Castration-Resistant Prostate Cancer: Locking Up the Molecular Escape Routes

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Abstract

The understanding of the key role that androgens play on the normal and pathological physiology of the prostate guided the development of different therapies for the treatment of locally advanced or metastatic prostate cancer (PCa). These so-called androgen deprivation therapies include surgical or chemical castration, by the administration of gonadotropin-releasing hormone analogs; inhibition of steroidogenic enzymes; and finally, blocking of the binding of androgens to their receptor (AR) by the use of antiandrogens. Despite an excellent initial response, in approximately 2 to 3 years, most of these patients will succumb to the castration resistant form of the disease. Remarkably, even in the presence of castration levels of circulating androgens, these tumors are still dependent on a functional AR, and several molecular mechanisms have been proposed to explain this phenomenon. These include: (1) gene amplification and increased expression of the AR mRNA and protein, (2) selection of mutations in the AR that confer broader ligand specificity, (3) changes in the ratios or expression between the AR and its coregulators, (4) increased expression of steroidogenic enzymes, and (5) up-regulation of cross-talk signal transduction pathways that can activate the AR in a ligand-independent manner. We will summarize how these molecular hypotheses are being tested in the clinic by the latest therapeutic modalities.

Background

The concept of androgen deprivation for the treatment of advanced prostate cancer was developed more than 50 years ago (1), and today, newer forms of androgen-deprivation therapies remain the first line treatment for this disease. The principle behind reducing the levels of circulating androgens for therapeutic purposes is based on the central role these hormones have on the development, differentiation, and maturation of male reproductive organs including the prostate.

Androgens are synthesized primarily in the testes, under the regulation of luteinizing hormone, which is itself regulated by the levels of gonadotropin-releasing hormone (GnRH), and in the adrenal glands, and secondarily in peripheral tissues including the prostate. Testosterone (T) is the principal androgen and circulates mostly (98%) bound to sex hormone-binding globulin and albumin. Intracellularly, T is enzymatically converted to the more potent metabolite dihydrotestosterone (DHT); both steroids bind to the androgen receptor (AR), a ligand-regulated transcription factor in the nuclear hormone receptor superfamily (2, 3). Liganded-AR binds to the androgen response elements present in the regulatory regions of a variety of genes, many of which are involved in the growth, survival, and differentiation of prostate cells (4).

The understanding of the endocrine regulation of androgens guided the development of several androgen deprivation therapies. Those include: surgical or chemical castration, the later achieved by the use of GnRH agonists or antagonists; inhibition of steroidogenic enzymes; and finally, the use of antiandrogens that block the binding of androgens to the AR (Fig. 1). More recently, complete androgen blockade was pursued by the use of antiandrogens in combination with GnRH agonists. Despite an excellent initial response, in most cases, the tumors will progress through treatment to a hormone refractory (HRPC), androgen independent (AIPC), or castration resistant prostate cancer (CRPC) stage, and once this occurs the median survival is between 18 and 24 months (5). As shown by a significant body of evidence recently accumulated, the first two designations (HRPC and AIPC) are misnomers because these tumors are clearly dependent on AR signaling. Analysis of tumor samples from patients with CRPC has revealed several mechanisms utilized by tumor cells to reactivate the AR signaling at subphysiological serum concentration of androgens or even in the presence of AR antagonists. These mechanisms are listed in Fig. 2 and include:

Increased expression of AR. At least six independent studies reported AR gene amplification in approximately 30% of patients with recurrent prostate cancer (6–9), and these patients have a 4.569 higher likelihood of responding to a second-line hormonal therapy as compared with patients with no AR amplification (10, 11). Higher levels of the AR protein can result from gene amplification, but also from increased transcription rates, or stabilization of the mRNA or protein. Regardless of the cause,
higher levels of AR protein do not seem to affect survival (7). Pre-clinically, the overexpression of AR has been shown to be involved in the progression to CRPC (12). Together with the AR, the expression of many AR-controlled genes are up-regulated in the castration resistant tumors, including the prostate specific antigen (PSA) and the recently discovered TMPRS2:ERG fusion genes, in which members of the ETS family of transcription factors, most commonly ERG, are placed under a strong androgen dependent regulation (13, 14). The biological functions of ERG overexpression in CRPC remain to be fully understood in their context to the pathobiology of the disease (15).

Selection of AR mutants. Several investigators identified different mutations in the AR that confer to it the ability to be activated by weak androgen precursors, progesterone, estradiol, cortisol, or even antiandrogens. Those include point mutations in the AR ligand binding domain that can result in activation by nonandrogenic ligands (16–19), or mutations in other regions such as the amino terminus (20) or the DNA binding domain that confer oncogenic properties to the AR (21). Although suspected to be low, the real frequency of AR mutations in CRPC is not known, mainly due to the difficult access to metastatic samples. However, it is well documented that some antiandrogens can lead to the selection of tumors expressing AR mutants that can be activated by the therapeutic agent. Some of those tumors can still be sensitive to a second-line antiandrogen (22, 23).

