Priority Report

Cancer Research

Distinct Transcriptional Programs Mediated by the Ligand-Dependent Full-Length Androgen Receptor and Its Splice Variants in Castration-Resistant Prostate Cancer

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Abstract

Continued androgen receptor (AR) signaling is an established mechanism underlying castration-resistant prostate cancer (CRPC), and suppression of androgen receptor signaling remains a therapeutic goal of CRPC therapy. Constitutively active androgen receptor splice variants (AR-Vs) lack the androgen receptor ligand-binding domain (AR-LBD), the intended target of androgen deprivation therapies including CRPC therapies such as abiraterone and MDV3100. While the canonical full-length androgen receptor (AR-FL) and AR-Vs are both increased in CRPCs, their expression regulation, associated transcriptional programs, and functional relationships have not been dissected. In this study, we show that suppression of ligand-mediated AR-FL signaling by targeting AR-LBD leads to increased AR-V expression in two cell line models of CRPCs. Importantly, treatment-induced AR-Vs activated a distinct expression signature enriched for cell-cycle genes without requiring the presence of AR-FL. Conversely, activation of AR-FL signaling suppressed the AR-Vs signature and activated expression programs mainly associated with macromolecular synthesis, metabolism, and differentiation. In prostate cancer cells and CRPC xenografts treated with MDV3100 or abiraterone, increased expression of two constitutively active AR-Vs, AR-V7 and ARV567ES, but not AR-FL, paralleled increased expression of the androgen receptor–driven cell-cycle gene *UBE2C*. Expression of AR-V7, but not AR-FL, was positively correlated with UBE2C in clinical CRPC specimens. Together, our findings support an adaptive shift toward AR-V–mediated signaling in a subset of CRPC tumors as the AR-LBD is rendered inactive, suggesting an important mechanism contributing to drug resistance to CRPC therapy. *Cancer Res; 72(14); 3457–62. ©2012 AACR.*

Introduction

Androgen deprivation therapy (ADT) for advanced prostate cancer is designed to disrupt the androgen receptor (AR) pathway (1). The intended therapeutic target is the full-length androgen receptor (AR-FL), complete with an intact ligand-binding domain (LBD). Prostate tumors that progress despite first-line ADT (e.g., LHRH analogues), generally termed castration-resistant prostate cancer (CRPC), frequently show continued androgen receptor signaling driven by intratumoral androgens as well as elevated levels of AR-FL (2, 3). In support of the importance of ligand-driven AR-FL signaling in CRPCs, a number of clinically effective endocrine therapies targeting AR-LBD were recently developed to treat patients with CRPCs (e.g., abiraterone, MDV3100; refs. 4, 5). Nevertheless, the majority of patients progress shortly after treatment, again with reactivated androgen receptor signaling (4, 5).

Androgen receptor splice variants that lack the functional LBD (AR-Vs) were recently decoded and characterized (6–11), with some (e.g., AR-V7, ARV567ES; refs. 7, 9, 10) but not all (8) facilitating ligand-independent androgen receptor signaling in CRPCs. AR-Vs originate from androgen receptor transcripts with insertions of cryptic exons downstream of the coding sequences for androgen receptor DNA-binding domain (DBD; refs. 6, 9–11), or with deletions of exons coding for AR-LBD (7, 8). These alterations in androgen receptor transcripts disrupt the androgen receptor open reading frame, leading to truncated androgen receptor proteins with the intact N-terminal domain (NTD), AR-DBD, and a short variant-specific peptide replacing the functional AR-LBD. AR-Vs are capable of

