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AR-V7 and prostate cancer: The watershed for treatment selection?

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ABSTRACT

The androgen receptor (AR) plays a key role in progression to metastatic castration-resistant prostate cancer (mCRPC). Despite the recent progress in targeting persistent AR activity with the next-generation hormonal therapies (abiraterone acetate and enzalutamide), resistance to these agents limits therapeutic efficacy for many patients. Several explanations for response and/or resistance to abiraterone acetate and enzalutamide are emerging, but growing interest is focusing on importance of AR splice variants (AR-Vs) and in particular of AR-V7. Increasing evidences highlight the concept that variant expression could be used as a potential predictive biomarker and a therapeutic target in advanced prostate cancer. Therefore, understanding the mechanisms of treatment resistance or sensitivity can help to achieve a more effective management of mCRPC, increasing clinical outcomes and representing a promising and engaging area of prostate cancer research.

Introduction

Androgen-deprivation therapy (ADT) represents the gold standard treatment for relapsed or advanced prostate cancer patients, given the androgen receptor-dependent nature of this tumor. However, disease is usually only temporarily controlled and progression typically occurs within 12–24 months of initial response, a state named castration-resistant prostate cancer (CRPC), and defined as a prostate cancer that has progressed despite castrate levels of serum testosterone (⩽50 ng/dL) [1]. The evidence that many genes known to be under androgen receptor (AR) transcriptional control in prostate cancer are re-expressed in CRPC shows that, despite ADT, AR pathway remains active and provides the source for the growth and survival of tumor cells. Based on these assumptions, AR may be considered the first example of a lineage oncogene [2], representing a critical and functionally important therapeutic target. Owing to this new understanding, two next-generation AR-targeting therapies (abiraterone acetate and enzalutamide) have been recently approved by the FDA for the treatment of metastatic CRPC (mCRPC) [3–6]. Abiraterone acetate is a potent and highly selective irreversible inhibitor of cytochrome P450 17A1, which impairs androgen synthesis in the adrenal glands, testes and in prostate tumor itself. Enzalutamide (formerly MDV3100) acts as an AR antagonist, binding competitively to the AR and therefore displacing the natural ligands and preventing nuclear translocation of the ligand–receptor complex. Although these agents represent breakthroughs in the treatment of mCRPC, almost all patients develop innate or acquired resistance with a widely variable duration of response. Constitutively active AR splice variants (AR-Vs), which lack the AR ligand-binding domain as a result of alternative splicing of the human AR gene, represent an emerging crucial mechanism responsible for castration resistance prostate cancer progression. Among several truncated AR-Vs identified, AR-V7 is the major clinically meaningful receptor variant characterized in prostate cancer samples, being involved in tumor progression and anti-cancer treatments

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resistance. In this review we analyzed the clinical relevance of AR-Vs, focusing on the prognostic value of AR-V7 and its emerging predictive role as a biomarker of resistance/sensitivity to next-generation hormonal therapies and taxane chemotherapy.

The androgen receptor structure

The human androgen receptor (AR) gene, mapped on chromosome Xq11-12, belongs to the steroid hormone receptor genes family. The structure of the AR gene, mature spliced mRNA, and protein domains resembles that of the family members estrogen receptors and progesterone receptor. The AR acts as ligand-activated transcription factor, by regulating the expression of definite genes. Eight different exons of the AR gene encode for corresponding functional regions forming the AR multidomain protein [7].

The N-terminal domain (NTD, also called activation function 1 [AF1]), coded by the first exon, is the main effector region of the AR, controlling its transactivation. It contributes in regulating the transcription of the AR gene, which is tissue-specific, steroid hormones-related and age-dependent [7,8]. The NTD can include a variable number of polyglutamine (17–29) and polyglycine repeats, encoded by polymorphic (CAG)n and (GGN)n repeat units, whose length influences the AR transcription activity. In particular, the receptor activity is augmented in case of shorter polyglutamine repeats, while the loss of amino acids 141–338 (especially residues 210–337) markedly impairs the AR function [9].

The DNA-binding domain (DBD), encoded by exons 2 and 3 and highly conserved among the steroid hormone receptors, consists of a globular body and two coordination zinc finger complexes, with four cysteine residues and a Zn^{2+} ion each [10]. The AR binds to the androgen-response element (ARE) of the major groove of DNA through these two zinc fingers, which also contribute to the binding complex stabilization [11] and the AR dimerization [12].

