Interplay between Cytoplasmic and Nuclear Androgen Receptor Splice Variants Mediates Castration Resistance

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Abstract
Androgen receptor splice variants (AR-V) are implicated in resistance of prostate cancer to androgen-directed therapies. When expressed alone in cells, some AR-Vs (e.g., AR-V7) localize primarily to the nucleus, whereas others (e.g., AR-V1, AR-V4, and AR-V6) localize mainly to the cytoplasm. Significantly, the latter are often coexpressed with the nucleus-predominant AR-Vs and the full-length AR (AR-FL). An important question to be addressed is whether the cytoplasmic-localized AR-Vs play a role in castration-resistant prostate cancer (CRPC) through interaction with the nucleus-predominant AR-Vs and AR-FL. Here, it is demonstrated that AR-V1, -V4, and -V6 can dimerize with both AR-V7 and AR-FL. Consequently, AR-V7 and androgen-bound AR-FL induced nuclear localization of AR-V1, -V4, and -V6, and these variants, in turn, mitigated the ability of the antiandrogen enzalutamide to inhibit androgen-induced AR-FL nuclear localization. Interestingly, the impact of nuclear localization of AR-V4 and -V6 on AR transactivation differs from that of AR-V1. Nuclear localization leads to an increased ability of AR-V4 and -V6 to transactivate both canonical AR targets and AR-V–specific targets and to confer castration-resistant cell growth. However, although AR-V1, which lacks inherent transcriptional activity, appears to activate AR-FL in an androgen-independent manner, it significantly antagonizes AR-V7 transactivation. Together, these data demonstrate that the complex interactions among different AR-Vs and AR-FL play a significant role in castration-resistant disease.

Implications: This study suggests important consequences for clinical castration resistance due to simultaneous expression of AR-FL and AR-Vs in patient tumors and suggests that dissecting these interactions should help develop effective strategies to disrupt AR-V signaling. Mol Cancer Res; 15(1); 59–68. ©2016 AACR.

Introduction
Prostate cancer is the second leading cause of cancer mortality in men in the United States (1). Androgen deprivation therapy (ADT), which disrupts androgen receptor (AR) signaling through androgen depletion or an antiandrogen, is the first-line treatment for advanced prostate cancer (2–4). ADT initially results in a favorable clinical response. However, prostate cancer invariably progresses to incurable castration-resistant prostate cancer (CRPC; refs. 2–4). Resurgent AR activity is increasingly recognized as a pivotal driver for castration-resistant progression (2–4). This led to the development of two next-generation agents of androgen-directed therapy, the androgen biosynthesis inhibitor abiraterone and the potent AR antagonist enzalutamide (5, 6). These agents prolong the survival of patients with metastatic CRPC, but both de novo and acquired resistance to the two drugs are common (7–10). One potential mechanism of AR reactivation after androgen-directed therapies, including abiraterone and enzalutamide, is the synthesis of C-terminally truncated AR variants (AR-V), through alternative RNA splicing (2, 4).

