The Role of Androgen Receptor Variant AR-V9 in Prostate Cancer

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ABSTRACT

The expression of androgen receptor (AR) variants is a frequent, yet poorly-understood mechanism of clinical resistance to AR-targeted therapy for castration-resistant prostate cancer (CRPC). Among the multiple AR variants expressed in CRPC, AR-V7 is considered the most clinically-relevant AR variant due to broad expression in CRPC, correlations of AR-V7 expression with clinical resistance, and growth inhibition when AR-V7 is knocked down in CRPC models. Therefore, efforts are under way to develop strategies for monitoring and inhibiting AR-V7 in castration-resistant prostate cancer (CRPC).

The aim of this study was to understand whether other AR variants are co-expressed with AR-V7 and promote resistance to AR-targeted therapies. To test this, we utilized RNA-seq to characterize AR expression in CRPC models. RNA-seq revealed the frequent coexpression of AR-V9 and AR-V7 in multiple CRPC models and metastases. Furthermore, long-read single-molecule real-time (SMRT) sequencing of AR isoforms revealed that AR-V7 and AR-V9 shared a common 3'terminal cryptic exon. To test this, we knocked down AR-V7 in prostate cancer cell lines and confirmed that AR-V9 mRNA and protein expression were also impacted. In reporter assays with AR-responsive promoters, AR-V9 functioned as a constitutive activator of androgen/AR signaling. Similarly, infection of AR-V9 lentiviral construct in LNCaP cells induced androgen-independent cell proliferation. In conclusion, these data implicate co-expression of AR-V9 with AR-V7 as an important component of constitutive AR signaling and therapeutic resistance in CRPC.

BACKGROUND

- Castration resistant prostate cancer (CRPC) develops when patients acquire resistance to androgen deprivation therapies (ADT).
- Expression of AR variants represents an important mechanism of the resistance to ADT.
- AR-V7 is considered the most clinically-relevant AR-V as the expression of AR-V7 in circulating tumor cells is associated with resistance to ADT in CRPC patients.
- It is not known whether other AR-Vs expressed in prostate cancer contribute to ADT resistance.

FIG 1. Frequent coexpression of AR-V9 and AR-V7 in CRPC models

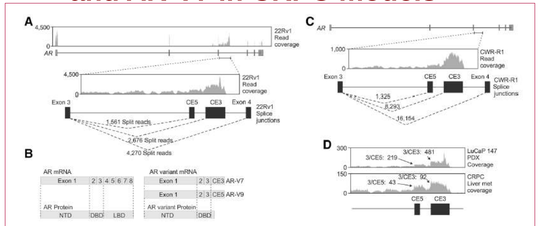

Fig 1. RNA-seq reveals frequent coexpression of AR-V9 and AR-V7 in prostate cancer. A and C. Illumina RNA-seq read coverage along discrete regions of the AR gene in 22Rv1 cell line (A) and CWR-R1 cell line (C). B. Schematic depicting the relationship between AR and AR-V mRNA species and modular domains in AR and AR-V proteins. D. RNA-seq read coverage within intron 3 of the AR gene in the LuCaP 147 PDX and a liver metastasis biospy.

FIG 2. AR-V7 and AR-V9 share a common 3'terminal cryptic exon

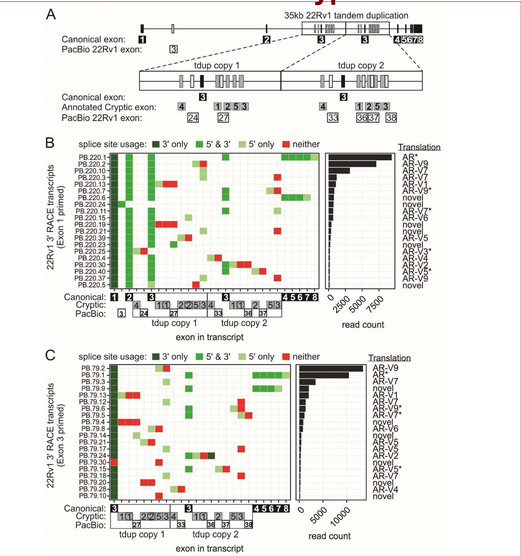

Fig 2. SMRT sequencing of AR isoforms in CRPC. A. Schematic of AR gene structure in 22Rv1 cells, which harbor a 35-kb AR intragenic tandem duplication. B. Exon composition and quantification of the 20 most abundant AR transcripts isolated from 22Rv1 cells by 3' rapid amplification of cDNA ends (RACE) using a forward primer anchored in AR exon 1. C. Exon composition and quantification of the 20 most abundant AR transcripts isolated from 22Rv1 cells by 3' RACE using a forward primer anchored in AR exon 3.

