Androgen Receptor Splice Variants Dimerize to Transactivate Target Genes

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Abstract

Constitutively active androgen receptor splice variants (AR-V) lacking the ligand-binding domain have been implicated in the pathogenesis of castration-resistant prostate cancer and in mediating resistance to newer drugs that target the androgen axis. AR-V regulates expression of both canonical AR targets and a unique set of cancer-specific targets that are enriched for cell-cycle functions. However, little is known about how AR-V controls gene expression. Here, we report that two major AR-Vs, termed AR-V7 and ARv567es, not only homodimerize and heterodimerize with each other but also heterodimerize with full-length androgen receptor (AR-FL) in an androgen-independent manner. We found that heterodimerization of AR-V and AR-FL was mediated by N- and C-terminal interactions and by the DNA-binding domain of each molecule, whereas AR-V homodimerization was mediated only by DNA-binding domain interactions. Notably, AR-V dimerization was required to transactivate target genes and to confer castration-resistant cell growth. Our results clarify the mechanism by which AR-Vs mediate gene regulation and provide a pivotal pathway for rational drug design to disrupt AR-V signaling as a rational strategy for the effective treatment of advanced prostate cancer. Cancer Res; 75(17): 3663–71. ©2015 AACR.

Introduction

Recurrence with lethal castration-resistant prostate cancer (CRPC) after androgen deprivation therapy remains the major challenge in treatment of advanced prostate cancer (1, 2). Significant advances in our understanding of continued androgen receptor (AR) signaling in CRPC have led to the development and FDA approval of two next-generation androgen-directed therapies, including abiraterone and enzalutamide, by signaling through constitutively active alternative splicing variants of AR (AR-V; refs. 5–10). They localize primarily to the nucleus, androgen-independent manner. We found that heterodimerization of AR-V and AR-FL was mediated by N- and C-terminal interactions and by the DNA-binding domain of each molecule, whereas AR-V homodimerization was mediated only by DNA-binding domain interactions. Notably, AR-V dimerization was required to transactivate target genes and to confer castration-resistant cell growth. Our results clarify the mechanism by which AR-Vs mediate gene regulation and provide a pivotal pathway for rational drug design to disrupt AR-V signaling as a rational strategy for the effective treatment of advanced prostate cancer. Cancer Res; 75(17): 3663–71. ©2015 AACR.

To date, 15 AR-Vs have been identified (18). Structurally, AR-Vs have insertions of cryptic exons downstream of the exons encoding the DNA-binding domain (DBD) or deletions of the exons encoding the ligand-binding domain (LBD), resulting in a disrupted AR open reading frame and expression of LBD-truncated AR (6, 7, 9, 15, 19, 20). Because the N-terminal domain, which contains the most critical transactivation domain of the receptor (AF1), and the DBD remain intact in the majority of the AR-Vs, many AR-Vs display ligand-independent transactivation. AR-V7 (aka AR3) and ARv567es (aka AR-V12) are two major AR-Vs expressed in clinical specimens (7–10, 15, 17). They localize primarily to the nucleus, activate target gene expression in a ligand-independent manner, and promote castration-resistant growth of prostate cancer cells both in vitro and in vivo (7, 9, 15, 19–21). Strikingly, patients with high levels of expression of AR-V7 or detectable expression of ARv567es in prostate tumors have a shorter survival than other CRPC patients (8). Moreover, AR-V7 expression in circulating tumor cells of CRPC patients is associated with resistance to both abiraterone and enzalutamide (17). These findings indicate an association between AR-V expression and a more lethal form of prostate cancer, and also highlight the importance of AR-Vs in limiting the efficacy of androgen-directed therapies.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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AR-V7 and ARV567es can regulate the expression of both canonical AR targets and a unique set of targets enriched for cell-cycle function independent of the full-length AR (AR-FL; refs. 7, 10, 15). AR-V7 and ARV567es can also activate AR-FL in the absence of androgen by facilitating AR-FL nuclear localization and coregulate the expression of canonical AR targets (5). It has long been appreciated that dimerization is required for AR-FL to regulate target gene expression (22), but little is known about AR-V dimerization. Coimmunoprecipitation of endogenous ARV567es and AR-FL (15) and co-occupancy of the PSA promoter by AR-V7 and AR-FL (5) suggest that AR-Vs may form heterodimers with AR-FL. However, whether AR-Vs homodimerize or heterodimerize with each other and whether the dimerization is required for AR-Vs to regulate target genes and to confer castration-resistant cell growth are currently unknown.