The full-length AR (AR-FL) is composed of an N-terminal domain, a central DNA-binding domain, a hinge region, and a C-terminal ligand-binding domain (11). Because of insertions of cryptic exons downstream of the DNA-binding domain, AR-V transcripts lack the reading frame for the ligand-binding domain (Supplementary Fig. S1; refs. 2, 4). Nonetheless, because the majority of the AR-Vs retain the DNA-binding domain and the N-terminal domain, which contains the most critical transactivation domain of the receptor (AF-1), many AR-Vs display constitutive activity (12–18). AR-V7 (aka AR3) and ARV567es (aka ARV12) are two major AR-Vs expressed in clinical specimens (14, 15, 17, 19–26). They activate target gene expression in a ligand-independent manner and promote castration-resistant growth of prostate cancer cells in vitro and in vivo (12, 14–18). Strikingly, patients with high levels of AR-V7 mRNA or nuclear AR-V7 protein or detectable expression of ARV567es mRNA in prostate tumors have a shorter survival than other CRPC patients (20, 23, 25). Moreover, the expression of AR-V7 in circulating...
tumor cells of patients with metastatic CRPC is associated with resistance to both abiraterone and enzalutamide (19). AR-V7 and AR-V1/2/3/2b, AR5, AR-V6, and AR-V9, in general, localize mainly to the cytoplasm (12, 16, 18), and AR8 localizes primarily to the plasma membrane (31). We previously reported that dimerization is essential for the nucleus-predominant AR-Vs, AR-V7, and AR-V6 when expressed alone, AR-V7 and AR-V6 localize predominantly to the nucleus (12, 15–17, 30), AR-V1 (aka AR4), AR-V4 (aka AR 1/2/3/2b, AR5), AR-V6, and AR-V9, in general, localize mainly to the cytoplasm (12, 16, 18), and AR8 localizes primarily to the plasma membrane (31). Importantly, all the AR-Vs have variable capability in nuclear localization. For example, when expressed alone, AR-V7 and AR-V6 localize predominantly to the nucleus (12, 15–17, 30), AR-V1 (aka AR4), AR-V4 (aka AR 1/2/3/2b, AR5), AR-V6, and AR-V9, in general, localize mainly to the cytoplasm (12, 16, 18), and AR8 localizes primarily to the plasma membrane (31). It is known that AR-Vs have variable capability in nuclear localization, and the dimerization between these AR-Vs and AR-FL induces AR-FL nuclear localization and activates AR-FL in an androgen-independent manner (30, 32). Compared with AR-V7 and AR-V6, the non-nucleus-predominant AR-Vs are much less characterized in terms of their functions and clinical relevance. A recent single-cell RNA-Seq analysis of 73 circulating tumor cells from 11 CRPC patients showed that 78% and 43% of these cells express AR-FL and at least one AR-V, respectively (22). Importantly, all the AR-V-expressing cells were also found to express AR-FL (22). In addition, the non-nucleus-predominant AR-Vs were found to be always coexpressed with one of the nucleus-predominant AR-Vs (22). Moreover, the frequencies of expression of the lesser known AR-Vs in clinical prostate cancer specimens, although lower than that of AR-V7, are quite high (24). For example, at least seven of these variants have an incidence of >8% (16.6% ± 3.2%) in the hormone-naïve prostate cancer samples in the TCGA dataset, and at least 12 have an incidence of >25% (55.8% ± 4.8%) in the metastatic CRPC samples in the SU2C cohort (24). Given their high frequencies of expression, the simultaneous expression of the different types of AR-Vs and AR-FL, as well as AR-V7 being the most abundantly expressed AR-V in patient tumors (24, 26), we set out to investigate the influences of AR-V7 and AR-FL on the subcellular localization and transcriptional activities of three non-nucleus-predominant AR-Vs, AR-V4, -V6, and -V1 and the impact of these interactions on castration resistance.

### Materials and Methods

#### Cell lines and reagents

LNCaP, PC-3, DU145, 22Rv1, and HEK-293T cells were obtained from the ATCC and cultured as described previously (33). All the cell lines were authenticated on April 1, 2015, by the method of short tandem repeat profiling at the Genetic DNA Laboratories. Enzalutamide was purchased from Selleck Chemicals. The following antibodies were used in Western blot analyses: anti-GAPDH (Millipore), anti-AR (N-20, Santa Cruz Biotechnology), anti-AR-V7 (Precision Antibody), and anti-FLAG (M2, Sigma).

#### Plasmid construction

The coding regions of AR-V1, -V4, and -V6 were PCR amplified from 22Rv1 cell cDNA and cloned into the pGEM-T EASY TA-cloning vector separately (Promega). Their expression constructs were generated by subcloning the respective coding region from the TA-plasmids into the pLVX-puro vector (Clontech). The FLAG-tagged AR-V constructs were generated by adding three tandem FLAG epitopes (DYKDDDDK-DYKDDDDK-DYKDDDDK) in front of the AR-V genes, and the NLS-AR-V6 construct was generated by adding the nuclear localization signal sequence (PKKKRKV) before the AR-V6 gene. Bioluminescence resonance energy transfer (BRET) fusion constructs of AR-V1, -V4, and -V6 were generated by subcloning the AR-V1, -V4, and -V6 cDNA from the respective TA-plasmids into the BamHI and XbaI sites of the pCMV3-ARpcDNA3.1-RLuc8.6 and TurboFP635 vectors (34). All plasmids were sequence verified. The sequences of the primers used for PCR cloning are listed in the Supplementary Table S1.

