of growth-related processes [5, 48]. Chen et al. discovered partial agonist characteris-

tics of anti-androgens sufficient to activate the AR in the setting of AR overexpression secondary to changes in coactivator recruitment to the AR promoter region (rather than AR gene mutations)[44, 49]. Initiation of newer second-line anti-androgens without agonist potential, such as enzalutamide, prevents the conversion of androgen antagonists to agonists with AR overexpression [48].

AR Promiscuity: Mutations in the AR gene occur in approximately 20% of CRPCs[50] Hundreds of mutations in the AR have been identified after ADT, of which 90% are nonsense and missense mutations resulting in nonfunctional ARs that are clinically insignificant[44]. Of mutations occurring in the AR gene, 49% occur in the LBD, 40% in the amino-terminal domain, 7% in the DBD, and 2% in the hinge domain [19]. Most significant AR mutations occur in the LBD, which increase the sensitivity and decrease the specificity of the ligand binding [18, 47]. ARs become more promiscuous allowing activation with binding of other steroid hormones and AR antagonists [5, 7, 51]. The most common point mutation identified, T877A, occurs in the LBD, which allows activation of the receptor with hydroxyflutamide, an anti-androgen, and other steroids such as cortisol and estradiol [47]. AR activation by anti-androgens whether consequential to AR overexpression or AR gene mutations is clinically manifested by androgen withdrawal induced-PSA reduction [2, 7, 14].

AR Splice variants: AR splice variants (AR-Vs) that lack an LBD have been discovered recently [7, 10]. The insertion of cryptic exons in the AR gene leads to premature stop codons that produce truncated AR proteins that are constitutively active, since they maintain the domains critical to transcription [7, 10, 47, 52]. The nuclear localization of AR-Vs with immunohistochemical staining supports their existence in a permanently active conformation [53]. Seven AR-Vs have been described [48]. AR-V7, like other the other AR variants lacks an LBD, and via its nuclear localization binds DNA independently, without androgen activation, regulating a unique set of target genes that facilitate mitosis, in addition to the regular androgen-dependent genes activated by full-length ARs[18] that promote disease progression [10]. The two most common AR-Vs, ARv567es and AR-V7, are detected in tumor cells only after exposure to ADT indicating they are produced as an adaptive response to ADT [42]. They are expressed in bone metastasis and predict a poor prognosis [18, 42]. AR-Vs are resistant to ADT because they are active transcription factors independent of ligand stimulation; moreover, AR antagonists, which target the LBD, have no effect on AR-Vs that lack LBDs. Ligand-independent activation of the AR pathway that is resistant to targeted

therapies is a critical mechanism in the development of CRPC and disease progression[42].

AR phosphorylation: Phosphorylation of the serine 81 (Ser-81) residue on AR is stimulated by mitotic cycline-dependent kinase-1 (CDK-1) after ligand-binding [54]. Chen et al. demonstrated that phosphorylation of Ser-81 is required for androgen-dependent DNA-binding [54, 55]. CDK-1 sensitizes AR rendering it responsive to lower concentration of androgens [55]. Increased CDK-1 activity in CRPC, provides an another molecular mechanism conferring resistance to ADT [56]. During progression to metastatic CRPC, persistent activation of AR signaling establishes the significance of intratumoral/extragonadal androgen production and ligand-independent AR activation. In a series of dynamic functional interactions, AR signaling engages distinct oncogenic pathways to evade apoptosis and promote tumor cell migration, invasion and metastasis.

AR takes Lead Role in PI3K/AKT Survival Signaling

Mutations in the PI3K/AKT survival signaling pathway have been found in approximately 40% of prostate cancers and 70% of metastatic prostate cancers [18]. Phosphatase and tensin homolog (PTEN), the most commonly mutated tumor suppressor gene in prostate cancer, induces apoptosis through its interaction with PI3K/AKT [57-59]. Loss of PTEN leads to buildup of PIP3, which activates AKT, signaling cell survival and inhibition of apoptosis [60, 61]. Activation of AKT induces phosphorylation of AR resulting in inhibition of AR-induced apoptotic pathways [62]. The AR and PI3K/AKT pathways are linked by negative feedback inhibition. AR inhibition activates the PI3K/AKT survival pathway indicating ADT is involved in the escape of tumor cells from apoptosis and resistance to treatment [12, 61, 63]. Activation of AKT signaling in prostate cancer cells has been shown to functionally compromise AR activity; evidence directly implicating this mechanism as a contributor to the development of therapeutic resistance to ADT and emergence of CRPC. Protein effectors of the PI3K/AKT signaling pathway are pursued as therapeutic targets to reinstate apoptosis within the cancer cells. Selective inhibition of PI3K/AKT pathway with AKT inhibitor, AZD5363, retards cellular proliferation and increases apoptosis, delaying tumor progression in androgen-sensitive and CRPC xenografts [63]. Dual inhibition of AR and PI3K/AKT pathways disrupts the feedback loop between the two pathways enabling disease regression that endures longer than either monotherapy [63].

