Review

Current strategies for targeting the activity of androgen receptor variants

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Abstract Current therapies for advanced prostate cancer, such as enzalutamide and abiraterone, focus on inhibiting androgen receptor (AR) activity and reducing downstream signaling pathways to inhibit tumor growth. Unfortunately, cancer cells are very adaptable and, over time, these cells develop mechanisms by which they can circumvent therapeutics. One of the many mechanisms that have been discovered is the generation of AR variants. These variants are generated through alternative splicing of the full length AR and often lack the ligand binding domain. This leads to forms of the AR that are constitutively active that continue to promote prostate cancer cell growth even in the absence of ligand. The high prevalence of AR variants and their role in disease progression have prompted a number of studies investigating ways to inhibited AR variant expression and activity. Among these are the anti-helminthic drug, niclosamide, which selectively promotes degradation of AR variants over full length AR and re-sensitizes anti-androgen resistant prostate cancer cells to treatment with enzalutamide and abiraterone. Other AR variant targeting mechanisms include interfering with AR variant co-activators and the development of drugs that bind to the DNA or N-terminal AR domains, which are retained in most AR variants. The clinical efficacy of treating prostate cancer by targeting AR variants is under investigation in several clinical trials. In this review, we provide an overview of the most relevant AR variants and discuss current AR variant targeting strategies.

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1. Introduction

Prostate cancer is the second leading cause of cancer related deaths and the most commonly diagnosed cancer in men in the United States [1]. Androgen deprivation therapy (ADT) reduces circulating androgen levels to reduce tumor growth and is an early line of treatment for this disease. While ADT is initially effective at reducing prostate cancer growth, most patients will progress to castration-resistant prostate cancer (CRPC) after 2–3 years of treatment. CRPC is defined as progression of prostate cancer in the presence of castrate levels of circulating testosterone [2,3]. CRPC is often heralded by hyper-activated androgen receptor signaling leading to the transcription of downstream target genes and tumor growth. One of the primary identified mechanisms of aberrant AR signaling is the expression of AR variants.

The presence of AR variants has been detected in nearly all CRPC cell lines and CWR22Rv1 cells have similar expression levels of full length AR and AR variants. Prostate cancer bone metastases have been found to have high AR variant expression [4]. AR splice variants are formed through a number of mechanisms including genome rearrangement and alternative splicing that involves splicing factors such as hnRNPs [5,6]. Other studies have demonstrated that calpain-mediated cleavage of full length AR can also promote expression of AR variants and androgen independance [7]. AR variants most commonly lack the C-terminal ligand-binding domain. These truncated versions of AR are often constitutively active because they do not rely on the presence of ligand to initiate downstream AR signaling [8–12].

Expression of these AR variants has been correlated with resistance to ADT clinically [13]. AR variant expression has been associated with resistance to enzalutamide and abiraterone, and, though there are conflicting data, to docetaxel resistance as well. AR-V7 is the most widely studied of these variants. AR-V7 expression in prostate cancer patients treated with enzalutamide or abiraterone has been related to significantly decreased prostate-specific antigen (PSA) response and shorter progression-free survival and overall survival compared to men who do not express AR-V7 [14]. While AR variant expression is associated with poorer prognosis and the development of CRPC, the functional roles of AR variants are not yet fully understood. This is in part due to the lack of accurate AR variant specific antibodies [4].

A number of recent studies have sought to uncover methods for targeting AR variants in order to improve treatment response due to the high prevalence of AR variant expression in treatment resistant prostate cancer. In this review, we will discuss the current understanding of the roles of the various AR variants and cover current and emerging AR variant targeting strategies to overcome treatment resistance in prostate cancer.

2. The AR variants

The existence of endogenous AR variants was first recognized in prostate cancer cell lines through the pronounced immunoblotting of a lower molecular weight band when blots were screened for full length AR [15]. Further characterization determined that the variants in the lower molecular weight band lacked the ligand binding domain [15,16]. More than 20 unique AR variants have been identified, however, only some of these have been studied in detail. In addition to AR-V7, the most highly researched AR variants are AR-V1, AR-V3, AR-V9, and ARv567es.