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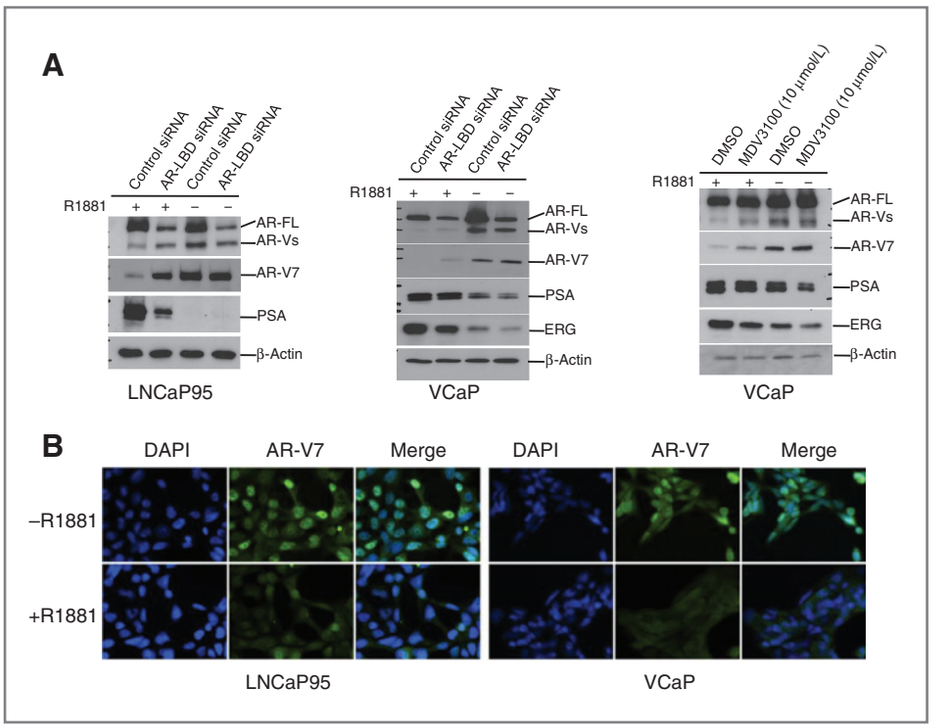

Figure 1. Regulation of AR-V expression by AR-FL signaling in LNCaP95 and VCaP cells. A, increased AR-Vs following suppression of AR-FL by ligand depletion (R1881–), siRNA targeting AR-LBD (AR-LBD siRNA), or MDV3100 (10 μmol/L). Protein levels of androgen receptor (by the N20 antibody that detects both AR-FL and AR-Vs), AR-V7, prostate-specific antigen (PSA), ERG, and β-actin were assessed by Western blotting. B, immunofluorescent images showing decreased or loss of AR-V7 nuclear staining in the presence of 1 nmol/L R1881. DAPI, 4′,6-diamidino-2-phenylindole.

activating canonical AR-FL–regulated genes (e.g., *KLK3*, *TMPRSS2*, *NKX3.1*) in the absence of AR-FL signaling (6–11), raising the possibility that AR-Vs and AR-FL may direct a similar transcriptional program and that elevated AR-Vs in CRPC may compensate for AR-FL signaling. However, previous studies also suggest that AR-Vs are not as potent as AR-FL in inducing the expression of AR-FL genes (9). Because AR-FL and AR-Vs are both overexpressed in CRPCs and AR-Vs are less abundant than AR-FL (6, 9), the role of ligand-independent androgen receptor signaling in the context of suppressed, yet still active, AR-FL signaling is not clear. In addition, regulation of expression of endogenous AR-Vs and their functional relationships with AR-FL signaling have not been discerned in the context of ADTs. In this study, we used a set of detection and targeting tools that differentiate the AR-FL and AR-Vs to investigate the functional interplay and distinctions between AR-FL and AR-V. The cumulative *in vitro* and *in vivo* evidence supports the importance of AR-V–mediated signaling in mediating responses to CRPC therapies targeting AR-LBD.

