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Review

Lessons from tissue compartment-specific analysis of androgen receptor alterations in prostate cancer

Mark Daniel[a,b], Scott M. Dehm[b,c,d,*]

[a] Graduate Program in Microbiology, Immunology, and Cancer Biology, University of Minnesota, Minneapolis, MN, USA
[b] Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA
[c] Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA
[d] Department of Urology, University of Minnesota, Minneapolis, MN, USA

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ABSTRACT

Androgen receptor (AR) splice variants (AR-Vs) are constitutively active transcription factors that function in the absence of ligand. AR-Vs represent one of several AR re-activation mechanisms utilized by prostate cancer to circumvent first-line androgen deprivation therapy. Second line therapies such as enzalutamide and abiraterone are treatments that re-target components of the androgen/AR axis. However, these second line therapies do not benefit all patients, and patients that do receive initial benefit can develop resistance rapidly. Alterations in components of the androgen/AR axis, including expression of AR-Vs, appear to be linked to primary as well as secondary resistance to second line therapies. However, some key conclusions appear to differ depending on the tissue compartment and measurement platform utilized for analysis. In this review, alterations in AR and the broader AR pathway will be examined in the context of primary prostate cancer tissue, metastatic castration-resistant prostate cancer tissue, circulating tumor cells, and circulating cell-free tumor DNA. Questions regarding the utility of AR-V measurements to provide prognostic information or predict patient responses to AR-targeted therapies will be addressed.

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Abbreviations: LHRH, luteinizing hormone releasing hormone; ADT, androgen depletion therapy; CRPC, castration-resistant prostate cancer; CYP17, cytochrome P450 c17; MR, mineralocorticoid receptor; ER, estrogen receptor; PR, progestins receptor; GR, glucocorticoid receptor; NTD, NH_2-terminal domain; AF-1, activation function 1; LBD, ligand bind domain; ARE, androgen response element; RIP, RNA co-immunoprecipitation; NCOA, nuclear receptor coactivator; NCOR, nuclear receptor corepressor; CTC, circulating tumor cell; ctDNA, circulating tumor DNA; FISH, fluorescence *in situ* hybridization; PSA, prostate-specific antigen; PMSA, prostate-specific membrane antigen.

* Corresponding author at: Masonic Cancer Center, University of Minnesota, Mayo Mail Code 806, 420 Delaware Street SE, Minneapolis, MN, USA.
E-mail address: dehm@umn.edu (S.M. Dehm).

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1. Introduction

Prostate cancer accounts for a quarter of all new cancer diagnoses, and one in seven men in the United States will develop the disease. Of those men, roughly 27,000 will die each year, making prostate cancer the second leading cause of cancer related death in the United States [1]. Prostate cancer is referred to as an androgen-dependent disease, which is based on the requirement of androgen-mediated activation of the androgen receptor (AR) for growth and survival of prostate cancer cells. As a result of androgen-dependence, the androgen/AR axis has been a target of treatment since the early 1940s when Huggins et al. established the beneficial effects of castration in men with metastatic prostate cancer [2]. Contemporary therapies for prostate cancer continue to rely on reducing the levels of circulating androgens and inhibiting AR function via orchiectomy or luteinizing hormone releasing hormone (LHRH) agonists. Antiandrogens such as bicalutamide are also used to competitively block the ability of androgens to bind and activate the AR. Patients that fail this first-generation of androgen depletion therapies (ADT) and progress to a disease stage referred to as castration-resistant prostate cancer (CRPC) are treated with second-generation AR-targeting therapies such as abiraterone acetate and enzalutamide (Fig. 1A). Abiraterone acetate is an inhibitor of the cytochrome P450 c17 (CYP17) testosterone synthesis enzyme [3], which can achieve a more robust inhibition of androgen production than castration. Enzalutamide is an antagonist that binds the AR with a higher affinity than bicalutamide, and can partially inhibit AR translocation to the nucleus, reduce binding of AR to DNA, and prevent recruitment of coactivators required for active transcription [4]. Despite these advances in treatment, CPRC remains a uniformly fatal disease. Importantly, persistent AR signaling can be maintained during and after treatment with these androgen/AR-targeted therapies. The focus of this review will be observations made in clinical tissues collected at various disease stages (Fig. 1B) that have illuminated key mechanisms of AR re-activation that can occur in CRPC (Fig. 1C), with an emphasis on AR splice variants (AR-Vs).