Exons 4–8 encode for the COOH-terminal region, which represents the ligand-binding domain (LBD). The LBD contains a ligand-binding pocket formed by 12 folded helices, which is responsible for hormone recognition and guarantees the specificity and selectivity of the signaling pathway. According to the nature of the bound ligand – agonist or antagonist – the C-terminal helix 12 acquires two different conformations. The agonists (dihydrotestosterone and testosterone) binding leads helix 12 to bend over and closes the pocket describing a groove that binds a region of NTD, which acts as an AR co-activator. Conversely, when an antagonist is bound to the AR, helix 12 opens the entrance to the LBD, interfering with co-activators binding [13]. Specifically, after agonist binding, the conformational change of helices 3, 4, 5 and 12 of the LBD constitute the activating factor (AF)-2, also involved in controlling the receptor transcription activity. AF-2 indeed recruits p160 coactivator proteins (members of the steroid receptor coactivator family) in a hormone-dependent way [14].

The portion between the DBD and the LBD is called the hinge region. The hinge region has a key role in controlling AR activity, regulating the nuclear translocation signal, DNA binding, coactivator recruitment, and the transcriptional activity of the receptor [15].

In the presence of intracellular androgen exposure, full-length AR (FL-AR) can dimerize, thereby overcoming inhibition due to the AR N-terminal domain [16]. After ligand binding and dimerization, the AR translocates from the cytoplasm to the nucleus, where it binds to the promoter of specific DNA regions (AREs), induces the assembly of a co-activator protein transcriptional complex [17], and stimulate transcription of genes (including PSA, TMPRSS2, and hK2) involved in cell growth, differentiation and survival [18].

The AR and prostate cancer

Similarly to the healthy prostate glandular epithelium, prostate cancer cells are strictly dependent on androgens. To support this reliance, virtually all prostate tumors express the AR. Androgen deprivation therapy (ADT), dropping serum testosterone levels under castration value (via surgical or pharmacological castration, or through AR-antagonists, both used alone or in combination) represents the mainstream therapeutic strategy for prostate cancer. Tumor progression despite androgen depletion defines an advanced stage of the disease, defined castration-resistant prostate cancer (CRPC). Interestingly, CRPC cells continue to be largely androgen-dependent and AR signaling-guided [19]. Indirect evidence of the persistent role of AR signaling in driving late-stage disease is the remarkable clinical efficacy of the second-generation anti-androgens (enzalutamide [3,4] and abiraterone [5,6]) in CRPC patients. Abiraterone, an inhibitor of the Cytochrome P450 17alpha-hydroxylase/17,20-lyase (CYP17) enzyme, avoids the tumor conversion of progesterone and adrenal androgens to dihydrotestosterone (DHT). Enzalutamide, a non-steroidal antiandrogen, blocks the receptor even in case of AR overexpression.

A deep understanding of mechanisms underlying castration resistance and sustained AR-signaling activity is still lacking. Several hypothesis, not mutually exclusive, have been proposed:

(1) The amplification and over-expression of wild-type AR gene, allowing androgen-dependent cancer cells to proliferate despite the low serum androgens values, has been described as a driving mechanism supporting CRPC progression [20,21].
(2) Somatic point mutations in the AR gene provide a growth advantage after androgen ablation therapy, both with first-generation [22–24] and second-generation [25–27] anti-androgen molecules. Moreover, gross deletions in the LBD of the AR leads to constitutively active receptors [28].
(3) Proliferative stimuli triggered by alternative pathways converge to modulate and stimulate the AR signaling cascade [29–31].
(4) The intratumoral de novo over-production of testosterone and DHT from adrenal androgens or cholesterol also represents a possible cause for androgen deprivation therapy failure [32,33].
(5) The activation of non-conventional pathways involved in DHT biosynthesis, as a result of mutations in steroid metabolism enzymes, can contribute to tumor progression [34].

Therefore, despite the absence of a thorough knowledge of the etiopathogenesis, targeting AR signaling remains an important treatment option for CRPC.

AR splice variants

Constitutively active ARs, resulted from alternative splicing of the human AR gene leading to truncated AR isoforms lacking the LBD, represent an emerging key mechanism responsible for castration resistance tumor progression.

Truncated AR variants (AR-Vs) wanting the LBD can be the result of somatic nonsense mutations that introduce stop codons in the AR gene [35], AR gene rearrangements, or post-translational AR proteolysis [36]. Furthermore, alternative splicing of the primary transcript, considering the complexity of the AR protein multi-domain structure, can actually impair the AR transcriptional activity by generating functionally distinct receptors from the same AR gene. Structurally, these AR variants lack the open reading frame of the LBD as a result of insertions of "intronic"