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Androgen Receptor Gene Rearrangements: New Perspectives on Prostate Cancer Progression

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Abstract

The androgen receptor (AR) is a master regulator transcription factor in normal and cancerous prostate cells. Canonical AR activation requires binding of androgen ligand to the AR ligand binding domain, translocation to the nucleus, and transcriptional activation of AR target genes. This regulatory axis is targeted for systemic therapy of advanced prostate cancer. However, a new paradigm for AR activation in castration-resistant prostate cancer (CRPC) has emerged wherein alternative splicing of AR mRNA promotes synthesis of constitutively active AR variants that lack the AR ligand binding domain (LBD). Recent work has indicated that structural alteration of the AR gene locus represents a key mechanism by which alterations in AR mRNA splicing arise. In this review, we examine the role of truncated AR variants (ARVs) and their corresponding genomic origins in models of prostate cancer progression, as well as the challenges they pose to the current standard of prostate cancer therapies targeting the AR ligand binding domain. Since ARVs lack the COOH-terminal LBD, the genesis of these AR gene rearrangements and their resulting ARVs provides strong rationale for the pursuit of new avenues of therapeutic intervention targeted at the AR NH_2-terminal domain. We further suggest that genomic events leading to ARV expression could act as novel biomarkers of disease progression that may guide the optimal use of current and next-generation AR-targeted therapy.

Keywords

Androgen Receptor; alternative splicing; castration resistance; genomic rearrangement; prostate cancer

INTRODUCTION

The AR is a 110 kDa protein with a modular domain organization found in members of the steroid hormone receptor superfamily [1, 2]. The NH_2-terminal domain (NTD), also referred to as transcriptional activation function (AF)-1, is a potent transcriptional activation domain in isolation and is responsible for the majority of AR transcriptional activity through the recruitment of diverse co-regulatory proteins. The central domain of the AR is the DNA binding domain (DBD), which is comprised of two zinc-finger motifs. The first zinc finger is responsible for making direct contact with the DNA major groove of an androgen

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response element (ARE) half-site, while the second zinc finger mediates dimerization with a second AR molecule bound to an adjacent ARE half-site [3]. The DBD is followed by a short, flexible hinge region which contains the bipartite nuclear localization signal. The COOH-terminal domain (CTD) of the AR houses both the ligand binding domain (LBD) and a secondary transcriptional activation domain termed AF-2.

The primary role of the AR is to sense and respond to circulating androgens, the most abundant of which are testosterone and dihydrotestosterone (DHT) [4, 5]. In the absence of ligand stimulation, AR is cytoplasmic, bound in a chaperone complex of heat shock proteins and high molecular weight immunophilins, which maintains AR protein in an inactive conformation with a high affinity for ligand binding [6]. Following ligand binding, the AR undergoes a conformational change, causing dissociation of a subset of chaperone proteins and exposing the nuclear localization signal in the hinge region. Upon translocation of the AR/DHT complex to the nucleus, the AR DBD engages with genomic AREs [7], mediating chromosomal looping and structural reorganization of the genome [8–10]. In order for productive gene transcription to occur, the AR is reliant on interactions with a wide variety of transcriptional co-regulators, of which nearly 200 have been identified to date [11]. These transcriptional co-regulators form large complexes that result in recruitment of the basal transcriptional machinery and a finely-tuned level of androgen-responsive gene transcription [12, 13]. In healthy prostate tissue, these androgen-responsive genes are important for normal prostate architecture, homeostasis, and physiological function. In prostate cancer (PCa), these target genes support ongoing proliferation and survival of tumor cells.

Structure and Function of the AR COOH-Terminal Domain

The CTD of the AR is the best understood functional domain by virtue of its structural homology and regulatory similarities with other steroid receptors [14]. The AR gene locus, located at Xq11-12, is approximately 180 kilobases in length and consists of eight coding exons separated by intronic segments of varying length. Exon 1 codes for the entire AR NTD, or approximately 60% of the total protein, while exons 2 and 3 code for the two zinc finger domains of the AR DBD. Exons 4–8 are located in close proximity to one another in the AR gene locus, and code for the hinge region and CTD/LBD of the AR (Fig. 1). Importantly, all AR-targeted therapeutics currently approved for clinical use modulate AR activity by exerting action on this domain [15]. The CTD contains 11 α-helices that form the binding pocket of the AR LBD, while a twelfth helix forms a “kickstand” which locks into place upon androgen binding [16–18]. This upswing of helix 12 stabilizes AR binding to DHT and forms the AF-2 protein interaction interface [19, 20]. AF-2 has been shown to exert transcriptional activity in the presence of bound agonist by binding to nuclear receptor (NR)-box motifs in coactivators, such as SRC-2 [19], and is also capable of mediating intramolecular interaction with FxxLF or WxxLF motifs in the AR NTD [21]. Furthermore, the CTD contains a ligand-regulated nuclear export signal which is dominant over the AR nuclear localization signal but is inhibited by ligand binding [22].