Alterations in the balance between AR and its transcriptional co-regulators. Several coactivators including TIF2, SRC1, and TIP60 have been shown to be overexpressed or accumulated in the nucleus of recurrent prostate cancer specimens (24, 25). Moreover, transfection of TIF2 increases AR-mediated gene transactivation in response to physiological concentrations of adrenals androgens or other steroids with low affinity for AR (24). In addition, it has been proposed that many kinase pathways can enhance the AR activity through phosphorylation of coactivators, as it was shown for the EGF-induced phosphorylation of TIF2 (26). The AR overexpression in prostate cells enhanced the DHT-stimulated expression of coactivators such as MAK, BRCA1, AIB1, and CBP (27).

Increased expression of enzymes involved in steroidogenesis. The intraprostatic conversion of adrenal precursors into androgens that can signal through the AR regardless of castrate levels of circulating androgens was proposed almost 15 years ago (28). Recent data have added to this hypothesis (29, 30); e.g., Montgomery and colleagues, convincingly showed that median T levels within metastases from castrated men are approximately threefold higher than levels within the primary prostate cancers.
from untreated eugonadal men (31). The authors further showed up-regulated expression of steriodogenic enzymes including FASN, CYP17A1, HSD3B1, HSD17B3, CYP19A1, and UGT2B17 ($P < 0.001$ for all). Indeed, several clinical studies have proposed minimizing levels of these extragonadal sources of T and its precursors by using combinations of inhibitors targeting different points of steriodogenesis such as ketoconazole and $5\alpha$-reductase inhibitors (32, 33).

Activation of cross-talk signal transduction pathways. As mentioned above, the activation of different signal transduction pathways in CRPC cells can enhance the activity of the AR or its coactivators in the presence of low levels or even in the absence of androgens. Examples of these include: epidermal growth factor, insulin-like growth factor, IL-6, Wnt signaling, and Stat5a/b (34–40).

**Clinical-Translational Advances**

Docetaxel administered with prednisone was approved in 2004 by the FDA for the management of patients with CRPC on the basis of its improved survival, pain reduction, PSA response, and quality of life, as compared with mitoxantrone plus prednisone (41). Currently, a variety of agents (molecularly targeted and others) are being tested in clinical trials both with and without docetaxel. Here we will focus on newer approaches that are most relevant to AR signaling pathways.

**Therapies aimed to block the activation of the AR.** The reactivation of the AR is a common consequence of several mechanisms of resistance in prostate cancer, therefore a logical point of intervention is to develop more potent AR antagonists as compared with the existing agents. One such molecule, MDV-3100, blocks the nuclear translocation and DNA binding of AR and showed in phase I/II studies, PSA declines of 44% to 87% for 19+ weeks in three of three patients in the 30 mg cohort, and 74% to 96% for 14+ weeks in three of three patients in the 60 mg cohort (42). Another intervention is the inhibition of enzymes involved in steriodogenesis. Abiraterone is a potent inhibitor of CYP17A1 currently in phase II and phase III clinical trials in CRPC patients previously treated with docetaxel-based chemotherapy. In phase I studies, abiraterone produced durable PSA responses in approximately half of the patients. It was well tolerated; although it often induced a syndrome of secondary mineralocorticoid excess that was improved by the use of mineralocorticoid receptor antagonists and the coadministration of chronic corticosteroids (43).

In preclinical models, the AR can be activated by other factors, such as the cytokine IL-6 (44). Increased serum levels of IL-6 have been reported in patients with HRPC (45, 46). CNTO 328, an anti-IL-6 antibody is being tested in a phase I/II, open label study evaluating safety, pharmacokinetics, and pharmacodynamics at three dose levels in combination with docetaxel in men with metastatic HRPC. Six of eight patients had at least 50% PSA reductions and all had stable or improved bone or CT scans (47).