AR Navigates EMT-MET Transitions

One of the most critical steps of tumor metastasis is the detachment of cancer cells from the primary tumor and extracellular matrix (ECM), invasion into the surrounding tissue, and migration via a chemoattractive path to a metastatic site [57]. Distinct molecular programs are responsible for the regulation of adhesion, migratory and invasive properties of disseminating tumor cells, all processes impacted by the cytoskeleton dynamics. During the process of oncogenic epithelial-mesenchymal transition (EMT), clusters of malignant cells lose their epithelial characteristics and acquire self-sustained migratory and highly invasive cell phenotypes via cytoskeletal remodeling. EMT is characterized by loss of proteins associated with the polarized epithelial phenotype and *de novo* synthesis of proteins associated with mesenchymal, migratory morphology of transitioning cells. Loss of epithelial proteins, such as E-cadherin and cytokeratin 18, in cells of epithelial units defines epithelial dedifferentiation. In contrast, *de novo* expression of mensenchymal markers, vimentin, N-cadherin, ZO1 and Snail, is correlated with downregulation of epithelial cytokeratins and has been proposed as canonical marker of the fibroblastoid state of transitioning cells [64, 65]. Table 1 is a collective summary of molecular markers associated with CRPC.

Growing evidence implicates the contribution of androgen signaling towards induction of EMT, which promotes the invasive potential of prostate glandular epithelial tumor cells [11, 66, 67]. Upon activation of the EMT process, tumor cells lose their epithelial cell markers, such as E-cadherin and B-catenin, which are replaced with mesenchymal cell markers, like N-cadherin and vimentin [68]. These changes allow for the characteristic loss of cellular adhesion and gain of migratory capacity, which promote the development of metastases and resistance to anoikis [11, 57, 66, 69]. During ADT, transcriptional repressors of E-cadherin, including zinc finger E-box binding homeobox 1 (ZEB1), ZEB2, and TWIST, are increased, which results in a predictable decrease in E-cadherin expression and increase in both N-cadherin and vimentin expression [11, 66]. Sun et al. demonstrated a negative feedback loop between AR and ZEB1 wherein ADT upregulates ZEB1 expression towards induction of EMT [66]. The androgen depleted microenvironment created by ADT forces adaptive changes in tumor cells to promote survival and, in this case, invasive potential. Directed by the AR, EMT can be reactivated in prostate cancer epithelial cells by TGF-β and androgens. Putative targets in cell adherence junctions mediated by E-cadherin can impact EMT outcomes under the control of AR signaling interactions with critical effectors of EMT.

Table 1. Molecular Markers Associated with Prostate Cancer Progression to Castration Resistant Disease.

Molecular Marker	Function and Role in CRPC	References
AR	Overexpression, increased transcriptional activity or aberrant signaling, promotes EMT, invasion and metastasis	Shafi et al. 2013[18] Linja et al. 2013[41]
PTEN/RAS/MAPK	Active signaling pathway accelerates cancer progression to metastasis	Carver et al. 2011[12]
PTEN/AKT	Signaling pathway alterations or constitutive activation promotes tumor growth and metastasis	Morgan et al. 2009[61] Lin et al. 2001[62] Thomas et al. 2013[63]
E-cadherin	Required for cell-cell adhesion and invasive properties, loss promotes EMT	Umbas et al. 1992[92] Paul et al. 1997[93] Pontes et al. 2010[94]
ZO-1	Regulation of cell migration by modulating tight junction assembly	Franke et al. 1982[65] Boyer et al. 1989[64]
N-cadherin	Overexpression promotes growth, migration, invasion and metastasis	Tanaka et al. 2010[95]
β- Catenin	Overexpression promotes cancer invasion and metastasis	Pontes et al. 2010[94]
Fibronectin	Promotes adhesion to ECM, regulates tumor invasion and confers resistance to anoikis	Fornaro et al. 2003[96] Jia et al. 2012[97]
Collagen I	Loss promotes ECM degradation and bone metastasis	Jin et al. 2011[98] Docheva et al. 2010[99]
Vimentin	Overexpression promotes cancer cell invasion	Franke et al. 1982[65] Boyer et al. 1989[64] Satelli et al. 2011[100]
Zeb1	Overexpression promotes EMT and tumor invasion	Kim et al. 2013[101]
Slug	Overexpression promotes EMT and tumor invasion	Behnsawy et. al 2013[102]
Twist	Overexpression promotes EMT and tumor invasion	Behnsawy et. al 2013[102]
Snail	Overexpression promotes EMT and tumor invasion	Behnsawy et. al 2013[102]
ETS-1	Overexpression promotes prostate cancer metastasis and increased transcriptional activity promotes castration resistant disease	Li et al. 2012[103] Smith et al. 2012[104]
aplhaII(b) beta 3integrin	Mediates cellular cytoskeleton/ECM attachment, loss promotes EMT, tumor invasion and metastasis	Trikha et al. 1998[105]
Syndecan-1	Cell surface protein regulates cell adhesion, loss correlates with cancer progression	Contreras et al. 2010[106]
Notch-1	Down-regulation inhibits cancer cell growth, migration, invasion, and induces apoptosis	Wang et al. 2010[107]
PDGF-1	Overexpression promotes cancer cell invasion and angiogenesis	Kong et al. 2008[108, 109] Kong et al. 2009[110]
DAB2IP	Modulates EMT via GsK-3β-catenin, loss facilitates EMT and metastasis	Xie et al. 2010[111]