Materials and Methods

Cell line models and treatments

LNCaP, CWR22Rv1, and VCaP human prostate cancer cell lines were obtained from the American Type Culture Collection. Early-passage cells were used in all experiments. LNCaP95 is an androgen-independent cell line derived from the parental LNCaP cells (provided by Dr. Alan K. Meeker, Johns Hopkins University, Baltimore, MD). LNCaP95 cells and the parental LNCaP cells show qualitatively similar androgen receptor transcriptional responses (Supplementary Fig. S1). Previous studies have evaluated and established the expression status for both AR-FL and AR-V in these 4 cell lines (9). Stable clones of AR-FL–positive and -negative LNCaP cells were developed by E. Bluemn (Supplementary Methods). In all comparisons involving the treatment of cells with or without R1881 (NEN), cells were cultured for indicated time periods in culture media without phenol red (Invitrogen) supplemented with 10% charcoal-stripped FBS (CSS; Invitrogen) with or without 1 nmol/L R1881 (NEN). For siRNA treatment, cells reaching approximately 70% confluence were treated with siRNA for 24 hours and then cultured for 48 hours with or without 1 nmol/L R1881. Target sequences for specific knockdown of different androgen receptor molecules were described previously (9). MDV3100 was obtained from Medivation. VCaP and LNCaP95 cells were treated with MDV3100 dissolved in dimethyl sulfoxide (DMSO) at the indicated concentrations for 24 hours with or without 1 nmol/L R1881 (NEN). Transient transfection with AR-Vs was conducted as described previously (7–9).

Western blot analysis

Antibodies used in this study include anti-AR N20 (Santa Cruz Biotechnology); anti-AR-V7 and anti-PSA (Santa Cruz Biotechnology); anti-ERG (C-17, Santa Cruz Biotechnology); anti-UBE2C (Boston Biochem); and anti-β-actin (Sigma-Aldrich). The anti-AR-V7 antibody is a mouse monoclonal