Dimerization of AR-FL is mediated mainly through N/C-terminal interactions, via the FxxLF motif in the N-terminal domain and the coactivator groove in the LBD, and DBD/DBD interactions, via the dimerization box (D-box; ref. 22). Because the FxxLF motif and the D-box (Fig. 1A) are maintained in the majority of ARVs identified, we hypothesize that these AR-Vs can form heterodimers with each other as well as homodimers via DBD/DBD interactions and that they can also form heterodimers with AR-FL via DBD/DBD and N/C interactions. In the current study, we tested this hypothesis by using the bimolecular fluorescence complementation (BiFC) and bioluminescence resonance energy transfer (BRET) assays, which have complementary capabilities for characterizing protein–protein interactions in live cells. BiFC allows direct visualization of subcellular locations of the interactions (23), while BRET allows real-time detection of complex formation (24, 25).

**Materials and Methods**

**Cell lines and reagents**

LNCaP, PC-3, DU145, VCaP, and HEK-293T cells were obtained from the ATCC, and cultured as described (26). C4-2 was provided by Dr. Shahriar Koochekpour (Roswell Park Cancer Institute, Buffalo, NY). All the cell lines were authenticated on April 1, 2015 by the method of short tandem repeat profiling at the Genetica DNA Laboratories. Enzalutamide was purchased from Selleck Chemicals.

**Plasmid construction**

To generate different BiFC fusion constructs of AR-FL, AR-V7, and ARV567es, we PCR amplified the AR-FL, AR-V7, and ARV567es cDNAs from their respective expression construct, and cloned the PCR amplicons separately into a TA-cloning vector (Promega). Fusion constructs of AR-FL, ARV567es, and AR-V7 with either VN or VC were generated by subcloning the cDNAs from the TA plasmids into the Sall and Xhol sites of the pBiFC-VN155 and pBiFC-VC155 vectors. The mutant BiFC-AR-V and BiFC-AR-FL constructs with mutations at the FxxLF motif (F23,27A/L26A) and/or D-box (A596T/S597T) were generated by site-directed mutagenesis by using the QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene). BRET-fusion constructs of AR-FL, AR-V7, and ARV567es were generated by subcloning the AR-FL, AR-V7, and ARV567es cDNA from the respective TA plasmids into the BamH1 and XbaI sites of the pCDNA3.1-RLuc8.6 and TurboFP635 vectors (24). The doxycycline-inducible ARV567es lentiviral construct was generated by subcloning the ARV567es cDNA from its TA plasmid first into the pDONR221 vector (Invitrogen) and subsequently into the doxycycline-inducible pHAGE-Ind-EF1a-DEST-GH lentiviral construct.

**Figure 1.**

AR-FL and AR-Vs in BiFC fusion proteins are functional. A, schematic representation of AR-FL, AR-V7, and ARV567es protein structure. The DBD is composed of two zinc fingers, NTD, N-terminal domain; H, hinge region; U, unique C-terminal sequence. D-box and FxxLF motif mediate AR-FL dimerization. B, a schematic of the principle of the BiFC assay. VFP, Venus fluorescent protein. C, schematic diagram of the constructs used in the BiFC assay. D, luciferase assay showing AR transactivating activity in PC-3 cells cotransfected with the indicated BiFC construct and the ARE-tuc plasmid. *, P < 0.05 from mock control. E, immunofluorescent (IF) staining showing protein fusion does not change subcellular localization of AR-FL, AR-V7, or ARV567es. The indicated expression construct or BiFC fusion construct was transfected into PC-3 cells, and immunofluorescent staining was conducted at 48 hours after transfection. DAPI, nuclear stain. Scale bars, 10 μm. Cells were cultured under androgen-deprived condition unless specified. DHT, 1 nmol/L, for 24 hours.
AR-V7 and AR$^{V567es}$ heterodimerize with AR-FL through both N/C and DBD/DBD interactions. wt, wild-type; F-mut, FxxLF-motif mutant; D-mut, D-box mutant; FD-mut, FxxLF-motif and D-Box double mutant. Hoechst, nuclear stain. Scale bars, 10 μm. *P < 0.05. A and B, dimerization was detected by the BiFC assay in PC-3 cells under androgen-deprived condition. Right, quantitation of BiFC signals by flow cytometry. C and D, pretreatment with androgen attenuates the dimerization between AR-V7 and wt AR-FL (C) but not the dimerization between AR-V7 and F-mut AR-FL (D). PC-3 cells were treated with 1 nmol/L DHT with or without 10 μmol/L enzalutamide (Enz) right after transfection with the indicated BiFC constructs, and BiFC signal was assessed at 48 hours after transfection. Right, quantitation of BiFC signals by flow cytometry. E, Western blotting with a pan-AR antibody showing expression of the BiFC-fusion proteins. Individual fusion construct was transfected into PC-3 cells cultured under androgen-deprived condition unless specified. DHT, 1 nmol/L for 24 hours.