AR Finds ERG-Fusions: The identification of TMPRSS2: ERG gene fusions by Arul Chinnayan and his group has been of fundamentally importance in advancing our molecular understanding of prostate cancer [70]. These gene fusions result in androgen-regulated expression of the transcription factor, ETS-related gene (ERG). This fusion is found in 50% of prostate cancers and causes co-overexpression of ERG and Frizzled4 (FZD4), a 7 pass transmembrane receptor in the Wnt signaling pathway [71]. Overexpression of ERG induces EMT in androgen responsive cells (VCaP), resulting in repression of E-cadherin and induction of N-cadherin [71, 72]. The effects of ERG overexpression are abrogated by modulation of FZD4, evidence suggesting that FZD4 mediates the oncogenic effects of ERG and impacts AR driven prostate cancer progression.

Taxane Chemotherapy in the Treatment of CRPC

Upon the inevitable progression to CRPC with metastases, systemic chemotherapy with taxanes replaces ADT as the treatment of choice. Taxanes are chemotherapeutic agents that bind the beta subunit of tubulin, stimulating polymerization into stabilized microtubules. Microtubule polymerization and de-polymerization are necessary for many cell processes. Stabilized microtubules inhibit the cell cycle from progressing through anaphase of mitosis, which leads to apoptosis [11, 73, 74]. Taxanes inhibit anti-apoptotic protein Bcl-2, leading to apoptosis. Taxane stabilization of microtubules has been shown to inhibit the translocation of the androgen-AR complex into the nucleus, which prevents the AR downstream pathway [74-76]. Taxanes lead to an increase in forkhead box 01 (FOXO01), which is a transcriptional repressor of AR, leading to inhibition of ligand-dependent and ligand-independent transcription [11, 77, 78]. Microtubule-targeted chemotherapy may provide the clinical benefit in improving survival by inhibiting AR nuclear translocation and transcriptional activity [11]. Docetaxel with prednisone is the current recommended treatment for men with minimally symptomatic metastatic prostate cancer with good performance status [2, 3], based on two landmark randomized control trials in 2004, TAX327 and SWOG 99-16. Mitoxantrone with prednisone had been the previous standard of therapy based on studies that demon-

strated palliation without survival benefit [79]. In the TAX327 trial demonstrated 24% improved survival with administration of docetaxel every 3 weeks plus prednisone compared to mitoxantrone and prednisone [80-82]. TAX327 also demonstrated a statistically significant improvement in pain and quality of life [81, 82]. The SWOG 99-16 trial compared docetaxel plus estramustine, an AR antagonist, to mitoxantrone and prednisone, which also demonstrated the survival advantage of docetaxel [83]. However, SWOG 99-16 did not demonstrate the same improvements in pain and quality of life as the TAX327 trial. The improved quality of life and pain in the TAX327 trial compared to SWOG 99-16 trial is due to the use of prednisone rather than estramustine [81]. Docetaxel resistance develops and disease progresses in approximately 7.5 months [73].

Docetaxel Resistance: Cellular Encounters Limit Therapeutic Options

Evidence-based mechanisms implicated in the development of docetaxel resistance include overexpression of P-glycoprotein drug efflux pump, mutational alterations in the tubulin gene and protein expression, and inhibition of apoptosis [11, 74, 76]. The overactivity of P-glycoprotein efflux pump limits the amount of drug able to accumulate in tumor cells. Cabazitaxel, a next generation taxane, was designed to bypass development of taxane-resistance related to the p-glycoprotein efflux pump [73, 74, 84]. Recent evidence from this laboratory suggests that docetaxel-resistant prostate cancer cells exhibit induced EMT (Martin et al, 2014; unpublished data). Puhr et al. demonstrated decreased E-cadherin expression in docetaxel-resistant cells [85]. Decreased E-cadherin promotes loss of cell adhesion and cell polarization, and gain of cell migration, which leads to invasion and metastases [85]. Moreover, E-cadherin expression is inversely related to patient survival. Collectively the evidence implicates EMT as a cellular mechanism conferring resistance to docetaxel, development of metastases, and contributing to mortality [85]. One may thus argue that docetaxel administration prior to ADT may result in improved outcomes via navigating EMT cycles [11].