Recent work has provided evidence for a second protein interaction domain within the CTD, termed binding function (BF)-3, which communicates allosterically with AF-2 [23, 24]. Interestingly, a host of mutations identified in both PCa and androgen insensitivity syndrome map to BF-3, supporting the concept that this domain may play an important role in allosteric regulation of AR function [25]. Another recent study provided evidence that FKBP52, a co-chaperone protein critical for maintaining AR in a conformation competent for ligand binding, may interact with AR through the BF-3 domain [26]. Importantly, these critical AF-2/BF-3 mediated functions are amenable to targeting with small molecules [23, 26–28] which could potentially lead to new avenues of AR-targeted therapy.

Therapeutic Targeting of the COOH-Terminal Domain in Prostate Cancer

PCa is the most frequently diagnosed male cancer and second leading cause of cancer deaths [29]. For tumors that are relapsed, locally advanced, and/or metastatic, the current standard of care is androgen deprivation therapy (ADT), achieved by suppression of AR signaling through the use of AR antagonists such as bicalutamide or flutamide, or by preventing production of testosterone by the testes using gonadotropin releasing hormone agonists such as leuprolide [15]. ADT initially provides a robust therapeutic benefit by blocking tumor cell proliferation and inducing apoptosis, resulting in clinical regression. Invariably, however, AR signaling is eventually reactivated via diverse mechanisms including AR amplification and/or AR protein overexpression, gain of function AR mutations [30, 31], enhanced uptake and conversion of adrenal androgens, or *de novo* androgen synthesis by tumor cells [32]. These mechanisms have been reviewed in detail elsewhere [33–35]. These molecular events mark a transition from the initial androgen-dependent PCa to a lethal castration recurrent phenotype, also referred to as “castration resistant PCa” (CRPC). Enzalutamide (formerly MDV3100) and abiraterone acetate, two next-generation AR-targeted therapeutics, were developed to address these mechanisms of disease progression [36, 37]. In Phase III trials, abiraterone and MDV3100 increased overall survival CRPC patients by 3.9 and 4.8 months, respectively [38, 39]. Clinical trials have demonstrated that both drugs provide significant therapeutic benefit to a high proportion of patients [38–41], but a subset of patients continue to experience disease progression, either through acquired resistance or through *de novo* insensitivity prior to treatment. Currently, it is of major interest to identify mechanisms that may drive these types of resistance to next-generation AR-targeted therapies. Importantly, unlike other steroid hormone receptors in which AF-2 is the dominant transactivation domain, the AR CTD plays a primarily regulatory role, and the AR NTD is responsible for the majority of AR transactivation [19]. Therefore, recent work describing the discovery and characterization of constitutively active, pathogenic AR splice variants which lack the CTD regulatory domain have generated significant interest, as these species may be capable of restoring AR signaling in PCa tissues following ADT through a mechanism of constitutive AR NTD transcriptional activity.

CONSTITUTIVELY ACTIVE AR SPLICE VARIANTS PROMOTE CASTRATION RESISTANCE IN PROSTATE TUMORS

Splice variants of the AR have been recognized for over two decades in the context of loss-of-function splicing alterations in androgen insensitivity syndrome, which is a topic that has been reviewed in detail elsewhere [42]. The first gain-of-function AR splice variant (ARV) was identified in 22Rv1 cells due to the presence of a smaller, 75–80 kDa AR immunoreactive species on western blot that was initially thought to be a proteolytic degradation fragment of full length AR [43]. This AR subspecies was shown to lack a ligand binding domain and was constitutively active in both the presence and absence of androgen. Similarly, a subsequently identified Q640Stop mutation resulted in premature truncation and constitutive AR signaling in bone metastases from a patient who relapsed following ADT with luprorelin and flutamide [44]. It was initially postulated that the smaller band observed in the 22Rv1 cell line resulted from calpain-mediated cleavage of full length AR at a consensus calpain recognition site in the AR hinge region [45]. However, later work demonstrated that RNA interference (RNAi) targeted against AR exon 7 (Fig. 1) had no effect on expression levels of the smaller species, despite robust ablation of full length AR. Conversely, RNAi targeted against AR exon 1 led to ablation of both the full length and the truncated species [46]. These data strongly suggested that the truncated ARV was not a product of full length AR mRNA or protein, but instead derived from an alternate mRNA species. The ability to differentially target full-length vs. truncated ARV species with

discrete RNAi reagents further revealed that the constitutive activity of the truncated ARV was the driving force behind the androgen independent proliferation of 22Rv1 cells.