#### Reporter gene assay

LNCaP and PC-3 cells were transfected by using the Lipofectamine 3000 (Invitrogen) and TransIT-2020 (Mirus), respectively, and TurboFect (Thermo) was used for the transfection of DU145 and HEK-293T cells. Reporter gene assay was performed as described previously (35) with either androgen-responsive element luciferase plasmid (ARE-luc) containing three ARE regions ligated in tandem to the luciferase reporter or a luciferase

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**Figure 1.**

AR-V7 facilitates AR-V4 and AR-V6 nuclear localization. A-C, Confocal fluorescence microscopy of AR-V4 and AR-V6 subcellular localization when expressed alone (A) or when coexpressed with AR-V7 in PC-3 cells (B and C). Right (B and C), quantification of the percentage of nuclear AR-V4 or AR-V6 expression. The FLAG-tagged AR-V4 or AR-V6 expression construct was transfected with or without the AR-V7-FLAG plasmid into PC-3 cells under androgen-deprived conditions, and immunofluorescence (IF) staining with an anti-FLAG antibody was conducted at 48 hours after transfection. DAPI was used for nuclear staining. *P < 0.05 from the control group.
construct driven by three repeats of an AR-V–specific promoter element of the ubiquitin-conjugating enzyme E2C (UBE2C) gene (UBE2C-luc ref. 32). To ensure an even transfection efficiency, we conducted the transfection in bulk and then split the transfected cells for luciferase assay.

**Immunofluorescence staining and confocal fluorescence microscopy**

Cells were transfected with indicated plasmids on Poly-D-lysine–coated chambered coverglass (Thermo Scientific) and cultured in phenol red–free medium supplemented with 10% charcoal-stripped FBS. For immunofluorescence staining, at 48 hours after transfection, cells were fixed with 4% paraformaldehyde and incubated with a pan-AR antibody (N-20, Santa Cruz Biotechnology; 1:500) or a FLAG antibody (M2, Sigma; 1:1,000) overnight at 4°C. Nuclei were then stained with 4',6-diamidino-2-phenylindole (DAPI). A Nikon ECLIPSE Ti system with a 40× oil immersion objective was used for confocal imaging. An average of 6 fields with approximately 10 cells per field was captured for each group, and image analysis was carried out with the use of the NIH ImageJ Software. The intensities of the nucleus and the whole-cell fluorescence signals were quantitated for calculation of the percentage of nuclear localization.

**qRT-PCR and cell growth assay**

The qRT-PCR procedure was described previously (36), and the qPCR primer–probe sets were from IDT. The sulforhodamine (SRB) assay was used to determine cell growth (37). To ensure an even transfection efficiency, we conducted the transfection in bulk and then split the transfected cells for qRT-PCR and SRB assays.

**BRET assay**

The BRET assay was performed as we described previously (32) in cells that were either transfected with an RLuc BRET fusion plasmid or cotransfected with an RLuc and a TFP BRET fusion plasmid. The BRET ratio was calculated as follows: BRET ratio = (emission at 635 nm)/(emission at 528 nm) – (emission at 635 nm RLuc only)/(emission at 528 nm RLuc only).

**Statistical analysis**

The Student two-tailed t test was used to determine the mean differences between two groups. P < 0.05 is considered significant. Data are presented as mean ± SEM.