AR-V1 arises from the splicing of a cryptic exon to exon 3. AR-V1 has been detected in bone metastasis, non-malignant prostate tissue, and primary prostate tumors. AR-V1 expression was increased in VCaP xenograft models following castration [17]. In general, AR-V1 localizes primarily in the cytoplasm and is only conditionally active, unlike many of the other AR variants. Zhan et al. [18] found that AR-V1 can homodimerize or heterodimerize with AR-V7 and full length AR. They observed that AR-V1 dimerized with full length AR in the absence of androgen and addition of androgen enhanced dimerization. When dimerized in the presence of androgen, AR-V1 was found to reduce full length AR translocation to the nucleus and antagonize AR-V7 transactivation. In soft agar assays, Watson et al. [19] observed that co-expression of AR-V1 with AR-V7 negated the gain in anchorage dependent growth seen with AR-V7 alone. Both groups therefore hypothesized that AR-V1 is a dominant-negative AR variant. AR-V3 is another truncated AR variant in which a cryptic exon has been added between exons 2 and 3. In CRPC patients treated with abiraterone, expression of AR-V3 is associated with shorter progression-free survival [20]. Some studies suggest that AR-V3 may be more abundantly expressed than AR-V7 in circulating tumor cells from CRPC patients [21].

AR-V9 shares a common 3’ terminal cryptic exon with AR-V7 and is conditionally active. In a recent study by Kohli et al. [20], AR-V9 was determined to respond to siRNA inhibition targeted at AR-V7, since these siRNAs were directed at the shared 3’ terminal cryptic exon. Previous AR-V7 studies utilizing these siRNAs may have reported effects from dual inhibition of AR-V7 and AR-V9 and not AR-V7 alone. This group also observed AR-V9 to promote androgen-independent AR transcriotional activity when over expressed in prostate cancer cells, even in the presence of enzalutamide. Furthermore, Kohli et al. [20] analyzed biopsies of metastatic CRPC tissues from patients about to undergo abiraterone therapy and found that AR-V9 levels in the highest quartile predicted primary resistance to therapy. Another study disputes this claim; To et al. [22] found no correlation between the variants and treatment response in an assay analyzing AR-V7 and AR-V9 in whole blood.

Exons 5, 6, and 7 of the AR have been deleted in ARV567es. This variant is constitutively active and frequently identified in CRPC xenograft tumors and human metastases [4,11,23,24]. Furthermore, ARV567es expression is increased in enzalutamide resistant xenograft tumors [25]. Other studies have noted that heat shock protein 90 (HSP90) inhibition can lead to accumulation of ARV567es, and AR-V7, and that these variants mitigated the effects of HSP90 inhibitors on transactivation of probasin-luciferase activity. Despite this, ARV567es expression did not induce resistance to HSP90 inhibitors on cell growth [26]. To investigate the functional role of ARV567es, Liu et al. [27] generated a transgenic mouse model with...
patients had inferior baseline PSA response rates and shorter progression-free survival rates when compared to patients who were AR-V7 negative when treated with enzalutamide or abiraterone [14]. Another study in AR-V7 positive metastatic castration-resistant prostate cancer (mCRPC) patients found that PSA response rates were increased and progression-free survival was prolonged in taxane treated men compared to anti-androgen treated men. No changes were observed based on treatment in AR-V7 negative mCRPC patients [39]. Furthermore, when Efstratiou et al. [41] analyzed AR-V7 protein levels in 60 patients with bone metastatic CRPC before and 8 weeks after enzalutamide treatment, AR-V7 expression was found to be associated with primary resistance to enzalutamide. However, To et al. [22] were unable to correlate baseline AR-V7 or AR-V9 in whole blood to PSA response rate or PSA progression-free survival. Overall, the varying results from these studies suggest that the potential of AR-V7 as a predictive biomarker is still ambiguous and more study in this area is needed.