Since their initial identification, nearly a dozen different ARV mRNA species have been identified in PCa cell lines, xenografts, and clinical samples [42]. ARVs arise as a result of the incorporation of alternative, or cryptic, exons coded for in the AR gene locus [46–49], or through an exon skipping mechanism in which non-contiguous AR exons are spliced together [50]. Characterization of these novel ARV mRNAs has revealed multiple alternative, or "cryptic" exons in the AR locus, most of which flank AR exon 3. For example, alternative exon 2b (also termed cryptic exon 4, or "CE4") is located upstream of exon 3, whereas many others are within AR intron 3 (CE1, CE2, CE3, CE5, and exon 3'). The products of these splicing aberrations generally incorporate canonical AR exons 1–3, which code for the AR NTD as well as the DBD. These three exons appear to form the minimum requirement for a transcriptionally active ARV [51]. However, ARVs differ in their utilization of exons 4–8, with most ARVs incorporating one of the seven currently identified cryptic exons coded for by the AR locus [42]. Problematically, multiple naming systems have been proposed to refer to the various ARVs. For example, the ARV encoded by contiguously spliced exons 1, 2, 3, and 2b has been alternately named AR-V4 [48], AR5 [47], and ARV6 [49]. Therefore, for the purposes of this review, we will refer to the variants by their exon composition, e.g. AR 1/2/3/2b, to alleviate confusion. To date, only three ARV transcripts have been mechanistically investigated in cell lines, xenografts, or clinical samples: AR 1/2/3/CE3, AR 1/2/3/2b, and AR 1/2/3/4/8.

The most studied and currently best characterized ARV is coded for by AR exons 1/2/3/CE3, alternatively termed AR-V7 and AR3 [47, 48]. AR 1/2/3/CE3 has been shown to be expressed at the mRNA and protein level in normal and cancerous prostate tissue, multiple commonly used PCa cell lines, and human tumor xenografts. Expression of this isoform was also demonstrated to be increased in locally recurrent and metastatic castration resistant PCa tissue compared to prostatectomy specimens from hormone naïve men [47]. A separate study found that 1/2/3/CE3 mRNA expression levels in prostatectomy specimens could predict the likelihood of biochemical recurrence after surgery [48]. Biochemically, 1/2/3/CE3 was shown to function as a constitutively active transcription factor independent of androgen ligand [47]. However, the exact transcriptional program mediated by this variant may differ slightly from full-length AR. In one study, transient transfection of LNCaP cells with an AR 1/2/3/CE3 expression vector was shown to effect a strikingly similar transcriptional program compared with ligand-activated full length AR [48]. On the other hand, a second study using targeted siRNA knockdown of endogenous full length AR versus 1/2/3/CE3 demonstrated that the CE3 isoform activated Akt expression, whereas full-length AR did not [48]. More recently, Hu and colleagues [52] have reported a unique role for AR 1/2/3/CE3 in the activation of M-phase specific cell cycle genes. For example, whereas full-length AR target genes appeared to be largely associated with pathways important for biosynthesis and metabolism, the 1/2/3/CE3 variant was able to activate transcription of pro-mitotic cell cycle regulators such as UBE2C, CDCA5, ZWINT, TPX2, and CDC25C. Taken together, these data strongly support a role for the AR 1/2/3/CE3 splice variant as a constitutively active AR isoform with significant clinical implications for biology and treatment of castration resistant PCa tumors.

The AR 1/2/2b and AR 1/2/3/2b mRNA variants were initially identified by 5' RACE experiments in the 22Rv1 cell line [46]. Specific knockdown of these ARVs using an exon 2b-targeted siRNA resulted in an expected reduction in the truncated 75–80 kDa AR species, suggesting that one or both of the 1/2/2b and 1/2/3/2b mRNAs were translated. However, our laboratory recently developed an antibody specific to the COOH-terminal extension encoded by AR exon 2b, which revealed that only the AR 1/2/3/2b variant is

productively translated to functional protein in 22Rv1 cells [51]. Importantly, siRNA knockdown of AR 1/2/3/2b significantly reduced the ability of 22Rv1 cells to proliferate in the absence of androgen, but had no effect on androgen dependent proliferation, supporting a role for this ARV as a driver of castration resistance in 22Rv1 cells [46].