**Results**

**AR-V7 induces nuclear localization of AR-V4 and -V6**

We first studied the effect of AR-V7 on AR-V4 and -V6 subcellular localization. To this end, we expressed FLAG-tagged AR-V4 or -V6 with or without AR-V7-turbo-red-fluorescent-protein

![Figure 2](https://www.aacrjournals.org/doi/10.1158/1541-7786.MCR-16-0236)

**Figure 2.**

Androgen-bound AR-FL induces nuclear translocation of AR-V4 and -V6. A, Confocal fluorescence microscopy of AR-V4 and AR-V6 subcellular localization when coexpressed with AR-FL in PC-3 cells. The FLAG-tagged AR-V4 or -V6 expression construct was transfected with or without the AR-FL-GFP plasmid into PC-3 cells under androgen-deprived conditions. At 40 hours after transfection, the cells were treated with or without 1 nmol/L R1881 in the presence or absence of 10 pmol/L enzalutamide (Enz) for 6 hours. B, Subcellular localization of AR-V4 and AR-V6 in LNCaP cells. The FLAG-tagged AR-V4 or -V6 expression construct was transfected into LNCaP cells under androgen-deprived conditions. At 40 hours after transfection, the cells were treated with or without 1 nmol/L R1881 for 6 hours. IF staining with an anti-FLAG antibody was conducted for AR-V detection. Bottom, quantitation of the percentage of nuclear AR-FL, AR-V4, or AR-V6 expression. *P < 0.05 from the no-R1881 control. ∆P < 0.05.
(AR-V7-TFP) in the AR-null PC-3 cells. Consistent with a previous report (16), AR-V4 and -V6 were found to localize mainly in the cytoplasm (Fig. 1A) with detectable signal in the nucleus (Fig. 1B and C), whereas AR-V7 localized predominantly to the nucleus. Coexpression of AR-V7 with AR-V4 or -V6 led to increased nuclear AR-V4 and -V6 accumulation without affecting AR-V7 localization (Fig. 1B and C). This observation suggests that AR-V7 facilitates nuclear localization of AR-V4 and -V6.

**Androgen-bound AR-FL induces nuclear localization of AR-V4 and -V6**

We next investigated the impact of AR-FL on the subcellular localization of AR-V4 and -V6 by expressing FLAG-tagged AR-V4 or -V6 with AR-FL-green-fluorescent-protein (AR-FL-GFP) in PC-3 cells or alone in the AR-FL–expressing LNCaP cells. Coexpression with AR-FL did not affect the subcellular localization of either AR-FL or AR-V4/V6 in androgen-deprived conditions (Fig. 2A). The addition of androgen in AR-FL–expressing cells, however, not only resulted in increased nuclear localization of AR-FL but also of AR-V4 and -V6 (Fig. 2), and this effect was inhibited by the antiandrogen enzalutamide (Fig. 2A). Importantly, the androgen-dependent nuclear localization of AR-V4 and -V6 was not observed in the absence of AR-FL (data not shown), suggesting that the effect of androgen on AR-V4 and -V6 localization was mediated through AR-FL. Moreover, although AR-V4 and -V6 could not retain AR-FL in the cytoplasm when cells were treated with androgen, they rendered enzalutamide less effective in inhibiting androgen-induced AR-FL nuclear translocation (Fig. 2A). Taken together, the data indicate that androgen-bound AR-FL can induce nuclear localization of AR-V4 and -V6, and this effect, in turn, can mitigate the ability of antiandrogens to inhibit androgen-induced AR-FL nuclear localization.

**AR-V4 and -V6 dimerize with AR-V7 and AR-FL**

We next used the BRET assay to determine whether the above-mentioned observations were due to direct dimerization between AR-V4 or -V6 and AR-V7 or AR-FL. BRET is based on energy transfer from an energy donor to an energy acceptor when the donor and acceptor are brought into close proximity.