Several more AR variants have been discovered, including AR45 and AR23, which are unique since they retain the ligand binding domain unlike the majority of the AR variants.

3. Targeting AR variants

A large body of work supports a role for AR variants in the development of treatment resistance in prostate cancer. This has led to increased interest in finding ways to inhibit their activity or expression in order to improve treatment response in prostate cancer patients. To date, a number of potential mechanisms have been identified that show promise. However, many of these treatment strategies are not specific to AR variants. The majority of the identified compounds dual-target multiple forms of the receptor due to the homology between full length AR and its variants. Therefore, it becomes difficult to delineate if the effects observed are due to inhibition of the AR variant alone or if the effects rely on both AR and the variants being inhibited. Regardless, several of these compounds show promise for reducing prostate cancer growth (Fig. 1).

3.1. AR variant degradation

A variety of drugs have been identified and developed that inhibit AR variant signaling by promoting degradation of the receptor. One drug that is well known for its ability to enhance AR variant degradation is niclosamide. Niclosamide is an FDA-approved anti-helminthic drug used for the treatment of tapeworm infections. Liu et al. [42] created an AR-V7 expression cell system that was used to screen the Prestwick Chemical Library to identify previously unknown inhibitors of AR-V7. Upon identification of niclosamide as a likely candidate, they determined that niclosamide induced AR-V7 protein degradation and reduced recruitment of AR-V7 to promoter regions of target genes. This action reduced transcriptional activity and resensitized anti-androgen resistant cells to enzalutamide and abiraterone treatment. Niclosamide had significant anti-tumor activity in a number of AR variant expressing CRPC cell lines, such as enzalutamide resistant C4-2B cells (C4-2B MDVR) and CWR22rv1 cells. The same effect was observed in an
enzoatalutamide and abiraterone resistant CWR22Rv1 xenograft model. In this model, the combination of niclosamide with either anti-androgen produced enhanced tumor inhibition over either compound alone [42,43].

A number of clinical trials have been instigated to test its efficacy in humans because of the promising pre-clinical data with niclosamide. Two ongoing clinical trials are underway at the University of California, Davis, CA, USA. The first of these is a phase II study with a lead-in safety phase investigating abiraterone in combination with niclosamide (NCT02807805). Recurrent or metastatic CRPC patients will receive abiraterone 1,000 mg daily and prednisone 5 mg twice daily plus escalating doses of oral niclosamide [44]. Early results from this trial report that the combination is well tolerated and therapeutic plasma levels of niclosamide are achievable [45]. Improved efficacy was observed with the combination treatment over abiraterone alone: 2/6 patients achieved complete PSA response compared to 0/30 in the historical abiraterone treatment control. Another two patients had partial PSA responses (>50% decrease). One was prematurely removed from the study due to rising PSA and the other had a 17.1% decrease in PSA, but biopsy of the only enlarged lymph node showed all necrotic tissue [45]. Future endpoints from this trial will include exploratory analysis of AR-V7 [44]. Another clinical trial from the same group (NCT03123978) will investigate niclosamide in combination with enzoatalutamide. This phase I trial will include exploratory analysis of AR-V7 [44]. Another clinical trial from the same group (NCT03123978) will investigate niclosamide in combination with enzoatalutamide. This phase I trial will determine the best dose and any potential side effects of niclosamide when given with enzoatalutamide [46]. A third clinical trial (NCT02532114) from a separate group was also investigating enzoatalutamide in combination with niclosamide. This study was discontinued when they failed to observe PSA reduction from the five patients enrolled following niclosamide administration. Furthermore, plasma niclosamide levels in the patients did not consistently remain at the level demonstrated to inhibit CRPC in vivo [47,48].

Other drugs have targeted AR variants for degradation: Yamashita et al. [49] were able to reduce CWR22Rv1 xenograft tumor growth by the addition of ASC-J9, a drug that degrades AR-V7 and full length AR. More recently, ASC-J9 has been shown to suppress enzoatalutamide resistant prostate cancer cell growth in vitro and in vivo [50].