DNA transfection and reporter gene assay

PC-3 and HEK-293T cells were transfected by using the TransIT-2020 (Minus Bio LLC) and TurboFect reagents (Thermo Scientific), respectively, per instruction of the manufacturer. DU145, C4-2, and LNCaP cells were transfected by using the Lipofectamine 2000 and Plus reagent (Invitrogen) as described (27). Reporter gene assay was performed as previously described (28) with either an androgen-responsive element-luciferase plasmid (ARE-luc) containing three ARE regions ligated in tandem to the luciferase reporter or a luciferase construct driven by three repeats of an AR-V-specific promoter element of the ubiquitin-conjugating enzyme E2C (UBE2C) gene (UBE2C-luc). To ensure an even transfection efficiency, we conducted the transfection in bulk and then split the transfected cells for luciferase assay.

Immunofluorescence staining

Cells were transfected with indicated plasmids on Poly-l-lysine–coated coverslips (neuVitro) and cultured in phenol red–free medium supplemented with 10% charcoal-stripped FBS. For the dihydrotestosterone (DHT) groups, 1 nmol/L DHT was added at 24 hours after transfection. At 48 hours after transfection, cells were fixed with 70% ethanol, and incubated with Alexa Fluor 488–conjugated secondary antibody (Invitrogen) and observed by using a Leica TCS SP2 system with a 40× oil-immersion objective on a Z-stage.

BIFC analysis

Cells were cotransfected with different BIFC fusion constructs. At 48 hours after transfection, cells were incubated with Hoechst33342 (Invitrogen) and observed by using a fluorescence microscope (Olympus). For flow cytometry quantitation of BIFC signals, the pDsRed2-C1 construct (Clontech) was cotransfected with the BIFC fusion constructs. At 48 hours after transfection, cells were trypsinized, and the Venus and DsRed fluorescence were analyzed by flow cytometry.
Western blot analysis
The procedure was described previously (29). The anti-GAPDH (Millipore), anti-AR (N-20, Santa Cruz Biotechnology), anti-HSP70 (Abcam), anti-Turbo-red fluorescent protein (Abcam), and anti-Renilla-luciferase (Thermo Scientific) antibodies were used.

Quantitative RT-PCR and cell growth assay
Quantitative RT-PCR (qRT-PCR) was performed as described (30), and the qPCR primer probe sets were from IDT. Cell growth was determined by the sulforhodamine (SRB) assay as described (31). To ensure an even transduction efficiency, we conducted the transduction of the cells with packaged lentivirus in bulk, and then split the transduced cells for qRT-PCR and SRB assays.

BRET assay
Cells were either transfected with an RLuc BRET fusion plasmid or cotransfected with an RLuc and a TFP BRET fusion plasmid. At 72 hours after transfection, cells were detached with 5 mmol/L EDTA in PBS and resuspended in PBS with 1% sucrose. Cells were counted and seeded in triplicate into a 96-well white-wall microplate (BioTek). The BRET ratio was calculated by subtracting the ratio of 635-nm emission and 528-nm emission obtained from cells coexpressing the RLuc and TFP fusion proteins from the background BRET ratio resulting from cells expressing the RLuc fusion protein alone in the same experiment. 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 \( \pm \) SEM.

Results
Characterization of AR-FL and AR-Vs in BiFC fusion proteins
For BiFC analysis of interaction between proteins A and B, the two proteins are fused separately to either the N- or C-terminal fragment of the Venus fluorescent protein (VN or VC, Fig. 1B). If the two proteins dimerize, the interaction allows regeneration of the Venus fluorescent protein to emit fluorescent signal (23). Because BiFC depends on the relative orientation of the fusion proteins (23), we generated all possible combinations of N- and C-terminal fusions by cloning the AR-FL, AR-V7, or AR-V567es cDNAs in front of VN or VC. Different pairs of fusion protein constructs were transfected into the AR-null PC-3 cells (to avoid confounding effect of endogenous AR), and the fusion protein constructs exhibited the highest BiFC signals (Fig. 1C) were chosen for further analysis. The transactivating abilities of the fusion proteins were tested by the reporter gene assay. Although the protein fusion affected the relative activities of the fusion proteins (Figs. 1D and Supplementary Fig. S1), all the fusion proteins can transactivate target genes. Immunofluorescence assay further showed that the AR-FL and AR-Vs in the fusion proteins have the same subcellular localizations as the respective nonfusion AR isoform (Fig. 1E). Collectively, the data indicated that AR-FL and AR-Vs are functional in the fusion proteins.