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**Figure 3.**

AR-V4 and AR-6 dimerize with AR-V7 and AR-FL and also homodimerize. A, Schematic diagram of the constructs used in the BRET assay. RLuc, RLuc8.6 luciferase; TFP, TurboFP635 fluorescent protein. B and C, Luciferase assay showing AR transactivating activity in HEK-293T cells cotransfected with the indicated BRET construct and the ARE-luc plasmid. Bottom, Western blotting confirmation of AR-V expression. D–I, BRET saturation curves showing AR-V4/AR-V7, AR-V6/AR-V7, AR-V4/AR-FL, and AR-V6/AR-FL heterodimerization as well as AR-V4 and AR-V6 homodimerization. Indicated BRET fusion constructs were cotransfected into HEK-293T cells at different ratios. Cells were cultured under androgen-deprived conditions unless specified. R1881, 1 nmol/L. *, P < 0.05 from mock control.
by their fused proteins. In the BRET6 system that we adopted in our study, the energy donor is the RLuc8.6 Renilla luciferase (RLuc) protein, and the energy acceptor is TFP (34). As BRET depends on the relative orientation of the fusion proteins, we generated all possible combinations of N- and C-terminal fusions through cloning the AR-V4, -V6, -V7, or -FL cDNA either in front of or after RLuc or TFP. Different pairs of the fusion protein constructs were transfected into the AR-null HEK-293T cells (to avoid confounding effect of endogenous AR), and the fusion constructs showing the highest BRET signals (Fig. 3A) were chosen for further analysis. The expression and transactivating abilities of the fusion proteins were validated by Western blotting and reporter gene analysis, respectively (Fig. 3B and C; ref. 32), indicating that the AR-Vs and AR-FL are functional in the BRET fusion protein context.

The BRET saturation curves for different combinations of the BRET fusion proteins in HEK-293T cells are shown in Fig. 3D–I. The BRET ratios increased hyperbolically and rapidly saturated with the increase in the ratio of the energy acceptor to the energy donor, indicating specific protein–protein interactions (38). Thus, both AR-V4 and -V6 can dimerize with AR-V7 and AR-FL. Interestingly, the dimerization between AR-V4 or -V6 and AR-FL is independent of androgen (Fig. 3F and G). In addition to heterodimerization with AR-V7 and AR-FL, AR-V4 and -V6 can also homodimerize (Fig. 3H and I).

**AR-V4 and -V6 nuclear localization promotes castration resistance**

To study the functional significance of AR-V4 and -V6 nuclear localization, we first analyzed the effect of androgen on the ability of AR-V4 and -V6 to transactivate the ubiquitin-conjugating enzyme E2C (UBE2C) promoter that we previously demonstrated to be specific for AR-V activation and not subjected to AR-FL modulation (32). As shown in Fig. 4A and B and Supplementary Fig. S2, both AR-V4 and -V6 displayed constitutive transactivating activity when expressed in LNCaP or C4-2 cells, and this activity was induced by androgen. On the other hand, androgen was not able to alter the transactivating activity of AR-V6 when AR-FL was not present (Supplementary Fig. S3), indicating that the increased

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**Figure 4.**

AR-V4/V6 nuclear translocation leads to induced AR transactivation and castration-resistant cell growth in LNCaP cells. A and B, Luciferase assay showing androgen inducing AR-V transactivation in LNCaP cells cotransfected with AR-V4 (A) or AR-V6 (B) and the UBE2C-luc construct. C, Confocal fluorescence microscopy of IF-stained AR-V6 or NLS-AR-V6 expressed in PC-3 cells. D and E, Luciferase assay showing the addition of NLS increasing AR-V transactivation. AR-V6 or NLS-AR-V6 were cotransfected with either the ARE-luc plasmid in PC-3 cells (D) or the UBE2C-luc construct (E) in LNCaP cells. F and G, qRT-PCR and SRB assays showing the addition of NLS increasing the ability of AR-V6 to induce target gene expression (F) and to promote castration-resistant growth of LNCaP cells (G). Western blotting confirmed AR-V expression. Cells were cultured under androgen-deprived conditions unless specified. DHT, 1 nmol/L for 24 hours. *, \( P < 0.05 \) from mock control; \( \Delta \), \( P < 0.05 \).
AR-V transactivation was induced by androgen-bound AR-FL. We then added a nuclear localization signal (NLS; ref. 39) to the 5’-end of AR-V6 (NLS-AR-V6) and found that this nucleus-localized AR-V6 (Fig. 4C) had improved the ability to transactivate both canonical and AR-V-specific targets (Fig. 4D–F and Supplementary Fig. S2B) and to promote castration-resistant growth of prostate cancer cells (Fig. 4G). In addition, consistent with the ability of NLS-AR-V6 to localize primarily to the nucleus constitutively, androgen was not able to further induce NLS-AR-V6 transactivation (Fig. 4E and Supplementary Fig. S2B). Taken together, the data indicate that AR-V4 and AR-V6 nuclear localization can induce AR transactivation and promote castration-resistant cell growth.

