
Pathophysiology of Castration-Resistant Prostate Cancer

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Abbreviations

CRPC	Castration resistant prostate cancer
PSA	Prostate specific antigen
ADT	Androgen deprivation therapy
DHT	Dihydrotestosterone
AR	Androgen receptor
DHEA	Dehydroepiandrosterone
AD	Androstenedione
HSP	Heat shock protein
IAP	Inhibitors of apoptosis proteins
PTEN	Phosphatase and tensin homolog
BTG1	B-Cell translocation gene 1
BCL2	B-Cell CLL/Lymphoma 2
IGF-1	Insulin like growth factor 1
KGF	Keratinocyte growth factor
EGF1	Epidermal growth factor 1
TIF2	Transcriptional intermediary factor 2
RTK	Receptor tyrosine kinase
MAPK	Mitogen activated protein kinase
STAT	Signal transducer and activator of transcription
AF-1, 2	Activating function
NTD	N-terminal domain

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DBD	DNA binding domain
LBD	Ligand binding domain
NLS	Nuclear localization signal
ARV	Androgen receptor variant
ASO	Antisense oligonucleotide
LHRH	Lutenizing hormone-releasing hormone
MAB	Maximum androgen blockade
CVD	Cardiovascular disease
IAD	Intermittent androgen deprivation
FDA	Food and Drug Administration
SWOG	Southwest Oncology Group
FOXO1	Forkhead box O1
HSET	Human kinesin-14
MCAK	Mitotic centromere associated kinesin
Src	Proto-oncogene protein tyrosine kinase Src

Background

Clinical Development of Castration Resistant Prostate Cancer (CRPC)

In the United States prostate cancer is the most commonly diagnosed malignancy, and remains the second most common cause of cancer related mortality second only to lung cancer. In 2014, there were an estimated 233,000 new cases of prostate cancer diagnosed and 29,480 deaths due to prostate cancer [1]. The incidence rate of prostate cancer declined -2.1% from 2000–2010, likely reflecting improved prevention and variable utilization of prostate specific antigen (PSA) screening methods nationwide, which remains controversial [1]. The historic studies by Huggins and Hodges in 1941 [2], established the role of androgens as the primary driving force in prostate tumor growth, with androgen removal resulting in dramatic suppression of tumor growth. Androgen-deprivation therapy (ADT) stems from the recognition that circulating androgen levels (primarily testosterone (T) and dihydrotestosterone (DHT)) are responsible for the embryonic development, differentiation, maturation, and consequently, malignant growth of the prostate [3]. Fortunately, improved screening and detection has resulted in roughly 80 % of new cases being diagnosed as early-localized disease. A large number of patients (33–40 %) diagnosed with early-localized disease treated with radical prostatectomy develop recurrence of disease or progress to metastases [4]. ADT remains the gold standard for patients with metastatic, advanced disease. Androgen deprivation therapy has been achieved via multiple methods including surgical orchiectomy, targeting the hypothalamic-pituitary axis via GnRH agonists and antagonists, blocking steroid production by enzymatic inhibition, and via antiandrogens that

inhibit binding to the androgen receptor (AR). Initial response to ADT is often dramatic, with 80–90 % of patients achieving rapid decline in serum PSA, and reduction of serum testosterone to ‘castrate’ levels (<50 ng/mL) [5, 6]. After an average remission time of 2–3 years, nearly all patients progress to castration resistant prostate cancer (CRPC), defined by rising serum PSA reflecting activation of AR transcriptional activity, or appearance of metastases via imaging [5, 7]. Approximately 90 % of patients with CRPC will develop bone metastases resulting in severe pain, pathologic fractures and/or bone marrow failure [7]. Advanced CRPC is ultimately lethal, with median survival of 18–24 months from initiation [3].