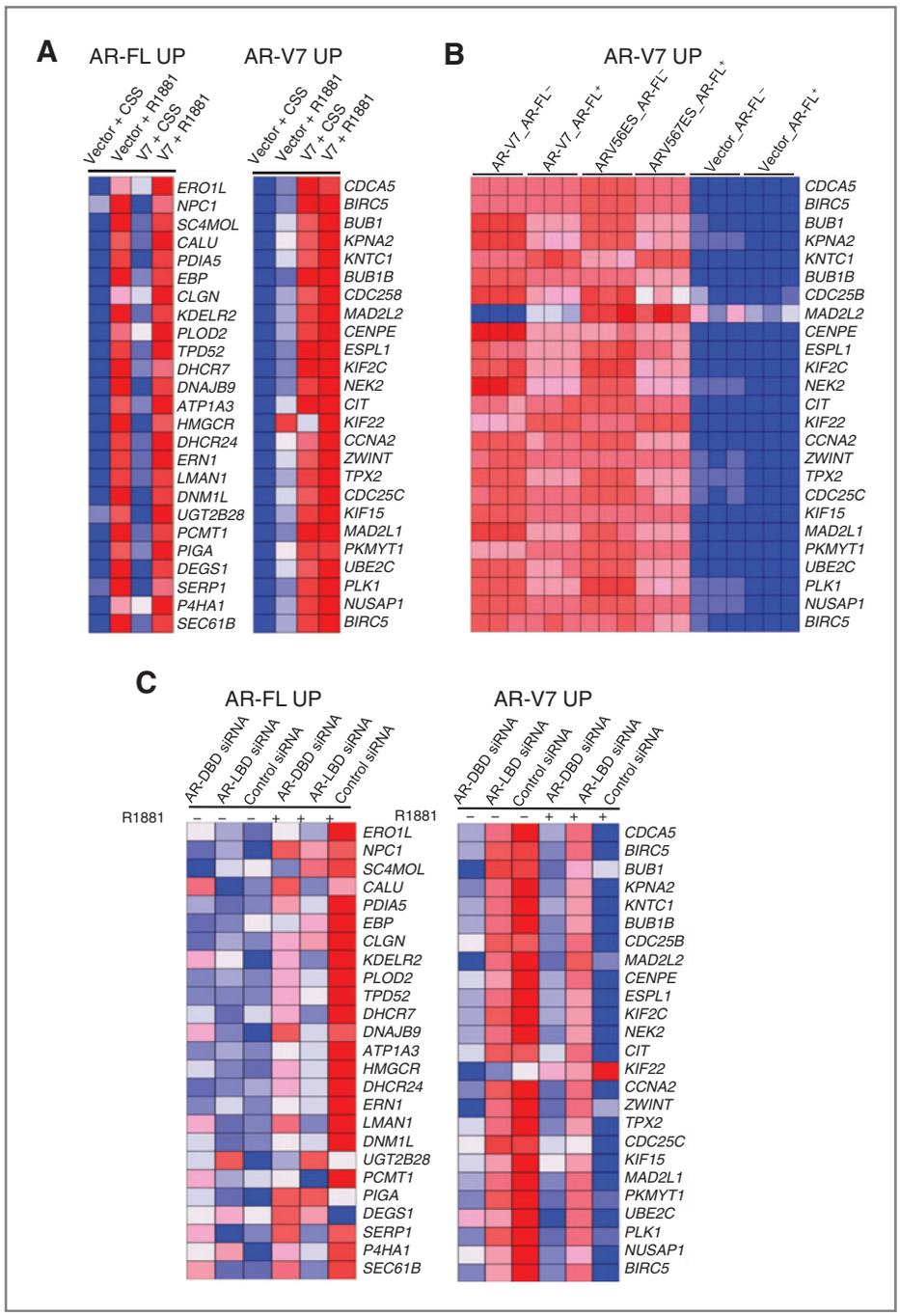

Figure 2. Distinctive expression patterns of gene sets representing the core transcriptional output of AR-V7 and AR-FL. A, expression of the AR-V7 UP and AR-FL UP gene sets in parental LNCaP cells transiently transfected with AR-V7 in the presence (R1881) or absence (CSS) of AR-FL signaling. B, expression of the AR-V7 UP gene set in stable clones of LNCaP cells with AR-FL^{+} or without AR-FL^{-} endogenous AR-FL following transient transfection with either AR-V7 or ARV567ES. Each experiment was repeated 3 times. C, expression profiles of the AR-FL UP and AR-V7 UP gene sets in LNCaP95 cells following suppression of AR-FL only (AR-LBD siRNA) or both AR-FL and AR-Vs (AR-DBD siRNA) in the presence or absence of 1 nmol/L R1881.

antibody developed using peptide sequences specific to AR-V7 (CKHLKMTRP; Supplementary Fig. S2).

Immunofluorescent staining

Cells were grown on chamber slides to approximately 80% confluence in culture media supplemented with 10% FBS. At 24 hours after the indicated treatments, cells were fixed for 10 minutes using freshly made 4% paraformaldehyde and then with 0.2% Triton X-100 in ×1 PBS for 5 minutes at room temperature. Fixed cells were incubated with the primary anti-AR-V7 antibody for 2 hours at room temperature. Secondary antibody was an Alexa Fluor 488–conjugated goat anti-mouse antibody (1:200 dilution; Invitrogen).

Gene set enrichment analysis

Expression data were generated using the Agilent Whole Genome Expression Arrays (Agilent Technologies) at Johns Hopkins University (by J. Luo) and the University of Washington (Seattle, WA; by P.S. Nelson), analyzed using GeneSpring GX 11.5 (Agilent Technologies), and subjected to Gene Set