AR-V7 and androgen-bound AR-FL also induce AR-V1 nuclear localization.

We next characterized the interactions between AR-V7 or AR-FL and AR-V1. We specifically chose AR-V1 to study because it localizes to both the nucleus and the cytoplasm (Supplementary Fig. S4; refs. 12, 16, 18), has been shown to abrogate the ability of AR-V7 to confer castration-resistant cell growth (18), and has an intact D-box, which we showed previously to mediate AR-V/AR-V dimerization (32). Similar to AR-V4 and -V6, AR-V1 can heterodimerize with AR-V7 and AR-FL and can also homodimerize (Supplementary Fig. S5). Interestingly, although AR-V1 was able to dimerize with AR-FL in the absence of androgen, androgen enhanced their dimerization (Supplementary Fig. S5D). This was slightly different from AR-V4 or -V6 dimerization with AR-FL, which was not modulated by androgen (Fig. 3F and G). As a result of the dimerization, AR-V1 was piggybacked to the nucleus by AR-V7 and androgen-bound AR-FL (Fig. 5A–C), and the latter can be attenuated by enzalutamide (Fig. 5B). Notably, although AR-V7 localization was not affected by AR-V1 (Fig. 5A), AR-V1 was able to moderately inhibit androgen induction of AR-FL nuclear translocation and significantly mitigate enzalutamide activity (Fig. 5B). This again slightly differs from AR-V4 and -V6, which could not retain AR-FL in the cytoplasm when cells were treated with androgen (Fig. 2A).

AR-V1 enhances constitutive AR transactivation but attenuates AR-V7 transactivation

AR-V1 has been shown to be inactive in the AR-null PC-3 and DU145 cells but possesses high constitutive transcriptional activity in the AR-FL–expressing LNCaP cells (16, 18). Concordantly, we found that, compared with AR-V4 and -V6, AR-V1 showed minimal transactivating activity in AR-null cells (Fig. 6A and B). However, expression of AR-V1 in LNCaP cells led to a significant activation of a canonical AR-responsive reporter and the expression of a canonical AR target, PSA (Fig. 6C and D). Interestingly, unlike AR-V4 and -V6, AR-V1 failed to transactivate the AR-V-
specific UBE2C promoter or induce UBE2C expression even when AR-FL was present (Fig. 6E–G and Supplementary Fig. S6A), indicating that AR-V1 might not have inherent transcriptional activity and that the increased canonical AR transactivating activity was likely due to androgen-independent activation of AR-FL by AR-V1. In contrast, when coexpressed with AR-V7 in LNCaP or C4-2 cells, AR-V1 antagonized the ability of AR-V7 to transactivate the AR-V-specific UBE2C promoter (Fig. 6H and Supplementary Fig. S6B). Taken together, the data indicate the potential of AR-V1 to selectively activate canonical AR signaling while inhibiting AR-V-specific gene expression.

**Discussion**

The current study represents the first to characterize the complex interactions of AR-FL as well as AR-V7 with three non-nucleus–predominant AR-Vs and the implication of these interactions on castration resistance. A schematic model of these interactions is presented in Fig. 7. We showed that AR-V7 heterodimerizes with AR-V4, -V6, and -V1 and that the dimerization facilitates nuclear localization of AR-V4, -V6, and -V1. Moreover, these non-nucleus–predominant AR-Vs also dimerize with AR-FL, and the dimerization can occur in the absence of androgen.

Although their dimerizations do not affect the subcellular localization of either AR-FL or any of the three AR-Vs in androgen-deprived conditions, AR-V4, -V6, and -V1 can be piggybacked into the nucleus by androgen-bound AR-FL. Significantly, the interactions between AR-V4, -V6, or -V1 and AR-FL mitigate the ability of the antiandrogen enzalutamide to inhibit androgen-induced AR-FL nuclear localization.