Ponnusamy et al. [51] identified several selective AR degraders (SARD). The authors created a library of small molecules using rational drug design based on molecular modeling of the AR LBD to identify the most potent SARDs. They selected two of these compounds to study further: UT-155 and UT-69. Both compounds where found to have lower inhibitory constants and inhibited R1881 induced full length AR transactivation with 6- to 10-fold higher potency compared to enzoatalutamide. UT-155 and UT-69 also reduced AR expression. Cycloheximide studies were used to determine that enhanced protein degradation was the likely cause of the decreased AR protein expression. Ponnusamy et al. [51] treated LNCaP cells with UT-155 in the presence of the proteasome inhibitor bortezomib to determine the mechanism of degradation. UT-155 significantly downregulated AR protein in LNCaP cells. In the presence of bortezomib, AR levels reverted to the level observed in R1881-treated cells. From these data, the authors concluded that it is likely that UT-155 downregulates AR through the proteasome pathway. To determine if their compounds also influenced AR splice variant expression, Ponnusamy et al. [51] utilized a number of cell lines that express AR variants, including 22Rv1 cells with endogenous AR-V7 expression, D567es cells, and LNCaP-95 cells. Treatment with UT-155 routinely leads to decreased expression of AR variants. UT-155 and cycloheximide both reduced the levels of AR variants and degradation was enhanced by the combination treatment. Functionally, UT-155 was more effective than enzoatalutamide in inhibiting enzoatalutamide resistant prostate cancer cell xenograft proliferation and growth [51].

A drug that showed early promise was galeterone. Galeterone was identified as a CYP17 inhibitor. Other studies demonstrated that galeterone acted as an AR antagonist to both full length and mutated AR and degraded the AR and its splice variants [52–54]. In vitro studies demonstrated that galeterone inhibited enzoatalutamide-resistant cells and blocked AR nuclear translocation and subsequent activation of androgen-dependent genes. However, a recent clinical trial with galeterone was ended prematurely when it was concluded that it was unlikely to reach its target goals possibly due to inactivation of galeterone metabolites through 5α-reductase activity [55–57].
3.2. Targeting the N-terminal domain and DNA binding domain of the AR

Since most AR variants retain the N-terminus, classes of drugs have been developed that target this region of the AR protein. Among these drugs are EPI-001 (EPI) and its derivatives. EPI covalently binds the N-terminal domain of AR and its variants and inhibits transcriptional activity. EPI has been demonstrated to inhibit prostate cancer cell growth in in vivo xenograft models [58, 59]. More recent studies using in vitro and in vivo models have demonstrated that EPI can inhibit the proliferation of enzalutamide resistant cells [60]. Recently, a phase 1/2 clinical trial (NCT02606123) investigating the use of EPI in men with metastatic CRPC who had progressed on enzalutamide or abiraterone was terminated early, at the end of phase 1, due to high pill burden [61]. Another class of drugs that target the N-terminus of the AR are niphatennones. Niphatennones were observed to inhibit trans-activation of AR and its variants, but they also promoted the formation of glutathione adducts and may not be useful for prostate cancer therapy [62].

As with the N-terminal domain, most AR variants also retain the DNA binding domain. VPC-14449 was identified as a small molecule capable of binding the DNA binding domain of the AR and its variants [63]. VPC-14449 reduced the ability of full length AR and AR variants to interact with chromatin, which reduced expression of full length AR and AR variant-specific target genes and enhanced the effectiveness of enzalutamide in vitro [63]. In vivo studies demonstrated that VPC-14449 reduced tumor volume and inhibited PSA production in aLNCaP xenograft model at a level similar to that of enzalutamide.

3.3. Inhibition of AR variant synthesis

Another way to reduce AR variant expression is to inhibit their synthesis. To date, a few mechanisms have been identified that achieve this.

Recent studies have determined that thailanstatins can significantly suppress the expression of AR-V7 mRNA and protein and, to a lesser extent, full-length AR expression. This was determined to be through an inhibition of AR-V7 genesplicing by altering the interaction between U2AF65 and SAP155. Furthermore, treatment of mice bearing 22rv1 xenografts with thailanstatins was shown to inhibit tumor growth and induce apoptosis and reduce proliferation [64].