**Therapies aimed to alter the stability of the AR and other relevant proteins in prostate cells.** In its inactive state, the AR is stabilized in the cytoplasm by a complex containing several chaperones including the heat shock protein 90 (Hsp90). In addition to the AR, Hsp90 client proteins also include Akt, Raf-1, src, Bcr-Abl, and HER2; therefore, inhibition of Hsp90 function theoretically should promote the degradation of its client proteins disrupting several pathways required for cell growth and the prevention of apoptosis (48). One such inhibitor of Hsp90 is being tested in patients with CRPC. Tanespamycin (17AAG) was assessed in a two-stage phase II study in 15 patients with prostate cancer. Several molecular mechanisms have been proposed to explain the dependency of these tumors on a functional AR. Those include: A, gene amplification and increased expression of the AR mRNA and protein; B, selection of mutations in the AR that confer broader ligand specificity; C, changes in the ratios or expression between the AR and its coregulators (GTF, general transcription factor); D, increased expression of steriodogenic enzymes; and E, up-regulation of cross-talk signal transduction pathways that can activate the AR in a ligand-independent manner.
Proteins tagged for degradation are recognized and eliminated by the proteasome complex (50), which constitutes another point of therapeutic intervention. However, a phase II study testing weekly docetaxel and the proteosome inhibitor bortezomib (VELCADE) as a first-line treatment for patients with CRPC showed no signs of improved efficacy over docetaxel alone (51). Additional evaluation of bortezomib in relapsed prostate cancer patients is ongoing.

**Therapies aimed to block mitogenic or prosurvival pathways.** A growing body of evidence supports the involvement of the insulin-like growth factor type I receptor (IGF-IR) in the progression to castration resistant disease (40, 52). Two monoclonal antibodies that block the IGF-IR are currently in clinical trials. The full human antibody CP-751,871 inhibits IGF-IR autophosphorylation and induces receptor internalization and is being tested in a randomized phase II, two-arm open label study, in combination with docetaxel and prednisone. IMC-A12, a fully human IgG1 monoclonal antibody, is being evaluated in a phase II randomized study in combination with mitoxantrone and prednisone following disease progression after docetaxel-based chemotherapy. IMC-A12 is also being evaluated in a separate phase II trial in asymptomatic chemotherapy-naive patients with metastatic CRPC.

Multiple studies provide evidence on the involvement of members of the Src family kinases (SFKs) in prostate cancer (53). In addition, Src signaling plays essential roles in both osteoclast (54) and osteoblast (55) activities, suggesting that inhibiting the pathway may also decrease the morbidity associated with bone metastases. There are two Src inhibitors being studied in clinical trials: Dasatinib (Sprycel), an oral tyrosine kinase inhibitor targeting BCR-ABL and the Src-family kinases, EphA2 and c-KIT; and PDGFR-β is being tested in phase II studies as a single agent in patients with progressive metastatic prostate carcinoma and a rising PSA. Using a composite endpoint incorporating PSA, bone scan, and RECIST response criteria, revealed a 67% disease control rate (ten of 15 had stable disease). Of the 27 patients with bone scans at 12 weeks, 16 were stable and one was improved. An improved PSA doubling time was seen in 29 of 36 patients, and one had a PSA response (55). Dasatinib is being tested in phase III studies in combination with docetaxel and prednisone in patients with CRPC. Another selective Src inhibitor AZD0530 is also currently in phase II testing in CRPC.

**Therapies aimed to block tumor-microenvironment interactions.** Bone metastases occur in the majority of patients with advanced prostate cancer (56). Most of the lesions are osteoblastic, and there is a complex interplay between osteoclasts, osteoblasts, stroma, and endothelium in both normal and pathological bone formation. Accordingly, several therapies targeting these different compartments are being investigated including vitamin D analogs, bisphosphonates (both radiolaabeled and free), and endothelial cell targeting agents such as endothelin A receptor inhibitors, thalidomide, and vascular endothelial growth factor axis inhibitors (57). New bone generation requires bone resorption, which is mediated by osteoclasts activated by RANKL. In phase II studies treatment with denosumab, a fully human antibody against RANKL, results in the decrease and in some cases normalization of elevated urine N-telopeptide (uNTx), indicative of a therapeutic effect on bone resorption. This occurs despite ongoing biphosphonate therapy (58). Based on these results, two phase III studies are ongoing in CRPC patients. One is evaluating the ability of single agent denosumab to prolong metastasis-free survival, with the primary endpoint being the time of first occurrence of bone metastasis or death from any cause. A second trial is comparing denosumab with the biphosphonate zoledronic acid (Zometa), and the primary outcome measure is the time to first on-study skeletal related event.

**Conclusion**

The dependency of prostate cancer cell growth on a single defined signaling pathway mediated by the AR strongly suggests that this disease may benefit greatly from a targeted therapeutic approach. Although many of these hypotheses-driven therapies are being tested in the clinic, there is still a clear need for a better identification and selection of the patient population that will experience maximal benefit from each particular therapy. As with any other cancer type, the development of a resistant phenotype is an evolutionary response of the cancer cell to the selective pressure applied. Hopefully in the near future, it will be possible to rapidly determine the mechanism of resistance predominant in a tumor as it progresses during treatment and apply that knowledge for the selection of the appropriate targeted therapy or therapies, with the goal of turning prostate cancer into a chronic disease.

**Disclosure of Potential Conflicts of Interest**


**Acknowledgments**

We thank Dr. Jeffrey Nemeth for his comments on this manuscript.

**References**


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