BiFC detection of AR-V/AR-FL heterodimerization
To assess the ability of AR-V7 and AR-V567es to heterodimerize with AR-FL, we cotransfected the AR-V- and AR-FL BiFC fusion constructs into PC-3 cells and quantitated the Venus fluorescent signal by flow cytometry. Both AR-V7 and AR-V567es dimerized with AR-FL, and the dimerization did not require androgen (Fig. 2A and B). To delineate the dimerization interface, we generated mutant BiFC-AR-V constructs with mutations at the FxxLF motif (F23,27A/L26A) and/or D-box (A596T/S597T). FxxLF motif and D-box mutant AR-V constructs (Fig. 2A) were chosen for further analysis. The transactivating abilities of the fusion proteins were tested by the reporter gene assay. Although the protein fusion affected the relative activities of the fusion proteins (Figs. 1D and Supplementary Fig. S1), all the fusion proteins can transactivate target genes. Immunofluorescence assay further showed that the AR-FL and AR-Vs in the fusion proteins have the same subcellular localizations as the respective nonfusion AR isoform (Fig. 1E). Collectively, the data indicated that AR-FL and AR-Vs are functional in the fusion proteins.
Figure 4. AR-FL and AR-Vs in BRET fusion proteins are functional. A, a schematic of the principle of the BRET assay. B, schematic diagram of the constructs used in the BRET assay. RLuc, RLuc8.6 luciferase; TFP, TurboFP635 fluorescent protein. C, Western blotting with a pan-AR antibody showing expression of the BRET-fusion proteins. Individual fusion construct was transfected into HEK-293T cells cultured under androgen-deprived condition. D, luciferase assay showing AR trans-activating activity in HEK-293T cells cotransfected with the indicated BRET construct and the ARE-luc plasmid. Cells were cultured under androgen-deprived condition. DHT, 1 nmol/L for 24 hours. *P < 0.05 from mock control.

BiFC detection of AR-V/AR-V dimerization

We further showed that, like liganded AR-FL (Figs. 3A and Supplementary Fig. S4), both AR-Vs can form a homodimer when expressed alone (Figs. 3B and C and Supplementary Figs. S2C, S2D, S3C, and S3D). The homodimerization can also occur when AR-V is coexpressed with AR-FL and even when it is expressed at a much lower level than AR-FL (Supplementary Fig. S5). Moreover, AR-V7 and ARv567es can heterodimerize (Fig. 3D). Mutating D-box, but not the FxxLF motif, abolished AR-V/AR-V interactions, indicating that AR-Vs homodimerize and heterodimerize with each other through DBD/DBD interactions. Interestingly, similar to AR-V7/AR-V7 dimerization, AR-V7/AR-V7 dimerization was detected primarily in the nucleus (Figs. 3B and C and Supplementary Figs. S2 and S3). However, ARv567es dimerization were observed in both the nucleus and the cytoplasm (Fig. 3C and D and Supplementary Figs. S2D and S3D).

Characterization of AR-FL and AR-Vs in BRET fusion proteins

We then used the newest BRET system, BRET6 (24), to confirm the BiFC results. BRET6 is based on energy transfer between the RLuc8.6 Renilla luciferase (Rluc) energy donor and the turbo red fluorescent protein (TFP) energy acceptor when the donor and acceptor are brought into close proximity by their fused proteins (Fig. 4A). Similar to BiFC, BRET also depends on the relative orientation of the fusion proteins. We therefore generated all possible combinations of N- and C-terminal fusions by cloning the AR-FL, ARv567es, or AR-V7 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 protein constructs exhibiting the highest BRET signals (Fig. 4B) were chosen for further analysis. The expression of these fusion proteins was confirmed by Western blotting (Fig. 4C). Furthermore, their abilities to transactivate were validated by luciferase assay with the cotransfection of the ARE-luc plasmid (Fig. 4D), indicating that AR-FL and AR-Vs are functional in the BRET fusion proteins.