Mechanisms Driving CRPC Progression to Metastasis

Identifying the hormonal and physiological mechanisms driving the transition to androgen-independent state has been a critical determinant to drug development and therapeutic outcomes during cancer progression to metastasis. While testosterone is the dominant circulating androgen, DHT is the primary intracellular androgen in the prostate gland, serving as the ligand for AR. DHT is produced by intraprostatic 5- α reductase enzyme from testosterone. About 10 % of circulating androgens are derived from the adrenal cortex [dehydroepiandrosterone (DHEA), androstenedione (AD)] and are also converted to testosterone within prostatic cells [8]. AR is present in the cytoplasm of secretory epithelial cells as well as surrounding stromal cells. In the absence of ligand binding, AR is bound to various chaperone molecules including heat shock proteins (HSP). Upon binding androgens, conformational changes occur within AR, allowing for dissociation from chaperone molecules and translocation of the AR-androgen complex to the nucleus where specific DNA sequences (androgen responsive elements) are bound, resulting in production of proteins that enable prostate epithelial cells and stromal cells to proliferate. Importantly, absence of androgen initiates apoptotic signaling within the stromal and epithelial compartments. The progression of tumor growth and development of metastases despite reduction of serum androgens to ‘castrate’ levels is dependent upon the utilization of adaptive cell-survival pathways [9–11]. Mechanisms contributing to CRPC progression include intratumoral production of androgens via increased expression of steroidogenic enzymes, apoptosis evasion, altered AR transcriptional coregulator expression, AR posttranslational modification (phosphorylation), ligand-independent pathways activating AR, amplification, and selection of genetically modified AR with constitutive active AR splice variants [3, 5, 6, 12].

Intratumoral Steroidogenesis

How can intratumoral androgen levels remain high after ADT? In the absence of gonadal androgen synthesis following ADT, the adrenal precursor DHEA is converted to androstenedione by 3 β -hydroxysteroid dehydrogenase within the prostate

[13]. Androstenedione is then converted to DHT via 5- α reductase (SRD5A1, SRD5A2). Inhibiting adrenal steroid production by administration of abiraterone acetate remains a cornerstone of CRPC treatment regimens. Abiraterone acetate inhibits CYP17A1, breaking the pathway to DHEA, T, and estradiol in both the testes and adrenal glands [6]. Recent work however, demonstrates that DHT synthesis is dominated by an alternative enzymatic pathway in CRPC. In this ‘back-door’ pathway, progesterone is converted to androstenedione in a series of enzymatic steps that does not require CYP17A1, and is then converted to 5 α -androstenedione after 5 α -reduction by SRD5A1, which is then converted to DHT, thus bypassing the action of steroid synthesis inhibitors [13, 14]. Compelling data suggests upregulation of a heterogeneous group of enzymes responsible for steroid synthesis from both adrenal precursors as well as cholesterol precursor (CYP17A1, HSD3B1, FASN, HSD17B3, CYP19A1, UGT2B17, etc.) in CRPC metastases [3, 15, 16]. Cholesterol is the primary substrate for the synthesis of steroid hormones, and its altered production is implicated in CRPC development. Increased cholesterol influx via SRD-1 and LDL, increased synthesis via upregulated HMG-CoA reductase, and increased formation of free cholesterol from intracellular cholesterol ester stores contribute to intratumoral androgen synthesis in CRPC [17]. Thus impaired cholesterol production via the use of HMG-CoA reductase inhibitors (statins) reduces intratumoral androgens in CRPC. The wealth of epidemiological evidence on the ability of statins to confer a reduced risk of developing advanced prostate cancer [18], directs an ongoing randomized trial on the use simvastatin in CRPC patients [19].

Dysregulation of Apoptotic Pathway

Programmed cell death is an intricately orchestrated cellular process that commences with activation of either the extrinsic (cell surface death receptor activation) pathway or the intrinsic (mitochondrial; release of cytochrome c) pathway with the end result being organized degradation of cellular organelles and machinery by proteolytic enzymes. The extrinsic pathway and intrinsic pathway converge at activation of the ‘effector’ caspase-3 [20]. The commitment to apoptotic pathways depends upon the Bcl2 protein family members, that functionally interact with inhibitors of apoptosis proteins (IAPs) to determine apoptotic outcomes and cell survival. Within the Bcl2 family resides both pro-apoptotic proteins (Bim, Bad, Bak, Bax, etc.) as well as anti-apoptotic proteins (Bcl-2, Bcl-cL, Bcl-xL, Mcl-1, etc.) whose balance is essential for both embryogenesis and normal tissue growth and maintenance [20]. Disruption of this molecular balance in favor of expression of anti-apoptotic proteins leads to apoptosis evasion, aberrant tumorigenesis, loss of androgenic control, therapeutic resistance, increased metastasis and shortened survival [20, 21]. Expression of anti-apoptotic Bcl-xL correlates with prostate cancer progression [21], and can be targeted by ADT, indicating dependence on AR signaling [21]. During progression to metastatic CRPC, Bcl-xL levels are significantly higher than primary tumors, correlating with activated ligand-independent AR signaling [21]. This outlaw pathway of AR activation