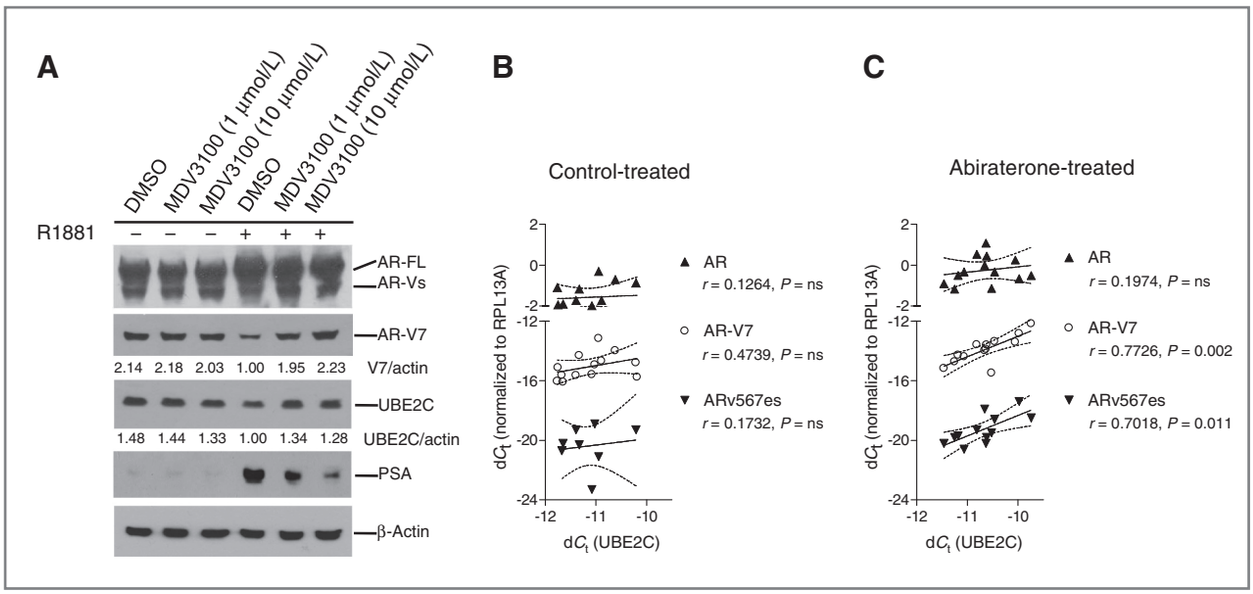

Figure 3. Increased expression of UBE2C parallels that of AR-Vs following treatment with MDV3100 and abiraterone. A, increased AR-V7 and UBE2C expression following MDV3100 treatment in the presence of 1 nmol/L R1881 in LNCaP95 cells. B, correlation of UBE2C and androgen receptor (AR-V7, ARV567ES, and AR-FL) expression in LuCaP35CR xenografts treated with control vehicles. C, correlation of UBE2C and androgen receptor (AR-V7, ARV567ES, and AR-FL) expression in LuCaP35CR xenografts treated with abiraterone. ns, not significant; PSA, prostate-specific antigen.

Enrichment Analysis (12). Other details are described in Supplementary Methods. Raw expression microarray data have been submitted to GEO (GSE36549).

CRPC xenografts

Abiraterone-treated xenograft tumor samples derived from the LuCaP35CR xenograft line was obtained from E.A. Mostaghel. Detailed methods for treatment, tumor collection, and mRNA analysis were fully described previously (13).

Tissue microarray analysis

CRPC tissues on the tissue microarray (TMA) were procured as previously described (14). Immunohistochemical staining for AR-V7, AR-FL, and UBE2C was optimized and conducted by J. Edwards. Deparaffinized TMA slides were placed in 10 mmol/L Tris, 1 mmol/L EDTA (pH 9.0) and steamed for 40 minutes for antigen retrieval. The primary antibodies were incubated with each slide for 1 hour at room temperature. The EnVision reagent (DAKO Corp.) was used for color development. Protein expression levels were scored by a semi-automated method as previously described (15). Scoring data were analyzed using SPSS (SPSS Inc.) and GraphPad (GraphPad Software, Inc.).

Results and Discussion

Regulation of AR-V expression by AR-FL

AR-FL and AR-V are both overexpressed in clinical CRPC specimens (9) and induced in castrate conditions in CRPC xenografts (6), suggesting that increased AR-V levels may be coupled with enhanced transcription of the androgen receptor gene and that AR-V function may require the presence of AR-FL (6). Because individual AR-V levels are typically lower than AR-FL (6, 8, 9), we used 2 cell lines, LNCaP95 and VCaP, that recapitulate the relative expression levels of AR-FL and AR-Vs in clinical CRPC specimens, that is, detectable but lower levels of AR-Vs than AR-FL (9). AR-FL signaling in the presence of R1881 was suppressed (Fig. 1A) by siRNA targeting the AR-LBD (AR-LBD siRNA), by ligand depletion (R1881–), or by MDV3100, a potent anti-androgen that targets the AR-LBD (4). We show that suppression of ligand-mediated AR-FL signaling by all 3 of these AR-LBD targeting strategies leads to an increase of the aggregate AR-V signal (AR-Vs; Fig. 1A). Increased AR-V protein expression following suppression of AR-LBD was confirmed by an increase in AR-V7, as shown by Western blot analysis (Fig. 1A) and immunofluorescent staining (Fig. 1B) using a variant-specific monoclonal antibody (Supplementary Fig. S2). We conclude that AR-V protein expression may not parallel expression of AR-FL following suppression of AR-FL signaling (Fig. 1A). Instead, AR-V protein levels are negatively regulated by ligand-mediated AR-FL signaling irrespective of the AR-FL protein levels, suggesting an adaptive shift toward AR-V–mediated signaling after therapy targeting AR-LBD.