**BRET confirmation of AR-V/AR-FL and AR-V/AR-V dimerization**

Figure 5 shows the BRET saturation curves for different combinations of the BRET fusion proteins in HEK-293T cells. The BRET ratios increased hyperbolically and rapidly saturated with the increase in the ratio of energy acceptor to energy donor, indicating specific protein–protein interaction (33). Similar to the BiFC data, mutating the FxxLF-motif and/or the D-box inhibited AR-V/AR-FL and AR-V/AR-V dimerization (Supplementary Fig. S6). Thus, the BRET data confirmed the BiFC results, showing the ability of AR-Vs to heterodimerize with AR-FL and to homodimerize. AR-V7/AR-V7 interaction was further demonstrated by coimmunoprecipitation assay (Supplementary Fig. S7).

**Dimerization is required for AR-V action**

To assess the requirement of dimerization for AR-V action, we first performed reporter gene assay with the wild-type or the dimerization mutants of AR-V. As shown in Fig. 6A, the dimerization mutants completely lost the ability to transactivate, indicating a requirement of dimerization for AR-V transactivation. We then analyzed the ability of the wild-type and dimerization mutants of AR-Vs to regulate target gene expression and castration-resistant growth of prostate cancer cells. To this end, we infected the AR-FL–expressing LNCaP cells with lentivirus encoding AR-V7 or doxycycline-inducible ARv567es. Mutation of the FxxLF motif alone or both the FxxLF motif and D-box attenuated AR-V induction of androgen-independent expression of the canonical AR target PSA and the AR-V–specific target UBE2C (Fig. 6B) as well as castration-resistant cell growth (Fig. 6C). The data indicated the requirement of dimerization for AR-Vs to regulate target genes and to confer castration-resistant cell growth.

**Discussion**

The current study represents the first to show the dimeric nature of AR-Vs in live cells. Using BiFC and BRET assays, we showed that AR-V7 and AR-V567es not only homodimerize and heterodimerize with each other but also heterodimerize with AR-FL. The dimerization does not require androgen. By mutating the FxxLF motif in the N-terminal domain and/or D-box in DBD of AR-Vs, we further showed that AR-V/AR-FL dimerization is mediated by both N/C and DBD/DBD interactions, whereas AR-V/AR-V dimerization is through DBD/DBD interactions. Because AR-Vs lack the C-terminal domain, the N/C interactions between AR-V and AR-FL is mediated presumably via the FxxLF motif of AR-V and the C-terminal domain of AR-FL. Significantly, dimerization mutants of AR-Vs lose the ability to transactivate target genes and to confer...
Dimerization mutants of AR-Vs lose ability to transactivate and to promote castration-resistant cell growth. A, wild-type or dimerization mutant of AR-V was cotransfected with the ARE-luc plasmid, and cells were cultured under androgen-deprived condition. B and C, LNCaP cells were infected in bulk with lentivirus encoding wild-type or dimerization mutant of AR-V7 (left) or doxycycline-inducible wild-type or dimerization mutant of ARv567es (right). At 24 hours after infection, cells were reseeded and treated with or without 200 ng/mL doxycycline and incubated for an additional 48 hours for qRT-PCR analysis of target genes (B) or for the indicated time for SRB assay of cell growth (C). Western blotting confirmed AR-V expression.* P < 0.05 from control cells.

Figure 6.

The localization of ARv567es/AR-FL dimerization. Intriguingly, AR-V7/AR-FL dimerization was also detected primarily in the nucleus, whereas AR\textsuperscript{567ex}/AR\textsuperscript{567ex} and AR-V7/AR\textsuperscript{567ex} dimerization were observed in both the nucleus and the cytoplasm. Whether this is also due to slower regeneration of the Venus fluorescent protein than AR-V7/AR-V7 nuclear translocation or AR-V7 entering the nucleus as a monomer requires further investigation. In addition, the majority of the posttranslational modifications sites of AR-FL are retained in AR-Vs (34). These post-translational modifications regulate AR-FL transactivating activity, possibly via the interaction of AR-FL with other proteins or with itself (34). It is very likely that these posttranslational modifications may impact AR-V dimerization and transactivation and therefore deserve further investigation.