navigated by apoptosis regulators can be overcome by inhibition of Bcl2 sensitizing CRPC tumors to chemotherapy [22]. Loss of tumor suppressors p53 and PTEN characterizes poorly differentiated tumors with treatment failure outcomes. Moreover loss of p53 contributes to apoptosis resistance by loss of its regulatory activation of Bax [23]. Functional loss of PTEN results in unregulated and constitutive activation of the PI3K/Akt pathway, which contributes to cell growth and survival [23]. The role of micro-RNAs in altering the molecular landscape of CRPC in the context of apoptosis regulation has recently been recognized with potential therapeutic value. Expression of miR-19a is associated with emergence to CRPC via inhibition of the BTG1 tumor suppressor gene which regulates Bcl2 expression [24]. Inhibition of miR-19a significantly induces apoptosis in CRPC cells, highlighting the functional relationship between miRNAs and apoptosis control in CRPC [24].

AR Bypass Pathways Navigated by Co-Regulators

The development of CRPC is due in large part to continued AR signaling made possible by aberrant and unregulated signaling of mutated or alternatively spliced AR that is no longer dependent upon androgen binding to ensure its activation. However, many other native cellular signaling cascades are altered in advanced disease that contribute to enhanced activity of AR and development of CRPC under conditions of castrate androgen levels. Insulin-like growth factor-1 (IGF1), keratinocyte growth factor (KGF) and epidermal growth factor (EGF) have been shown to contribute to the activation of AR in absence of androgen. Interestingly, when subjected to anti-androgen therapy (AR direct blockade) IGF1, KGF, and EGF1 were no longer able to induce AR activation and transcription of target genes, implying a direct link between these proteins with AR [25]. IGF1 induces AR signaling by upregulating expression of various AR co-activators including TIF2 [11]. Compelling evidence suggests that overexpression of p160 co-regulator proteins (SRC1, SRC2, TIF2, etc.) following ADT, can impact both androgen dependent and androgen independent effects on AR activation in CRPC under androgen-depleted conditions [14]. As these growth factors are ligands for receptor tyrosine kinases (RTKs), the cross talk between these signaling pathways in CRPC is prominent. Receptors for IGF1 and EGF (both RTKs) are well known to affect downstream signaling activation of various cell growth and survival pathways including AKT, MAPK, and STAT pathways, all of which are activated in CRPC [11]. One of the major RTKs that has been heavily involved in several human malignancies is HER-2/neu and its signaling.

Overexpression of HER-2/neu has been shown to increase transcription of PSA, even under conditions of androgen depletion, supporting a dynamic cross-talk between RTK and AR pathways in the absence of ligand [26]. The Wnt/ β -Catenin signaling pathway is implicated in a variety of cancers, by mechanistically contributing to cell self-renewal [27]. In the presence of androgens, stabilized β -Catenin co-localizes to the nucleus with AR and promotes its transcriptional activity, acting as an AR coactivator [27]. At castrate androgen levels mimicking

CRPC, AR signaling engages the Wnt/ β -Catenin pathway, and conversely, stabilized β -Catenin can in turn promote AR transcriptional activity, indicating the importance of the Wnt/AR crosstalk in CRPC development [27].

The cytokines interleukin-6 and -8 (IL-6) and (IL-8), under the regulation of NF- κ B signaling pathway, can also enhance the expression of AR target genes in a dose dependent, paracrine manner in androgen depleted conditions [11, 28]. Androgen independent MDA PCa 2b cells are growth inhibited in response to antiandrogens in the presence of IL-6 and IL-8 [28], supporting a dynamic exchange between AR and these cytokines. Additional signaling networks mediated by RAS/MAPK, TGF- β , FGF, c-MET, can causally interact with AR towards the emergence of CRPC, and their functional involvement and targeting consequences are being pursued.

Discussion

The Identity of the Androgen Receptor

The complexity of AR and its varied mechanisms and alterations exert an important role in embryogenesis, pubertal development, the physiologic dysregulation accompanying male pattern baldness and prostatic hyperplasia, and the development of prostate cancer. The androgen receptor is grouped into the steroid and nuclear receptor superfamily, which also consists of glucocorticoid, mineralocorticoid, estrogen, and progesterone receptors. The AR is transcribed from the AR gene, which is located on Xq11-12 and contains eight exons that encode a protein of roughly 919 amino acids, with its varying length in individuals afforded by variable length polyglutamine and polyglycine repeat sequences [14]. Genomic organization of AR has been highly conserved throughout mammalian evolution, and is characterized by presence of four functional motifs: an N-terminal domain (NTD) that regulates transcription via activation function-1 (AF1) units, a central DNA binding domain (DBD) comprised of two zinc fingers, a C-terminal ligand binding domain (LBD) that contributes to transcription regulation via activation function-2 (AF2) units, and a small hinge region between the DBD and LBD that contributes to nuclear localization and degradation [29–31]. Once translated, unliganded AR resides in the cytoplasm bound to chaperone proteins, most commonly heat shock protein 90 (Hsp90), and will inevitably undergo degradation by proteasomes in the absence of ligand (T or DHT) [30]. Once ligand binds AR LBD, conformational shifting of various helices releases AR from Hsp90 binding and results in the stabilization of bound ligand as well as the generation of a hydrophobic cleft motif responsible for subsequent binding of co-regulator proteins, of which more than 150 have been identified [14, 30]. The nuclear localization signal present in the hinge region (NLS) is revealed during this conformational shift, resulting in translocation of the dimerized AR-ligand complex to the nucleus (via ATP dependent dynein motor proteins) where binding of DNA at androgen