Distinctive transcriptional programs mediated by AR-FL and AR-Vs

To dissect the transcriptional programs induced by AR-V–mediated signaling, we took 2 approaches: first, we examined transcriptional changes driven by forced expression of AR-Vs in the presence or absence of AR-FL signaling by Gene Set Enrichment Analysis (12). Transient expression of exogenous AR-V7 in parental LNCaP cells (Supplementary Fig. S3) induced expression of cell-cycle genes, under both androgen-depleted and -stimulated conditions (Supplementary Fig. S4). On the other hand, top gene sets increased by

ligand-dependent AR-FL are dominated by those related to biosynthesis, metabolism, and secretion (Supplementary Fig. S5). For illustration, we generated 2 gene sets, AR-V7 UP and AR-FL UP, each containing 25 probes that contributed to the core enrichment of top ranked gene sets driven by AR-V7 and AR-FL. The 2 representative gene sets show clearly independent expression patterns (Fig. 2A). Other canonical AR-FL–regulated genes (*KLK3*, *TMPRSS2*, *NKX3.1*) follow the same pattern as those in the AR-FL UP gene set (not shown). To further determine whether expression of the AR-V genes requires the presence of endogenous AR-FL, we generated stable LNCaP clones with or without endogenous AR-FL protein (Supplementary Methods), and transiently transfected these clones with AR-V7 or ARV567ES in androgen-deprived conditions (Supplementary Figs. S6 and S7). In this independent series of expression profiles (Fig. 2B), the AR-V7 UP gene set remains as the top-ranked gene set induced by ARV567ES or AR-V7 (not shown), and the absence of endogenous AR-FL did not attenuate induction of the AR-V7 UP signature (Fig. 2B; Supplementary Fig. S8). Thus, the presence of AR-FL is not required for induction of cell-cycle genes by constitutively active AR-V7 and AR-V567ES.

As a second approach to further corroborate the functional distinctions between AR-FL and AR-Vs, we analyzed gene expression correlates of endogenous AR-Vs following suppression of canonical AR-FL signaling only or suppression of both AR-FL and AR-Vs (Fig. 2C; Supplementary Figs. S9–S12). In LNCaP95 cells, the AR-V7 UP signature is again the top gene set enriched for upregulation following an increase of endogenous AR-V7 induced by AR-FL suppression (Supplementary Fig. S11). Knockdown of both AR-FL and AR-Vs abrogated expression of the AR-V7 UP gene set (Fig. 2C), confirming the essential role of AR-Vs. In contrast, the canonical AR-FL target genes showed a change of direction opposite to those driven by AR-V, with the AR-FL UP gene set as the top-ranked gene set enriched for downregulation following suppression of AR-FL (Supplementary Fig. S11). Increased expression of the AR-V7 UP gene set was further confirmed in VCaP cells following suppression of AR-FL signaling by either AR-LBD siRNA or MDV3100 (Supplementary Fig. S12).

Correlation between AR-V and UBE2C after treatment with MDV3100 and abiraterone

The AR-V7 UP gene set included UBE2C (Fig. 2). Previous studies established a direct regulation of UBE2C expression by androgen receptor in LNCaP-abl cells under androgen-deprived conditions (16). Our findings (Fig. 2) suggest that UBE2C expression in our CRPC models may be driven by AR-Vs but not by AR-FL. To validate the relationship between UBE2C and AR-V expression following suppression of AR-FL signaling, we first show that in LNCaP95 cells treated with MDV3100, the expression of UBE2C parallels that of AR-Vs but not AR-FL (Fig. 3A). Both AR-FL and constitutively active AR-Vs are elevated in the LuCaP35CR xenografts following treatment with abiraterone (13). We further show that in those abiraterone-treated xenografts, AR-Vs, but not AR-FL, are significantly correlated with UBE2C and other cell-cycle genes at mRNA levels (Fig. 3B and 3C; Supplementary Table SI). From these findings (Figs. 1–3), we reason that an adaptive shift toward AR-V–mediated signaling may contribute to resistance to MDV3100 and abiraterone.