In a study by Van Etten at al. [65], AR variant synthesis was blocked by obstructing a polyadenylation signal in AR intron 3 with morpholinooligos or by silencing polyadenylation specificity factor 1 (CPSF1). The authors turned their sights to alternative polyadenylation after determining that splice acceptor site recognition was not a primary mechanism of AR-V7 generation in their cell culture models. They generated a novel morpholino, CE3 poly A mask (pAM), to block polyadenylation at AR CE3. CE3-pAM transfected into cells decreased expression of AR-V7, AR-V9, AR-V1, and AR-V6 at the protein and mRNA level. This was concomitant with an increase in full length AR. CE3-pAM inhibited growth of AR variant expressing prostate cancer cells in the absence of androgen. Aberrant polyadenylation facilitated the production of AR variants which could be blocked with CE3-pAM. Van Etten et al. [65] investigated alterations in genes encoding the cleavage and polyadenylation specificity factor complex. They determined that CPSF1 accrued the most frequent alterations in prostate cancer tissues. In addition, they found that inhibition of CPSF1 reduced expression of AR-V7 and reduced growth of AR variant expressing prostate cancer cells in the absence of androgen.

Other studies have found that inhibiting AR variant expression by limiting its production can improve prostate cancer cell response to enzalutamide. Ferraldeschi et al. [66] found that inhibition of HSP90 with onalesib altered AR splicing and lowered the expression of AR-V7. Nadiminty et al. [5] determined that downregulation of the splice factor hnRNPA1 reduced AR-V7 expression and sensitized cells to enzalutamide treatment. A naturally occurring compound, quercetin, which is found in many fruits and vegetables, was capable of reducing the expression of hnRNPA1 and subsequently AR-V7 and full length AR [67]. When quercetin and enzalutamide were used as a co-treatment in enzalutamide-resistant cells, mRNA and protein levels of hnRNPA1, AR, and AR-V7 were reduced more than by either compound alone. Dual treatment with quercetin and enzalutamide re-sensitized enzalutamide resistant prostate cancer cells to treatment as observed by a reduction of cell growth and clonogenic ability in vitro and by a reduction of tumor size in vivo [67].

3.4. Targeting AR variant co-activators

Another way to impede AR variant activity is to target their co-activators. Studies have shown that a variety of co-activators can enhance AR variant signaling. Vav3 was demonstrated to directly interact with AR-V7 and enhanced transcriptional activity of AR-V7 and ARV567es. Vav3 was determined to mediate ligand-independent AR activity and regulate nuclear levels of AR-V7. Knockdown of Vav3 or AR-V7, but not full length AR, promoted death of prostate cancer cells [68]. Other studies on Vav3 demonstrated that disrupting the interaction between Vav3 and AR-V7 decreased CRPC cell proliferation and anchorage-independent growth, reduced migration, upregulated apoptosis, and caused morphological changes associated with a less aggressive prostate cancer phenotype [69].

The DBC1 protein was identified as an AR variant coactivator. Moon et al. [70] observed direct interaction between endogenous AR-V7 and DBC1 in RV1 cells. DBC1 knockdown caused reduced binding of AR-V7 at the CDH2, PMEPA1, and FKBP5 enhancers, reduced AR-V7 specific target gene expression, enhanced AR-V7 degradation, increased ubiquitination, and reduced tumor growth.

4. Conclusion

The role of AR variants in promoting drug resistance in prostate cancer, particularly to anti-androgens, is indisputable. Numerous studies have correlated higher expression of AR-V7 and other variants as prostate cancer cells
progress towards resistance. Targeting AR variants is a valuable approach for advanced prostate cancer and both preclinical and clinical data support continued investigation. A more in depth understanding of the functionality of AR variants must guide targeting strategies.

Authors contributions

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Conflicts of interest

All authors declare no conflict of interest.

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