Although all of these three AR-Vs can be piggybacked into the nucleus by AR-V7 and androgen-bound AR-FL, the impact of nuclear localization of AR-V4 and -V6 on AR transactivation differs significantly from that of AR-V1. Nuclear localization leads to an increased ability of AR-V4 and -V6 to transactivate both canonical AR targets and AR-V-specific targets and to confer castration-resistant cell growth. In contrast, AR-V1 lacks the ability to transactivate the AR-V-specific UBE2C promoter either in the presence or absence of AR-FL but is efficient in inducing a canonical AR-responsive reporter in androgen-deprived conditions when AR-FL is present. On the other hand, it antagonizes significantly the transactivating activity of AR-V7, indicating the potential of AR-V1 to selectively activate canonical AR signaling while inhibiting AR-V-specific gene expression.

Our finding of AR-V7 facilitating AR-V4 and -V6 nuclear localization and inducing their abilities to transactivate target genes...
and to confer castration-resistant cell growth is reminiscent of our previous report on its ability to activate AR-FL in an androgen-independent manner (30). AR-V7 is the most abundantly expressed AR-V in clinical specimens (24, 26), and its role in mediating castration resistance is strongly supported by accumulating clinical evidences (19, 20, 23, 25). Its potential to confer mediating castration resistance is strongly supported by accumulated expression of AR-V in clinical specimens (24, 26), and its role in independent manner (30). AR-V7 is the most abundantly previous report on its ability to activate AR-FL in an androgen- and to confer castration-resistant cell growth is reminiscent of our data on AR-V1 point to the existence of another scenario abrogate AR-V7 induction of castration-resistant cell growth (18), further investigations, especially in different model systems, are warranted.

Like AR-V7, AR-V1, -V4, and -V6 are all truncated after exon 3, and all contain the D-box, which mediates AR-V/AR-V and AR-V/AR-FL dimerization (Supplementary Fig. S1; ref. 32). They differ only in the amino acid sequence of the short C-terminal extension (Supplementary Fig. S1). However, they are distinctive from AR-V7 in subcellular localization and transcriptional activity, and they also differ among themselves in biological functions. AR-V4 and -V6 appear to resemble each other but are functionally distinct from AR-V1. Although all three AR-Vs can homodimerize, AR-V4 and -V6, but not AR-V1, possess inherent constitutive transcriptional activity. When interacting with AR-V7, AR-V4 and -V6 increase, but AR-V1 suppresses, AR-V transactivation. In addition, although all three AR-Vs can dimerize with AR-FL in the absence of androgen, androgen does not affect the dimerization between AR-V4/-V6 and AR-FL but enhances AR-V1 and AR-FL dimerization. This also differs significantly from AR-V7 in that androgen disrupts AR-V7 and AR-FL dimerization, that is, only unliganded AR-FL can dimerize with AR-V7 (30). Nonetheless, all four AR-Vs can mitigate the ability of the antiandrogen enzalutamide to inhibit androgen-induced AR-FL nuclear localization. Taken together, although differing only in the C-terminal peptide sequence, these truncated AR-Vs can have distinct biological properties. A detailed functional characterization of their unique C-terminal peptide sequences and the impact on their protein structures, nucleocytoplasmic trafficking, DNA-binding capacities and specificities, as well as interactions with molecular chaperones and coregulators would help explain these distinctions.
In summary, we demonstrated the complex interactions among different AR-Vs and AR-FL in mediating castration resistance. As different types of AR-Vs are often simultaneously expressed and also coexpressed with AR-FL in patient tumors, our findings underscore the important role of their interactions in clinical castration resistance. The clinical relevance of certain AR-Vs may be neglected if they are viewed in isolation. Careful annotation of the expression of different AR-Vs in prostate cancer patients after initiation of androgen-directed therapies and at relapse should help clarify the role of different AR-Vs in driving the progression of the disease and help develop effective therapeutic strategies to disrupt AR-V signaling.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: Y. Zhan, H. Zhang, O. Sartor, P. Lee, Y. Dong

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References


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