Finally, AR 1/2/3/4/8 was shown to arise through the skipping of exons 5–7 in the mRNA transcript [50]. This exon skipping event places exon 8 out-of-frame, resulting in formation of a premature translation termination codon in exon 8. This variant, originally named AR^{v567es}, is expressed at the mRNA level in a wide range of normal and cancerous prostate tissues, although protein expression has not yet been confirmed using variant-specific antibodies. Sun and colleagues also demonstrated that AR 1/2/3/4/8 mRNA is expressed endogenously in the LuCaP 86.2 and 136 xenografts, and inferred that this protein was expressed endogenously due to its molecular weight. Interestingly, upon castration, mRNA levels of AR 1/2/3/4/8 increased in these xenografts compared with intact hosts. When expressed ectopically in LNCaP cells, AR 1/2/3/4/8 displayed constitutive transcriptional activity and could interact directly with full length AR, resulting in enhanced ligand dependent and -independent activity of the full length receptor in these cells. A later study by Hu and colleagues demonstrated that a novel ninth exon, located downstream of AR exon 8, was incorporated into the mRNA transcript of this AR 1/2/3/4/8 variant in VCaP cells [52]. However, because incorporation of exon 9 does not affect the premature translation stop codon in exon 8, this exon simply alters the 3’ untranslated region of this mRNA. Therefore, AR 1/2/3/4/8/9 mRNA codes for exactly the same protein as AR 1/2/3/4/8 mRNA, and thus it is not surprising that this variant displayed constitutive, ligand independent activity in promoter-reporter assays. Interestingly, in this study, the strength of AR 1/2/3/4/8/9 transcriptional activity appeared to depend on which cell line it was tested in, with higher activity apparent in PC-3 vs. LNCaP PCa cell lines.

Although all active gain of function ARVs identified to date consist of the AR NTD and DBD, they harbor unique COOH-terminal extensions encoded by the various exons that can be spliced into the 3’ mRNA termini of AR variant transcripts. This has been proposed to bear significant implications for ARV biochemistry because the bipartite nuclear localization signal (NLS), $RKx_{10}RKLKK$, spans the exon 3–4 junction in wild type AR [53]. Therefore, since most of the ARV mRNAs identified to date do not harbor exon 4, the bipartite NLS would be disrupted in these variants. However, recent work has demonstrated that the AR NTD/DBD core displays a high level of constitutive nuclear localization in the absence of ligand that is independent of both HSP90 and the nuclear import adapter protein importin-β, resulting in transcriptional activation of endogenous AR targets [51]. Moreover, this study demonstrated that differences in ARV transcriptional activity that have been observed are promoter-dependent phenomena as opposed to arising from differential rates of nuclear access.

GENOMIC REARRANGEMENTS PROMOTE DISEASE PROGRESSION AT MULTIPLE STAGES OF PROSTATE CANCER DEVELOPMENT

Gene Rearrangements Prior to ADT: From PIN to PCa and Beyond

Beginning with the discovery of recurrent Ets-family gene rearrangements in 2005 [54], it has become increasingly clear that structural alterations are frequent events in the PCa genome and underpin many aspects of tumor biology and disease progression. These events include the highly prevalent TMPRSS2-Ets family of gene fusions [54] as well as fusions involving Raf family members [55]. A number of other rearrangements have also been identified in primary prostate tumors, which may represent novel mechanisms for driving tumor invasiveness, proliferation/survival, and anchorage-independent growth in PCa [56,

57]. These gene rearrangements have been reviewed in detail elsewhere [58]. More recently, chromosomal alterations involving the PTEN locus have been shown to cooperate with allelic loss to drive PCa progression [59]. Interestingly, this mechanism of PTEN inactivation, as well as the rearrangements reported by Pflueger *et al.* [57] was highly correlated with underlying ERG rearrangement, supporting a role for Ets family rearrangements as a genome-destabilizing event early in prostate tumorigenesis. This may also explain the observation that patients with fusion-positive PCa experience more aggressive and lethal disease compared with fusion-negative cases [60, 61], though a number of studies have reported that TMPRSS2:Ets rearrangement may alternatively correlate with low Gleason grade [62], favorable prognosis [63], or may not be predictive of disease outcome at all [64]. Regardless, clinical samples from men with metastatic castration-recurrent PCa exhibit a wide range of mutations, deletions, and rearrangements as determined by exome sequencing [65]. Overall, these genomic rearrangements have been shown to be associated with and predictive of PCa genesis and/or progression, such that molecular subtyping of based on these criteria may result in improvements in patient management and/or clinical trial designs [58].

Rearrangements of the AR Locus: a New Paradigm for ARV Expression and Activity Following ADT?