response elements (ARE) results in formation a multi-protein complex after recruitment of multiple co-activators and co-repressor proteins that serve to regulate target gene transcription [14, 29, 32]. Many of the co-regulators are enzymes serving to remodel tightly bound chromatin structures to enable efficient transcription of DNA [29].

In the adult prostate, AR is located in luminal cells of prostate glandular tissue and surrounding stromal cells, and in a minority of basal epithelial cells and intermediate cell types of the epithelial compartment. Prostate glandular development and proliferation is dependent upon the paracrine effects of stromal cells. Once bound by circulating androgen, stromal AR induces the production of soluble paracrine factors termed ‘andromedins’ which diffuse across the epithelial basement membrane and mediate epithelial compartment proliferation [8, 33]. Interestingly, AR is growth stimulatory in luminal cells while it is inhibitory in basal cells highlighting its important regulation of normal prostate growth [34].

The AR Addiction: “Friend or Foe” in CRPC Treatment

AR Amplification

Amplification of the AR gene with resultant increased expression of AR target genes is a primary mechanism driving uncontrolled prostate tumor growth under conditions of androgen depletion. This state of “AR addiction” increases the probability of binding ligand in an androgen-depleted environment. In approximately one third of CRPCs treated with androgen deprivation therapy, amplification of AR is present [35]. In CRPC not treated with ADT, AR amplification is not found, pointing to clonal selection of those cells capable of AR amplification under conditions of very low androgen to retain AR signaling [8, 35, 36]. Amplification of AR is achieved by X chromosome rearrangements and polysomy in roughly 60 % of CRPC initially [36, 37]. Amplification of AR contributes to dramatically increased sensitivity of AR to very low levels of androgen, especially DHT. The concentration of DHT required for growth stimulation in CRPC tissues has been shown to be four orders of magnitude lower than that of primary tumors naïve to hormonal ablation [38]. AR amplification coincides with increased AR stability, increased AR nuclear localization and amplification in recurrent tumors does not appear to affect survival [36, 38]. Intriguingly, Chen et al. revealed that in the setting of AR amplification, administration of the antiandrogen bicalutamide, as well as other androgen receptor antagonists, led to increased AR target gene expression suggesting that in the setting of elevated AR, antagonists are converted to weak agonists [39].

AR Mutations and Promiscuity

Mutations of the androgen receptor are quite rare in early stage, untreated prostate cancer but are very common in CRPC, occurring in roughly 10–30 % of cases, suggesting clonal selection as an adaptive response to androgen ablation and

antiandrogen therapy [40, 41]. The highest frequency of AR mutations in CRPC occurs in patients treated with antiandrogens such as flutamide (~30 % of cases vs. ~5 % treated with castration alone) [41]. More than 660 mutations of AR have been reported, most of which are single base substitutions that have varying effects (gain of function, loss of function, null) of AR function depending on their location [42]. Roughly 49 % occur in the LBD, 40 % in the NTD, 7 % in the DBD, 2 % in the hinge region, and very rarely in untranslated regions [43]. The commonly occurring AR mutations in CRPC affect the ligand binding, reducing specificity and increasing promiscuity of binding to non-androgen ligands. The first AR point mutation identified in prostate cancer was identified in the LNCaP cell line, and occurs at codon 877, resulting in substitution of alanine for threonine (T877A). This mutation remains the most frequently occurring point mutation in CRPC AR, and results in an altered binding pocket that facilitates binding to various other hormones including estrogen, progesterone, various corticosteroids, and a select few antiandrogens (cypoterone and hydroxyflutamide) which confers a survival advantage within an androgen scarce environment [41, 43]. In pre-clinical models of CRPC, treatment with enzalutamide, an AR inhibitor, has been shown to a AR mutation F876L, resulting in the conversion of enzalutamide into an AR agonist (antagonist-to-agonist switch) [44]. Treatment resistance was demonstrated both in vitro and in vivo [44]. Several other mutations within the LBD result in increased AR transcriptional activity in the presence of various steroid hormones and include H874Y, L701H, V715M, V730M, [40, 41, 43, 45] etc. Importantly, point mutations within the NTD (G142V, M523V, G524D, and M537V) have been shown to induce development of constitutively active, mutant ARs with ligand independent activation, most likely due to increased interaction with p160 family of coregulator proteins [46].