Alterations in AR signaling interactions and transcriptional activity stimulate resistance to both ADT and taxanes. Understanding the mechanisms driving the development of therapeutic resistance would provide a new platform for exploring new targeted therapies and combination strategies to overcome those mechanisms, restore therapeutic sensitivity, and prolong patient survival. A phase II trial of docetaxel and prednisone combined with DN-101, a high dose calcitriol shown to upregulate apoptosis,

was terminated early due a greater number of deaths in the treatment group [77]. A much anticipated combination regimen of docetaxel and prednisone with bevacizumab, an angiogenesis inhibitor, did not produce a significant difference in overall survival compared to the control group [77]. Several new agents are being evaluated in combination with docetaxel and prednisone, such as medications targeting endothelin A receptor, the Src family of kinases and the cell membrane protein clusterin[77].

The surge of new CRPC treatments have required pursuit of the most effective sequencing of molecular therapeutics. However, therapeutic regimens with overlapping mechanisms of action may develop cross-resistance. Indeed several recent studies have established that microtubule-targeting chemotherapy such as docetaxel and cabazitaxel, and androgen signaling blocking agents, such as abiraterone and enzalutamide, have been shown to affect the cellular localization and nuclear translocation of AR[75, 86, 87]. Cross-resistance was demonstrated between taxanes, between abiraterone and docetaxel, and between abiraterone and enzalutamide, and responses to treatment varied with sequence [75, 88]. However, the addition of certain AR targets in combination with taxanes may improve patient survival. The AFFIRM trial revealed a 37% reduction in risk of death in men with docetaxel-resistant prostate cancer treated with enzalutamide compared to placebo [4, 89]. Mutations in tubulin that result in taxane resistance may be responsible for persistent AR nuclear translocation. AR translocation inhibited by enzalutamide, a second-generation androgen antagonist that also inhibits co-activator recruitment, may improve survival. Combination of enzalutamide with docetaxel is being investigated in a clinical trial, NCT01565928 [90].

Summary and Future Directions

The castration-resistant state during prostate cancer progression develops under the selective pressures of the androgen-depleted microenvironment. The tumor cells select for adaptive changes resulting in increased extragonadal androgen production and AR ligand-independent activation. Extragonadal androgen production can be inhibited by androgen synthesis inhibitors, such as abiraterone, although, a backdoor pathway to DHT persists. AR overexpression sensitizes the AR to low androgen levels and can be stimulated by the weak agonist properties of anti-androgens. The AR variants allow ligand-independent constitutive activity of AR axis despite androgen axis and AR antagonists. AR signaling may cooperate with other oncogenic pathways associated with EMT, anoikis and cell survival to

promote progression to metastatic CRPC. For patients with metastatic disease, taxane-based chemotherapy provides temporary disease regression via apoptosis, inhibition of AR nuclear translocation and ultimately blocking AR transcription activity.

Our knowledge of the most effective sequencing of combination of treatments for CRPC is currently limited. Stratifying treatment using Gleason score, PSA, and metastases are too non-specific and are poor surrogate markers for disease progression. There is a great need for validation of predictive biomarkers to personalize therapy and monitor efficacy [13, 47, 73]. Patient profiling would allow identification of individual genetic signatures that would predict susceptibility to a specific treatment modality [91], which would improve survival and limit adverse effects of ineffective treatments [85]. Thus patients exhibiting AR gene amplification, which is present in 30% of CRPCs, are 4.569 times more likely to respond to second line hormone therapies [5]. Studies in multiple prostate cancer models will improve assessment of the ability to genetically manipulate models to determine the functional contribution of gene products on therapeutic response and the impact of tumor heterogeneity on therapeutic efficacy. This will enable development of personalized-based platforms for targeted strategies specific to individual patients, with their tumor genomic signatures adjusted based on validated biomarkers.

Abbreviations

ADT, androgen deprivation therapy; AR, Androgen Receptor; AR-V, androgen receptor splice variant; CRPC, castration-resistant prostate cancer; DHEA, dihydroepiandrosterone; DHT, dihydrotestosterone; EMT, Epithelial mesenchymal transition; ERG, ETS-related gene; FOXO01, forkhead box 01; FZD4, Frizzled4; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; PSA, prostate specific antigen; SHBP, sex hormone binding protein; T, testosterone; PTEN, Phosphatase and tensin homolog; ZEB1, zinc finger E-box binding homeobox 1. Cyclin-dependent kinase-1, CDK-1.

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Competing Interests

The authors have declared that no competing interest exists.

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