We reported previously that AR-V binds to the promoter of its specific target UBE2C without AR-FL, but co-occupies the promoter of the canonical AR target PSA with AR-FL in a mutually dependent manner (3). Furthermore, knockdown of AR-FL and AR-V both result in reduced androgen-independent PSA expression, but only AR-V knockdown downregulates UBE2C expression (3). The data, together with the findings from the current study, indicate that AR-Vs regulate their specific targets as an AR-V/AR-V dimer but control the expression of canonical AR targets as an AR-V/AR-FL dimer. Interestingly, while mutating D-box alone of PSA and UBE2C as well as to promote castration-resistant cell growth. A plausible explanation is that, although D-box–mutated AR-V can dimerize with AR-FL, the dimer cannot bind to DNA to regulate the expression of target genes. This, together with the finding that D-box–D-box interactions are required for the formation of androgen-induced AR-FL intermolecular N/C

Our finding on AR-V/AR-FL interaction is in accordance with the previous reports on AR\textsuperscript{567ex} and AR-FL coimmunoprecipitation (15) as well as on AR-V7 and AR-FL co-occupancy of the PSA promoter (5), providing a direct evidence for their dimerization. Interestingly, we found that the androgen-independent dimerization between AR-V and AR-FL may mitigate androgen induction of AR-FL homodimerization. This could constitute a mechanismic basis for the ability of AR-Vs to attenuate androgen transactivation. In addition, the majority of the posttranslational modifications sites of AR-FL are retained in AR-Vs (34). These post-translational modifications regulate AR-FL transactivating activity, possibly via the interaction of AR-FL with other proteins or with itself (34). It is very likely that these posttranslational modifications may impact AR-V dimerization and transactivation and therefore deserve further investigation.

We and others showed previously that AR-V7 and AR\textsuperscript{567es} localize constitutively to the nucleus and can facilitate AR-FL nuclear entry (5, 15), indicating that the initial interaction between AR-V and AR-FL is likely to be in the cytoplasm. This is supported by our data showing both cytoplasmic and nuclear localization of AR\textsuperscript{567es}/AR-FL dimerization. Intriguingly, AR-V7/AR-FL dimerization is detected primarily in the nucleus in the vast majority of the cells. This may be due to the regeneration of the Venus fluorescent protein from the VN and VC fragments being slower than AR-V7/AR-FL nuclear translocation. Interestingly, AR-V7/AR-V7 dimerization was also detected primarily in the nucleus, whereas AR\textsuperscript{567es}/AR\textsuperscript{567es} and AR-V7/AR\textsuperscript{567es} dimerization were observed in both the nucleus and the cytoplasm. Whether this is also due to slower regeneration of the Venus fluorescent protein than AR-V7/AR-V7 nuclear translocation or AR-V7 entering the nucleus as a monomer requires further investigation. In addition, the majority of the posttranslational modifications sites of AR-FL are retained in AR-Vs (34). These post-translational modifications regulate AR-FL transactivating activity, possibly via the interaction of AR-FL with other proteins or with itself (34). It is very likely that these posttranslational modifications may impact AR-V dimerization and transactivation and therefore deserve further investigation.

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interactions (32), indicates that disrupting D-box–D-box interactions could lead to inhibition of not only ARV/AR-V dimerization and transactivation but also AR-FL activation induced by either AR-Vs or androgens. Thus, disrupting D-box–D-box interactions may represent a more effective means to suppress AR signaling than targeting the LBD of AR.

In summary, we demonstrated the dimeric nature of AR-Vs in live cells and identified the dimerization interface. Significantly, we showed that proper dimerization is required for AR-V functions. The research therefore represents a key step in delineating the mechanism by which AR-Vs mediate gene regulation. This is vital for developing effective therapeutic strategies to disrupt AR-V signaling and provide more effective treatments for prostate cancer.

Disclosure of Potential Conflicts of Interest

O. Sartor is a consultant/advisory board member for Astellas, Janssen, and Medivation. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: D. Xu, Y. Zhan, Y. Qi, B. Cao, H. Zhang, Y. Dong

Development of methodology: D. Xu, Y. Zhan, Y. Qi, S.S. Gambhir, H. Zhang

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D. Xu, S. Bai

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Xu, Y. Qi, S. Bai, P. Lee, Y. Dong

Writing, review, and/or revision of the manuscript: D. Xu, Y. Zhan, B. Cao, W. Xu, S.S. Gambhir, P. Lee, O. Sartor, E.K. Flemington, H. Zhang, Y. Dong

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Xu

Study supervision: Y. Qi, W. Xu, Y. Dong

Other (provided BiFC plasmids and suggestions to conduct the BiFC experiments. Also, reviewed the manuscript with comments): C.-D. Hu

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