AR Splice Variants

Androgen deprivation therapy for CRPC relies on the presence of a full-length AR with an intact LBD. The novel antiandrogen therapy approved for advanced disease, enzalutamide, exerts its effect by binding to and blocking the C-terminal LBD of intact AR, silencing transcriptional activity. The explosive evidence accumulating during the last few years on elucidation of the AR splice variants and the characterization of their clinical relevance in CRPC progression to advanced disease, has enhanced our understanding of the adaptive responses of prostate tumor cells to antiandrogen therapies. AR splice variants (AR-Vs) are the result of insertion of cryptic exons downstream of sequences encoding DBD, or deletions within the LBD that lead to disruptions in the AR open reading frame, and production of truncated AR lacking LBD, rendering them impervious to commonly utilized antiandrogen agents including enzalutamide [43, 47]. These ARVs are constitutively active mutants capable of regulating target gene expression in the absence of full length AR or androgen [48]. The exact mechanisms that lead to variable gene splicing of AR are poorly understood [41]. A loss of LBD, which normally functions as a repressor for the rest of the receptor, results in exposed and

functioning transactivation domains enabling initiation of gene transcription in the absence of ligand [41]. Among the family of newly identified AR-Vs, AR-V7 and ARv567 are the two most commonly occurring and clinically relevant. Both of these variants are induced by castration, and in men with CRPC bone metastases, their presence is a marker of particularly poor prognosis [48]. In a landmark study, Antonarakis et al. have recently demonstrated that prostate cancer patients harboring AR-V7 variants in circulating tumor cells showed no appreciable benefits from enzalutamide or abiraterone therapy, highlighting the AR-V7 as an important predictor of CRPC resistance [49]. Multiple strategies to target the various other domains (NTD, DBD, etc.) with novel agents are currently being investigated [37]. Targeting exon 1 of AR with antisense oligonucleotide approaches suppresses both the full length AR and AR-Vs in enzalutamide resistant pre-clinical CRPC models [50].

Therapeutic Challenges in CRPC

Androgen Deprivation Therapy

In men with locally advanced and symptomatic metastatic prostate cancer, ADT remains the treatment of choice. In advanced prostate cancer, ADT has been demonstrated to delay progression of disease by reducing extraskelatal metastases, spinal cord compression, and ureteral obstruction, although it has not been shown to significantly increase overall survival [51]. ADT has been achieved in the past with surgical orchiectomy, but today, is achieved with equally efficacious administration of luteinizing hormone-releasing hormone (LHRH) agonists, LHRH antagonists, and anti-androgens. ADT was initially used as a primary treatment in symptomatic metastatic disease, or localized disease in patients in which radiation therapy or surgery is contraindicated, as adjunct treatment in high-risk disease treated with radiation therapy, and as a salvage therapy following biochemical failure after surgery or radiotherapy [52]. The 1967 Veterans Administration Cooperative Research Group study, a randomized controlled trial with 2052 men with clinically advanced prostate cancer receiving either ADT, revealed no significant difference by ADT in Five-year overall survival [53]. Co-administration of LHRH agents with antiandrogens provides a modest improvement in overall survival, but significantly impairs quality of life [54]. ADT increases CVD incidence and mortality, increases bone loss and fracture risk, while impairing erectile function, and memory function [55]. ADT has no significant impact in the treatment of localized prostate cancer [56]. In men with locally advanced and metastatic cancer, whose tumors associated with rapid PSA doubling times and an initial PSA >50 ng/mL, there is a benefit from early ADT by delaying progressive disease [57]. However, overall survival is not affected by the timing of ADT either in men with locally advanced asymptomatic disease, or in men with biochemical recurrence (rising PSA) after radical prostatectomy [54]. Importantly, intermittent androgen deprivation (IAD) therapy with periods allowing for hormonal recovery, versus continuous androgen deprivation (CAD), is associated with significant improvements in quality of life while

maintaining similar survival (8.8 years vs. 9.1 for IAD and CAD respectively) [52, 54]. ADT provides rapid biochemical response with dramatic decline in PSA in roughly 90 % of patients, and can offer remission from clinically symptomatic disease for 2–3 years [10]. And then the inevitable progression to resistant disease despite castrate androgen levels resulting in CRPC.