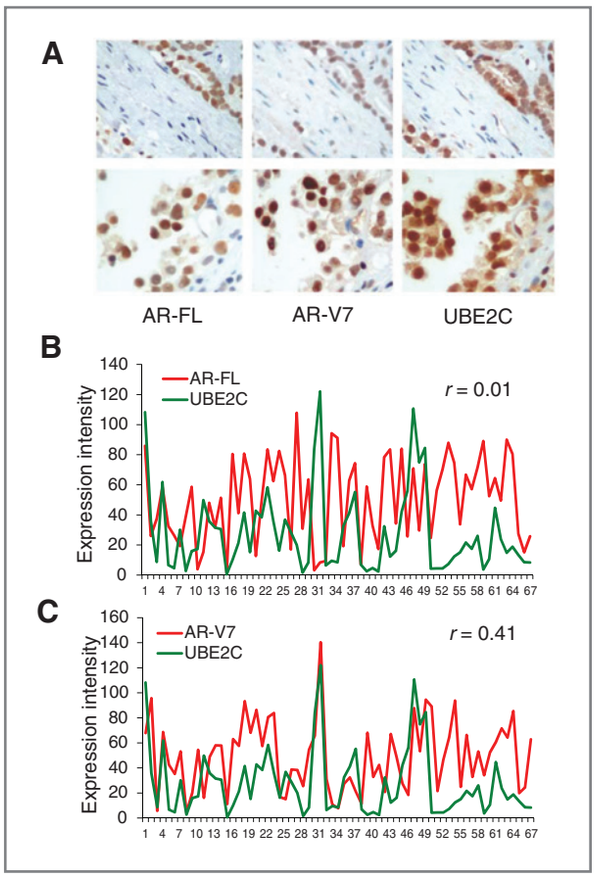

Figure 4. TMA analysis of correlation between androgen receptor (AR-FL and AR-V7) and UBE2C in castration-resistant prostate specimens ($n = 67$). A, representative high power TMA images showing nuclear staining for AR-FL, AR-V7, and UBE2C. B, no correlation between AR-FL and UBE2C ($r = 0.01$, $P > 0.05$) in 67 CRPC specimens. C, significant correlation between AR-V7 and UBE2C ($r = 0.41$, $P < 0.05$) in 67 CRPC specimens.

In vivo correlation between AR-V and UBE2C in CRPC

Suppression of AR-FL does not induce AR-V expression in the parental LNCaP and CWR22RV1 cells (Supplementary Fig. S13), in which high levels of AR-V7 may be caused by genomic alterations not frequently seen in clinical specimens (17). Consistent with the role of AR-V, we did not observe increased cell-cycle gene expression after suppression of AR-FL signaling in these 2 cell lines (not shown). Thus, cell-type–specific regulation of endogenous AR-V expression may explain why suppression of AR-FL inhibits tumor growth in some models of CRPC but not the others (6). Our results nevertheless suggest that AR-Vs, rather than AR-FL, may play a key role in supporting castration-resistant growth in at least a subset of CRPC. We investigated the relative importance of AR-FL and AR-V *in vivo* by immunohistochemistry analyzing the correlation between AR-FL and UBE2C, and between AR-V7 and UBE2C, in

transurethral resection of the prostate (TURP) specimens derived from patients who developed obstructive urinary symptoms after hormone therapy (Fig. 4A; Supplementary Fig. S14 and Supplementary Table SII). AR-V7, but not AR-FL, is significantly correlated with UBE2C (Fig. 4B and C), further supporting that AR-Vs, rather than AR-FL, mediate cell-cycle gene expression in at least a subset of CRPC specimens. This protein expression data are consistent with findings from an mRNA-based study showing elevated cell-cycle gene expression that are detected in bone metastasis express high levels of AR-Vs (18).