These recent whole genome studies have also supported the long-held fundamental concept that the AR signaling axis is a critical master regulator in PCa. Foremost, this axis has been shown to be the most frequently-altered pathway in hormone-naïve PCa, and 100% of castration-resistant PCa metastases display genomic and/or mRNA expression alterations in this pathway, most frequently in the AR gene itself [66]. The observation that AR exon 2b is incorporated into the 1/2/3/2b transcript downstream of exon 3 in 22Rv1 cells, despite the fact that 2b is located 5' of exon 3 in the normal reference genome [46, 48] (Fig. 2), raised an intriguing question: what is the molecular basis for this splicing pattern? One clue came from the observation that the full-length AR in 22Rv1 cells is slightly larger due to an extra zinc finger in the DBD encoded by tandem duplication of AR exon 3. Interestingly, in addition to these unanticipated splicing patterns, it was demonstrated that the 22Rv1 cell line exhibits significantly increased mRNA expression of the AR 1/2/3/CE3 variant [67]. In the same study, the androgen-dependent CWR22Pc cell line, which was derived from the same original CWR22 xenograft model as 22Rv1, was found by quantitative RT-PCR analysis to express extremely low but detectable transcript expression of these ARVs. These observations suggested that the observed splicing patterns may not be true “alternative splicing” events in 22Rv1 cells, but may instead be due to an underlying alteration in AR gene structure. Indeed, interrogation of AR gene structure demonstrated that the region harboring exon 2b, 3, and CE1-3 was present in the genome at two-fold higher copy number in castration-recurrent 22Rv1 cells, but not CWR22Pc, suggesting the presence of a tandem duplication [67]. More detailed analysis confirmed that a ~35kb segment, comprised of exon 3 and its flanking cryptic exons, was involved in a tandem duplication event within 22Rv1 cells (Fig. 2). Importantly, long term culture of the lineage-related CWR22Pc cell line in the absence of androgen resulted in the outgrowth of a castration resistant population of cells that harbored the exact same break fusion junction and repair signature as 22Rv1, and displayed increased expression of truncated ARVs mRNAs and proteins including 1/2/3/2b and 1/2/3/CE3. These data indicate that a subset of cells within the original CWR22 tumor harbor this rearrangement and are driven by constitutive, ligand-independent ARV activity prior to androgen deprivation. In this cell line, ADT simply results in selective outgrowth of these ARV-driven cells harboring the 35kb tandem duplication. Importantly, complex patterns of AR gene copy imbalance were also observed in metastatic CRPC samples, but not in hormone-naïve primary tumors [67], suggesting that generation of constitutively

active ARV through genomic rearrangements may be a recurring theme in human disease progression.

Interestingly, ARV have also been described in the mouse PCa cell line Myc-CaP [68], in which the AR is amplified through genomic copy number gain. Mouse AR (mAR)-V2 was shown to result from splicing of exons 1–3 together with a novel cryptic exon located ~250 kb downstream of the AR gene locus. Perhaps even more compelling, a second ARV termed mAR-V4 was generated by splicing of exons 1–4 and a novel cryptic exon located nearly 1 Mb upstream of the AR transcriptional start site. Whereas mAR-V2 showed little activity in functional assays, mAR-V4 was constitutively active and localized to the nucleus, similar to ARVs identified in human cell lines and tissues. Though the molecular basis for splicing of mAR-V4 was not addressed in this study, it is likely contingent upon the known amplification of the AR gene in Myc-CaP cells. Following this rearrangement of the AR gene, the V4 cryptic exon could be situated downstream of the AR open reading frame, thus accounting for the incorporation of the V4 exon at the 3' terminus of the transcript.

Further investigation of genomic copy number imbalance in additional models of CRPC progression has confirmed AR gene rearrangements as an important mechanism involved in the generation of constitutively active ARVs [69]. Multiplex ligation dependent probe assays (MLPA) were employed to query the copy number of AR exons in a variety of PCa cell lines and tissues. Interestingly, LuCaP 86.2 cells displayed reduced copy number of AR exons 5–7, which was shown subsequently to result from an 8.5kb intragenic deletion of this genomic segment (Fig. 2). Clearly, deletion of AR exons 5–7 provides an attractive mechanistic explanation for synthesis of the AR 1/2/3/4/8 variant in this xenograft model [50]. Interestingly, deletion of exons 5–7 prevents synthesis of full-length AR, indicating that this CRPC tumor would no longer be driven by androgen/AR signaling, but rather depends exclusively on the AR 1/2/3/4/8 variant for ongoing growth and survival.