Chemotherapy for Advanced and Metastatic Disease

Microtubule-targeting taxane based chemotherapy is the treatment option for patients with metastatic CRPC. Taxanes, derived from molecules present in the bark of yew trees, exert potent cytotoxic effects against cancer cells via their ability to bind and stabilize interactions among β -tubulin subunits, which interact to form microtubules, a major component of the cytoskeleton. Stabilization of β -tubulin subunits prevents depolymerization of microtubules which leads to cell cycle arrest in metaphase-anaphase, and leads to apoptosis of rapidly dividing cells [32, 58]. The clinical benefit of taxanes in CRPC was first recognized in 1996 after a Canadian phase III RCT demonstrated that prednisone administration plus mitoxantrone (taxane) provided palliative benefit to 30 % of symptomatic men that led to the Food and Drug Administration (FDA) approval for CRPC [59]. Although palliative benefit was achieved there was no change in survival [59]. Results from the TAX327 and Southwest Oncology Group (SWOG) 99–16 trials established docetaxel as the first agent conferring a survival benefit in men with mCRPC. The first TAX327 study revealed an improved overall survival for docetaxel administration every 3-wks plus prednisone versus mitoxantrone plus prednisone (median overall survival 18.9 mos vs. 16.5 mos respectively) [60]. TAX327 survival improvements were revisited in a 2008 study, demonstrating similar results: 19.2 mos for docetaxel every three weeks plus prednisone versus 16.3 months for mitoxantrone plus prednisone [61]. These results were similarly observed in the SWOG 99–16 trials, revealing a 2–3 month improvement in median survival with docetaxel versus mitoxantrone [59]. Progression after first line chemotherapy is inevitable in CRPC patients with a median PFS for patients treated with docetaxel of 7.5 months, before resistance emerged [61]. After progression on docetaxel, second line cytotoxic therapy with Cabazitaxel, a second generation taxane, is initiated. Cabazitaxel shares a mechanism of action similar to docetaxel, however, a rational approach to its design resulted in bulkier side chains preventing it from being utilized as a substrate for the multi-drug resistance P-glycoprotein efflux pump, which contributes to docetaxel resistance in CRPC [61]. The pivotal trial determining the approval of Cabazitaxel was the phase III multi-national TROPIC trial examining Cabazitaxel versus mitoxantrone after resistance to docetaxel; there was a 2.4 month median overall survival advantage for Cabazitaxel, and that secured the approval of the taxane for clinical use by the FDA in 2010 [62].

AR Transport by Microtubules: Value of “Cargo” Targeting

Efforts to identify the mechanisms of resistance to docetaxel in CRPC have provided insight into novel actions of taxanes. While it is well established that taxanes inhibit the cell cycle by preventing transition between metaphase-anaphase in rapidly dividing cells *in vitro*, it is argued that this action alone does not account totally for clinical action in *in vivo* models, in which prostate cancer cells characteristically divide slowly [63]. Multiple studies now demonstrate that taxanes inhibit AR signaling in addition to inhibiting the mitotic process. Microtubules efficiently mediate transport of multiple substances intracellularly, playing a role in critical endocrine signaling pathways [64]. Once AR conformational changes occur after ligand binding, the dimerized AR/ligand complex forms and must be physically transported to the nucleus. Work by Zhu et al. revealed that ATP dependent transport along microtubules facilitated AR nuclear translocation [65]. Moreover analysis of clinical specimens from patients treated with docetaxel revealed a significant reduction in nuclear AR compared to untreated patients (38% vs. 50 % respectively), which coincided with a marked increase in cytoplasmic AR in these treated patients [65]. Work by others confirmed these initial findings by our group, demonstrating that paclitaxel substantially influenced the AR cytoplasmic/nuclear localization ratio, reducing the percentage of cells with nuclear AR (70 % to less than 30 %) [63]. This effect is dependent upon stabilized, non-mutated microtubules and directly coincides with a dose dependent inhibition of AR transcriptional activity. Full length AR association with the microtubule associated motor protein dynein was inherent and increased upon ligand induced AR translocation, navigating its nuclear transport [63]. Both docetaxel and paclitaxel have been shown to induce nuclear accumulation of forkhead box O1 (FOXO1), a potent repressor of AR transcriptional activity [66]. FOXO1 can inhibit AR by binding and sequestering it in the nucleus thus rendering it unable to activate AREs [58, 66]. Mechanistically the ability of FOXO1 to inhibit both androgen-dependent and androgen-independent AR transcriptional activity, is highly significant in defining the impact of microtubule-targeting chemotherapy on therapeutic resistance in CRPC via targeting AR variants [58, 67]. The recognition that microtubule-targeting taxane chemotherapy can also inhibit AR signaling via disruption of microtubule transport together with intranuclear inhibition by FOXO1, has shed new light into the therapeutic value of the combination of taxanes with anti-androgens against the molecular landscape of CRPC, since they target different components of the AR signaling axis. Recent clinical evidence however revealed that in CRPC patients treated with abiraterone acetate followed by docetaxel, there was a >50 % PSA decline in only 26 % of patients (compared to 54 % in TAX327 trial) and an OS of only 12.5 months (18.9 mos OS in TAX327 trial) [68], pointing to cross-resistance. Dissection of the interactions of AR variants with the microtubule network revealed that differential association with microtubules and dynein by ARVs could affect taxane sensitivity in CRPC cells [69]. This pre-clinical study established that cells harboring ARv567 exhibited inhibition of AR nuclear