1.1. Brief introduction to AR structure/function

The AR is a member of the class I nuclear steroid receptor family. Within this family of steroid receptors are transcription factors regulated by ligands including mineralocorticoids (MR), estrogens (ER), progestins (PR) and glucocorticoids (GR). All of these steroid receptors are activated upon binding ligand, after which they engage their cognate hormone response element DNA sequences located in promoter and enhancer regions throughout the genome. The AR gene is located on the X chromosome at cytogenetic position Xq11-12. This gene contains eight exons and encodes a modular 110 kDa protein [5]. Exon 1 of AR encodes the NH_2-terminal domain (NTD), which is intrinsically disordered and harbors the transcriptional activation function-1 (AF-1) domain essential for the bulk of AR transcriptional activity. Exons 2 and 3 encode the DNA-binding domain. The 5′ section of exon 4 encodes the hinge region, which is a flexible domain containing a nuclear localization signal (NLS) that becomes accessible upon ligand binding. The 3′ section of exon 4 along with exons 5–8 encode the AR ligand-binding domain (LBD) and an AF-2 domain that is transcriptionally active but with weaker activity than AF-1 [5]. Upon binding to androgen, AR undergoes a conformational change, which exposes the NLS, and results in translocation to the nucleus. Once in the nucleus, AR binds to androgen response elements (AREs) and recruits a wide variety of coregulatory proteins with various scaffolding, enzymatic, and chromatin-modifying functions, ultimately leading to a finely-tuned level of transcriptional output for target genes. These features of canonical AR signaling are illustrated in Fig. 1C.

1.2. Brief introduction to AR splice variants (AR-Vs)

Over the past 8 years, AR-Vs have emerged as an important component of resistance to therapies targeting the androgen/AR axis. Approximately 20 discrete AR-Vs have been identified in cell- and animal-based models of CRPC progression, as well as tissues from patients with prostate cancer [6–9]. AR-Vs share the same NTD/DBD modular domain structure as the full-length AR, but commonly lack the COOH-terminal LBD/AF-2 module. Fig. 1C illustrates the structure of select AR-Vs that will be detailed in this review. Truncation of the AR LBD results in constitutive transcriptional activity of the AR NTD/DBD core in the absence of ligand. Thus, most AR-Vs have been reported to function as ligand-independent transcription factors, although discrepancies have been noted [10]. Originally, AR-Vs were reported to arise from proteolytic cleavage of full-length AR by calpains, which are calcium-dependent proteinases [11]. These early studies found that the AR hinge region harbored a calpain cleavage site, which could be targeted to eliminate the AR LBD. However, subsequent studies in the 22Rv1 cell line, which was the same model used to develop the proteolysis mechanism, revealed that truncated AR protein species were differentially sensitive to small interference RNAs (siRNAs) compared with full-length AR. This finding of differential siRNA sensitivity formed the basis for the concept that AR-Vs were encoded by mRNAs that were separate from the mRNA encoding full-length AR [12]. Subsequent studies demonstrated that different AR-V proteins were encoded by mRNAs containing exons 1–3 or exons 1–4 of the AR gene, but variable 3′ terminal exons harboring in-frame translation termination signals [13]. Subsequent studies defined an additional class of AR-V proteins that were encoded by skipping of exons encoding the AR LBD, but terminating with an out-of-frame 3′ terminal AR exon 8 [13].

One mechanism thought to underlie AR-V expression in prostate cancer is alternative splicing. RNA co-immunoprecipitation (RIP) assays using enzalutamide-treated VCaP cells revealed that splicing regulatory factors ASF/SF2, and U2AF65 were recruited to pre-mRNA sequences aligning with a splice acceptor site in AR exon CE3, which is the 3′ terminal exon in the mRNA encoding AR-V7 [14]. In this scenario, it was concluded that increased recruitment, but not expression, of splicing factors contributed to AR-V7 in these cells. In line with this, the AR-V7-negative LNCaP cell line did not display the same pattern of

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