In summary, this study reveals the functional interplay between AR-FL and AR-Vs when AR-LBD is rendered inactive by ADTs. Importantly, the combined *in vitro* and *in vivo* data predict an adaptive shift toward AR-V–mediated signaling with effective CRPC therapies targeting AR-LBD. These studies indicate that therapeutic efficacy of agents targeting AR-LBD may be compromised as a consequence of this adaptive shift and that early detection of this shift may be used to guide treatment decisions. In addition, novel agents for CRPCs (19) may be designed to suppress the activation of transcriptional programs directed by the AR-Vs. Overall, given the recently expanded therapeutic options for metastatic CRPCs (20), the present findings may stimulate efforts to target adaptive AR-V signaling for treatment selection and to overcome resistance to CRPC therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R. Hu, C. Lu, P.S. Nelson, S.R. Plymate, J. Luo
Development of methodology: R. Hu, C. Lu, S.R. Plymate, J. Luo
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Hu, C. Lu, E.A. Mostaghel, J. Edwards, P.S. Nelson, E. Bluemn, S.R. Plymate, J. Luo
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Lu, E.A. Mostaghel, S. Yegnasubramanian, M. Gurel, J. Edwards, W.B. Isaacs, P.S. Nelson, S.R. Plymate, J. Luo
Writing, review, and/or revision of the manuscript: R. Hu, C. Lu, E.A. Mostaghel, E. Bluemn, S.R. Plymate, J. Luo
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Tannahill, S.R. Plymate, J. Luo
Study supervision: J. Luo

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References

1. Attard G, Cooper CS, de Bono JS. Steroid hormone receptors in prostate cancer: a hard habit to break? Cancer Cell 2009;16:458–62.
2. Mostaghel EA, Nelson PS. Intracrine androgen metabolism in prostate cancer progression: mechanisms of castration resistance and therapeutic implications. Best Pract Res Clin Endocrinol Metab 2008;22:243–58.
3. Cai C, Chen S, Ng P, Bubley GJ, Nelson PS, Mostaghel EA, et al. Intratumoral de novo steroid synthesis activates androgen receptor in castration-resistant prostate cancer and is upregulated by treatment with CYP17A1 inhibitors. Cancer Res 2011;71:6503–13.
4. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science 2009;324:787–90.
5. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995–2005.
6. Watson PA, Chen YF, Balbas MD, Wongvipat J, Socci ND, Viale A, et al. Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. Proc Natl Acad Sci U S A 2010;107:16759–65.
7. Sun S, Sprenger CC, Vessella RL, Haugk K, Soriano K, Mostaghel EA, et al. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. J Clin Invest 2010;120:2715–30.
8. Hu R, Isaacs WB, Luo J. A snapshot of the expression signature of androgen receptor splicing variants and their distinctive transcriptional activities. Prostate 2011;71:1656–67.
9. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res 2009;69:16–22.
10. Guo Z, Yang X, Sun F, Jiang R, Linn DE, Chen H, et al. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. Cancer Res 2009;69:2305–13.
11. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res 2008;68:5469–77.
12. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545–50.
13. Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. Clin Cancer Res 2011;17:5913–25.
14. Edwards J, Krishna NS, Grigor KM, Bartlett JM. Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. Br J Cancer 2003;89:552–6.
15. Wei S, Dunn TA, Isaacs WB, De Marzo AM, Luo J. GOLPH2 and MYO6: putative prostate cancer markers localized to the Golgi apparatus. Prostate 2008;68:1387–95.
16. Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, et al. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell 2009;138:245–56.
17. Li Y, Alsagabi M, Fan D, Bova GS, Tewfik AH, Dehm SM. Intragenic rearrangement and altered RNA splicing of the androgen receptor in a cell-based model of prostate cancer progression. Cancer Res 2011;71:2108–17.
18. Hörnberg E, Ylitalo EB, Crnalic S, Antti H, Stattin P, Widmark A, et al. Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival. PLoS One 2011;6:e19059.
19. Sadar MD. Small molecule inhibitors targeting the "Achilles' Heel" of androgen receptor activity. Cancer Res 2011;71:1208–13.
20. Antonarakis ES, Eisenberger MA. Expanding treatment options for metastatic prostate cancer. N Engl J Med 2011;364:2055–8.