translocation in response to docetaxel, while there was no effect on ARv7 nuclear localization. Furthermore, the ARv7, unlike ARv567, lacks the hinge region and part of the NLS, which contains the minimum microtubule-binding domain, rendering it independent of microtubule binding [69].

Close examination of the second line taxane chemotherapy Cabazitaxel's effect on AR localization has yielded intriguing insights into the action of the drug. Pre-clinical studies from this laboratory demonstrated that Cabazitaxel treatment of *in vivo* models of advanced prostate cancer, androgen sensitive and CRPC), resulted in sustained AR nuclear localization while reducing AR activity, which contrasts directly with the observed effects of docetaxel on inhibiting AR nuclear translocation (Martin et al., Cancer Res., 2015 [70]). As illustrated on Fig. 2.1, the sensitivity of CRPC cells to Cabazitaxel is dependent on neither the AR nuclear localization nor the AR variant status, a result recently corroborated by van Soest et al. who reported that Cabazitaxel maintains a potent anti-tumor and anti-PSA effect in both enzalutamide naïve and resistant cell lines, regardless of AR nuclear localization status [71]. Further evidence from this laboratory demonstrated that Cabazitaxel induced significant multinucleation as well centrosome clustering and amplification in CRPC cell lines containing full length and variant AR. Clustering

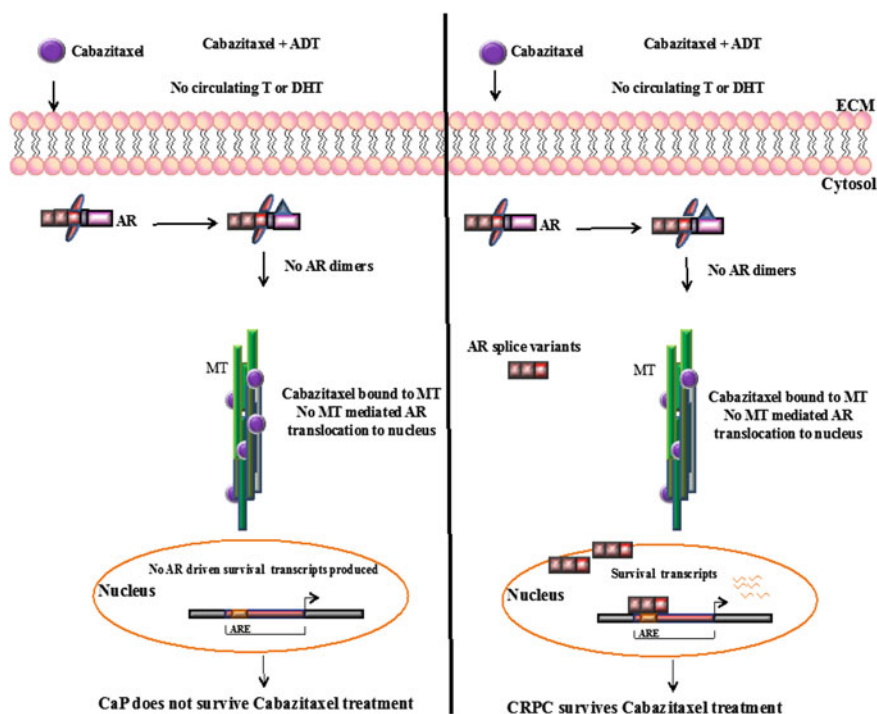


Fig. 2.1 Impact of cabazitaxel on microtubule transport network is short-circuited by the AR splice variants in CRPC