An additional model of CRPC that has been shown to express high levels of truncated ARVs is the CWR-R1 cell line. To determine the basis for the splicing alterations in this model, Illumina paired-end massively parallel sequencing was employed to determine the sequence and structure of the AR locus [69]. This approach detected copy number loss spanning a 48 kb region of AR intron 1 (Fig. 2), which was supported by MLPA data querying copy number throughout AR intronic sequences. Interestingly, this deletion was initially observed only within a subpopulation of CWR-R1 cells. However, long term culture of CWR-R1 cells in androgen-depleted growth medium resulted in the outgrowth of the deletion-positive population. Importantly, the outgrowth of this subpopulation was accompanied by a corresponding increase in the protein expression of the constitutively active AR 1/2/3/CE3 variant. This finding supports the possibility that within at least some prostate tumors, subpopulations of ARV-driven cells with underlying rearrangements in the AR gene may exist prior to administration of AR-targeted therapies and, by virtue of constitutively active ARV expression, be able to overcome any drug in the current arsenal of AR-based therapies to repopulate the tumor. Based on the finding that ARV expression is an important feature of CRPC progression [47, 48, 50, 70, 71] and these recent data demonstrating AR gene rearrangements as a mechanism for altered AR splicing [67, 69], it is possible that AR gene rearrangements may represent a new class of genomic markers with predictive and/or prognostic value in CRPC.

STRUCTURE AND FUNCTION OF THE AR NTD

The role of ARVs in clinical PCa and castration resistance highlights the need for a greater understanding of NTD structure and function to aid in the design of AR-targeted therapeutics that do not require an intact AR LBD. The principal role of the NTD is to serve

as a docking site for AR transcriptional co-regulators [72], and it is well established that the NTD is the predominant transcriptional activation domain of the AR [2, 19, 73]. This stands in contrast to other steroid hormone receptors, in which the CTD harbors the primary transcriptional activation domain [19]. The NTD is divided into two primary transcriptional activation units (TAUs) termed TAU-1 and TAU-5, which have been shown to have distinct roles in AR-mediated transcription [2, 74] (Fig. 1). The TAU-5 domain maps to amino acids 361–490 of the AR NTD, and has been shown to promote AR activity specifically under conditions of low/no androgens [74, 75]. Deletion of TAU-5 causes near-complete loss of AR function in the absence of DHT in both androgen dependent LNCaP [74] and castration resistant C4-2 cells [75]. Further work mapped TAU-5 activity to a conserved Trp-His-Thr-Leu-Phe (WHTLF) motif, and deletion or mutation of the hydrophobic W/L/F residues to alanine significantly inhibited androgen independent AR activity [76]. Interestingly, however, a recurring W435L mutation found in metastatic PCa tissue from patients relapsing after ADT was shown to increase ligand-dependent AR transcriptional activity, possibly through stabilization of an N/C intramolecular interaction between this domain and the AF-2 region [31].

The other major domain within the NTD, TAU-1, maps to amino acids 101–360 and houses two smaller subdomains, known as activation function (AF)-1a (amino acids 101–211) and AF-1b (amino acids 252–360). Deletion of either of the AF-1 sub domains causes complete loss of AR transcriptional activity in both androgen dependent LNCaP cells as well as castration resistant C4-2 cells, whereas deletion of the internal spacer region between AF-1a and -1b actually enhances AR transcription [75]. The transcriptional activity of TAU1 has traditionally been ascribed to an LxxLL-like motif, LKDIL, located within AF-1a [77]. Deletion or mutation of this sequence causes significant loss of AR activity, similar to deletion of the entire AF-1a fragment [72]. Intriguingly, the LKDIL motif overlaps an Lx_7LL motif described by Zhu and colleagues [78], which is critical for mediating interaction with the transcriptional co-repressor NCoR and its binding partner TAB2. When TAB2 was phosphorylated by MEKK1, the NCoR/TAB2 complex was released, resulting in AR de-repression. However, no transcriptional co-activators have yet been identified that specifically bind to the LKDIL motif following co-repressor dissociation [79]. Furthermore, attributing TAU-1 activity exclusively to LKDIL does not account for the transcriptional loss observed upon deletion of AF-1b, suggesting that other elements within the TAU-1 domain may be important to the activity of this region [75].

Therapeutic Targeting of the AR NTD

The AR represents a nearly ideal drug target, in that pharmacologic targeting of the AR signaling axis produces profound results on prostate tumor biology with relatively minimal toxic side effects. While therapies that require an intact AR LBD have proven effective in treating PCa, mounting evidence suggests that the CTD may be ultimately dispensable for AR function in the context of CRPC. Observations of CTD-truncated ARVs, which function as potent, constitutively active transcription factors independent of the CTD, suggest that the NTD itself could be an alternative target for inhibition of AR transcriptional activity. Despite significant progress in defining the structural and functional composition of the AR NTD with the goal of therapeutic targeting, one principal challenge is the inherent flexibility and lack of tertiary structure throughout the NTD [80]. These structural characteristics are likely to be of fundamental importance to the transcriptional activity of the AR, but they have also proven to be a major obstacle to crystallographic analysis of AR structure and subsequent intelligent drug design. Nonetheless, two promising classes of drugs have recently been identified that seem to interact specifically with the NTD to mediate its inhibition. EPI-001 is a chlorinated bisphenol A diglycidyl ether (BADGE) identified in a high-throughput screen for compounds that could inhibit AR NTD activity. EPI-001 was