FIG 3. AR-V9 protein expression is inhibited by RNAi targeted to exon CE3

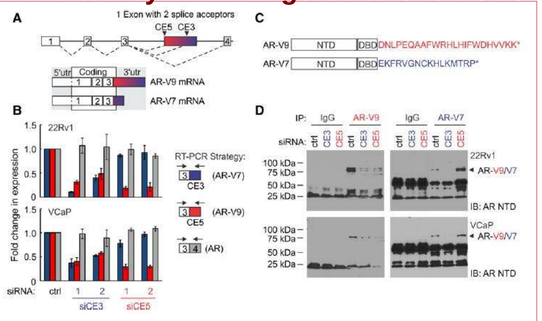

Fig 3. AR-V7 and AR-V9 contain overlap in 3'untranslated regions. A. Model for splicing of AR-V9 and AR-V7 derived from analysis of long-read sequence data. The model proposes that AR-V9 and AR-V7 originate from splicing of a common 3' terminal cryptic exon harboring two splice acceptor sites. B. siRNA targeting AR exon CE3 reduced mRNA expressions of both AR-V7 and AR-V9 in prostate cancer cell lines. In contrast, siRNA targeting AR exon CE5 reduced mRNA expression of AR-V9 but not AR-V7. C. Schematic of AR-V9 and AR-V7 proteins. D. siRNA targeting AR exon CE3 or CE5 reduced the protein expression level of AR-V9. In contrast, protein levels of AR-V7 was only reduced by siRNA targeting CE3.

FIG 4. AR-V9 is constitutively active and promotes androgen-independent growth

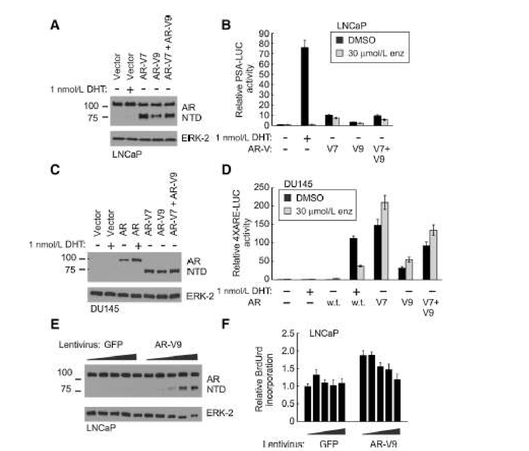

Fig 4. AR-V9 is constitutively active and promotes androgen-independent growth. A and B. AR-positive LNCaP cells were transfected with a PSA-driven luciferase reporter and luciferase assay was performed. C and D. AR-negive DU145 cells were transfected with an ARE-driven luciferase reporter. E and F. LNCaP cells were infected with GFP or AR-V9 lentivirus and assayed for proliferation by BrdU incorporation assay.

CONCLUSIONS

- AR-V9 was frequently coexpressed with AR-V7 in CRPC models.
- Both AR variant species were found to share a common 3' terminal cryptic exon.
- siRNA targeting AR exon CE3 reduced expression of both AR-V7 and AR-V9 mRNA and protein expression while siRNA targeting AR exon CE5 reduced expression of AR-V9 but not AR-V7.
- Similarly, siRNA targeting AR exon CE3 or CE5 reduced the protein expression level of AR-V9. In contrast, protein levels of AR-V7 were only reduced by siRNA targeting CE3.
- AR-V9 is constitutively active and promotes androgen-independent growth of CRPC cells.
- Co-expression of AR-V9 with AR-V7 may be an important component of constitutive AR signaling and therapeutic resistance in CRPC.

ACKNOWLEDGEMENTS

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