of chromosomes and supernumerary centrosomes contribute to chromosomal instability and may play a role in the regulation of the cell cycle as well [72]. The mitotic centromere associated kinesin (MCAK) and human kinesin-14 (HSET) expression are required for proper cytokinesis, and their overexpression may contribute to taxane resistance in CRPC cells via their ability to depolymerize microtubules; overexpression of both kinesins has indeed been detected in docetaxel resistant tumors [73, 74]. Cabazitaxel downregulates both kinesins, an effect coinciding with severe multinucleation and centrosome clustering, implicating cytokinesis disruption in a ligand-independent manner (Martin et al., *Cancer Res.*, 2015 [70]). In an intriguing functional twist, androgens contribute to rapid microtubule regrowth in an AR-dependent context as suggested by evidence that androgen activates ERK signaling by complexing with the non-receptor protein tyrosine kinase Src, resulting in γ -tubulin recruitment to the centrosome and microtubule nucleation, leading to rapid regrowth of microtubules after nocodazole-induced depolymerization [75]. Moreover, functional loss of AR resulted in inhibition of microtubule regrowth in the presence of androgens, supporting the critical role for AR in promote microtubule growth from centrosome clustering [75]. One might argue that impairing AR nuclear transport and activity by microtubule targeting chemotherapy and concomitant antiandrogen therapy, may impair centrosome mediated cytokinesis adaptations that contribute to therapeutic resistance in CRPC.

Conclusions and Future Directions

The continually evolving pattern of phenotypic resistance in prostate cancer, driven by mutations and functional alterations of AR has spurred rational drug design to target both mutated and wild-type AR, as well as cross- signaling pathways interacting with AR. Crystallography-guided approaches examining the binding of various ligands and drugs to AR have been challenging to date [76]. Via ligand docking and molecular dynamics simulations, the Sawyer's group, demonstrated that drug binding to F876L mutant AR LBD leads to a lack of displacement of helix 12 due to presence of leucine at position-876, which when non-displaced assumes an agonist conformation able to recruit coactivators and drive transcription [76, 77]. The rational design of an enzalutamide analog with a bulkier B-ring moiety to prevent the agonist-like conformation of helix 12 led to DR103, that potentially antagonizes F876L in prostate tumors harboring the mutation [77]. Modification of the enzalutamide backbone to reduce off-target interactions has been achieved by the novel AR inhibitor ARN509; in pre-clinical models, ARN509 displayed greater anti-tumor effects than enzalutamide [78]. Rational drug design has enabled the development and testing of the novel antiandrogen ODM-201, structurally distinct from other antiandrogens, and functionally capable to fully antagonize F876L AR, as well as T877A and W741L known to confer resistance to antiandrogens [79]. ODM-201 impairs proliferation of androgen-sensitive VCaP cells overexpressing

AR more effectively than ARN-509 or enzalutamide, without crossing the blood brain barrier [79]. ODM-201 has shown good safety and tolerability profiles and significant antitumor activity (86 % PSA response in chemotherapy naïve patients) in both phase 1 and phase 2 clinical trials [80], with phase III trials ongoing (ClinicalTrials.gov, NCT02200614). Targeting of the NTD with the novel AR inhibitor EPI-001 discovered by Marianne Sadar's group is also under clinical development. The AR NTD is a relevant target for drug development due to the prevalence of ARVs lacking argetable LBD. To date, no other compound has been demonstrated to be more efficacious in targeting AR NTD and inhibiting growth of both PCa and CRPC cell lines in vitro, as well as in LNCaP xenografts [76, 81]. Development of high-efficacy EPI-001 analogs [82] is ongoing towards clinical validation of these NTD inhibitors.

This is the year 2016, marking the initiation of the precision medicine era in cancer treatment. How can anyone dispute that computer modeling of genetically altered AR structures can define new therapeutic landscapes into the effective targeting of AR signaling by attractive combination strategies of androgen agonists, antagonists and taxanes in advanced metastatic hormone-sensitive prostate cancer and CRPC? The outpouring evidence identifying clinically-relevant AR splice variants not only as therapeutic targets but also as predictive markers of advanced disease delivers promise and raises expectations. Exploitation of the rational administration of antiandrogen and chemotherapeutic agents after scrutinizing individual patient's molecular landscape for AR mutations, gene splicing, epigenetic changes, will be instrumental in maximizing efficacy and increasing survival, while minimizing the risk for emergence of treatment resistance in CRPC patients.

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