demonstrated to function by preventing binding of the CBP/p300 histone acetyltransferase to the AR NTD, thereby preventing AR activity at target gene enhancers and preventing outgrowth of castration recurrent tumors in a xenograft model [81]. More recently, a similar drug screen for compounds isolated from the marine sponge *Niphates digitalis* identified a class of drugs termed Niphatenones [82], which were shown by click chemistry to covalently bind to an unknown portion of the AR NTD. This binding was shown to rely on a glycerol ether substructure and an extended saturated alkyl chain flanking the central ketone of Niphatenone B, which was shown to mediate growth inhibition in AR-expressing LNCaP cells but not AR-negative PC3 cells. These data suggest that the effect is AR specific, though no studies were performed to rule out effects on other steroid hormone receptors. Importantly, these compounds have not yet been tested against cell or xenograft models bearing truncated ARV, a critical experiment that will likely determine their impact and usefulness in the long-term maintenance of PCa. Taken together, however, the recent identification of these AR NTD inhibitors provides strong proof of principle that the NTD remains a vital and viable therapeutic target, and may be a key to containing the progression of late-stage PCa.

SUMMARY AND FUTURE DIRECTIONS

The discovery and characterization of ARVs has indicated that tumors driven by these species may represent a clinically relevant molecular subtype of CRPC that may require different therapeutic intervention than tumors only harboring full length AR. Whereas Ets family gene rearrangements have been proposed as a specific biomarker of PCa, high levels of ARV expression may serve as a marker of true androgen independence: ARV-positive tumors are highly unlikely to respond to any currently available antiandrogens or ADT strategies. Even the potent next-generation therapeutics enzalutamide and abiraterone fail in a significant fraction of patients, and it is tempting to hypothesize that these patients might progress due to AR gene rearrangements and/or expression of ARVs that lack the domain targeted by these new drugs. Used in conjunction with the classification schema suggested by Rubin and colleagues [58], ARV status could serve as an additional biomarker to inform the optimal use of current and next-generation ADT, as well as non-AR based therapies, in the treatment of PCa.

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LIST OF ABBREVIATIONS

ADT	androgen depletion therapy
AF	transcriptional activation function
AR	androgen receptor
ARV	androgen receptor splice variants
BF	binding function
CRPC	castration resistant prostate cancer
CTD	COOH-terminal domain
DBD	DNA binding domain

LBD	ligand binding domain
NTD	NH_2-terminal domain
PCa	prostate cancer
RNAi	RNA interference
TAU	transcriptional activation unit

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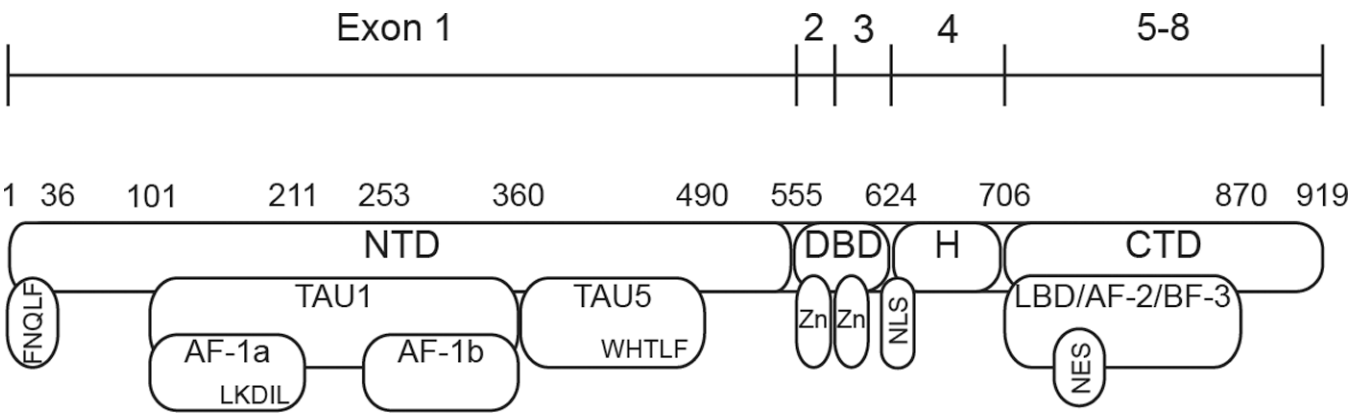

Figure 1. Androgen Receptor Functional Domains

The AR possesses a modular domain organization common to members of the steroid hormone receptor family of nuclear receptor transcription factors. The amino-terminal domain (NTD) harbors transcriptional activation unit (TAU)-1 and TAU-5. Transcriptional activation function (AF)-1a and AF-1b are subdomains of TAU-1. The DNA binding domain (DBD) is comprised of two zinc finger motifs (Zn), and a flexible hinge (H) region containing the AR nuclear localization signal (NLS). The COOH-terminal domain (CTD) harbors the ligand binding domain (LBD), a ligand-regulated nuclear export signal (NES), and the protein interaction domains AF-2 and binding function (BF)-3.

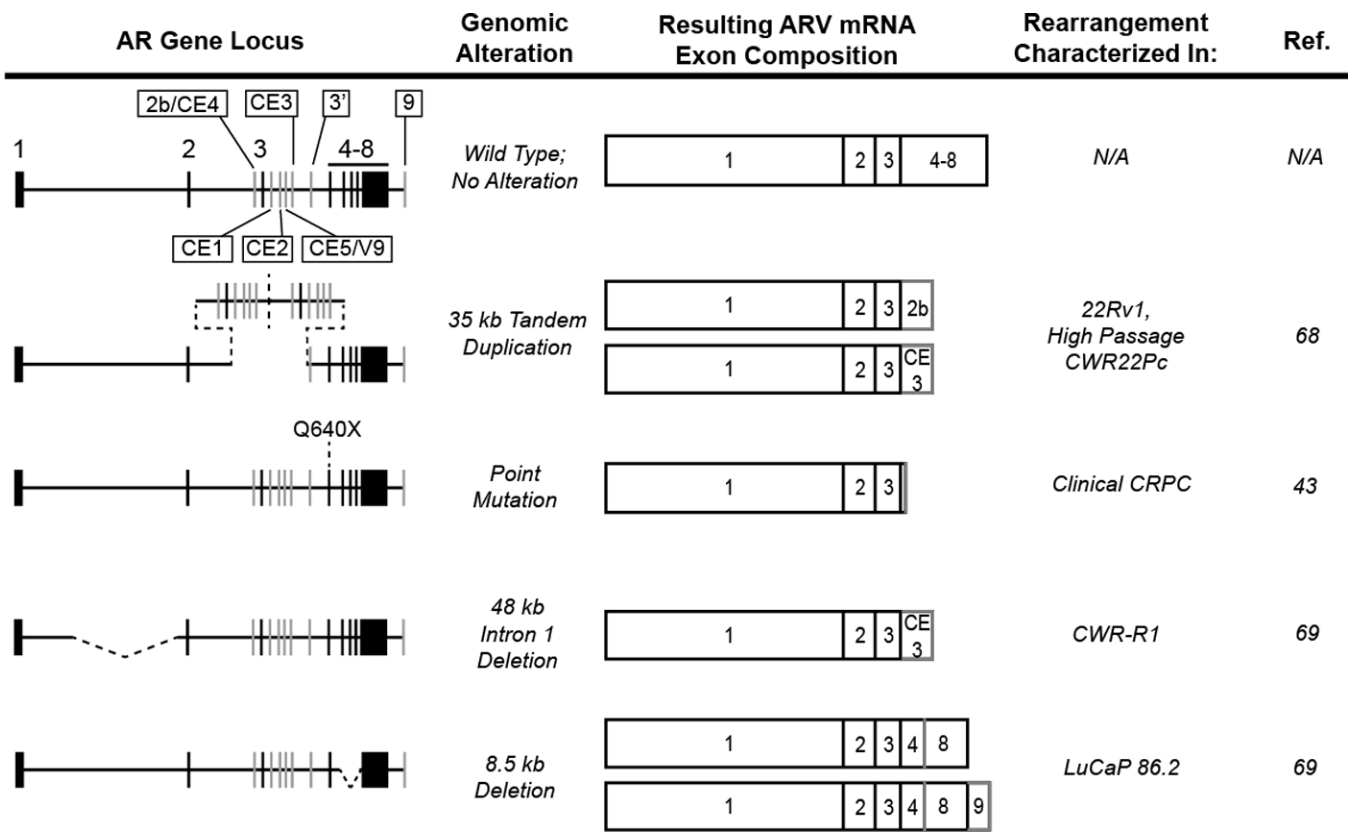

Figure 2. AR Genomic Alterations and Altered Splicing Patterns Leading to ARV Expression

The 180 kb androgen receptor gene locus harbors eight canonical exons (black vertical hashes) that code for the wild type AR mRNA and protein (black boxes). Seven alternative, or cryptic, exons have also been identified (gray vertical hashes) that can be incorporated into the AR transcript upon activation of alternative splicing pathways (gray borders/boxes). Four discrete AR gene rearrangements or mutations, depicted as dashed black lines, have been shown to disrupt AR splicing and favor the expression of AR variants in PCa cell lines and xenografts.