#1553

Spatial Profiling of Androgen Receptor Splice Variant 7 Transcriptional Activity in Prostate Cancer Metastases

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BACKGROUND

Androgen receptor splice variant 7 (AR-V7) is expressed in metastases from patients with castration resistant prostate cancer (CRPC) and shows a high level of inter- and intra-tumoral variability. However, the downstream activity generated by AR-V7 in tissue has not been shown. Whether AR-V7 is active in tissue or whether AR-V7 is a non-functioning biomarker with full-length AR has not been demonstrated.

METHODS

We constructed a tissue microarray (TMA) of 56 metastases from 27 patients with CRPC to analyze spatial gene expression using the GeoMx Digital Spatial Profiler. Immunostaining was performed to define epithelial, vascular, and stromal compartments. The stained tissues were then hybridized with barcoded tagged oligonucleotides targeting 2093 unique genes, which included those representing AR, AR-V cryptic exons, AR and neuroendocrine activity, and immune cell markers. One 500 mm region of interest (ROI) was assessed per tissue core (approximately 1200 cells). A sequential section from the same TMA was then stained with AR-V7 and AR-C-terminal specific antibodies. ROIs for RNA and protein were selected to be similar between slides. In addition to DSP, each metastasis was assessed by RNA-seq on the bulk tissue.

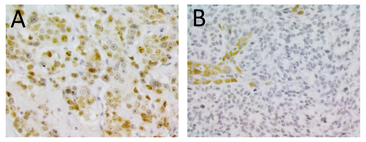
QUICK FACTS

> DSP counts and IHC AR-V7 correlate

> IHC and IF both demonstrate upregulation of AR transcriptome in AR-V7 expressing regions compared to regions with just AR-FL

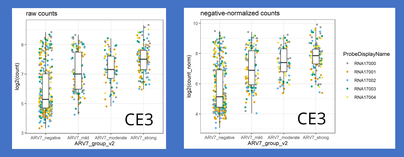
> AR sparse regions have upregulation of NE markers

AR-V7 IHC on Prostate Cancer Metastases

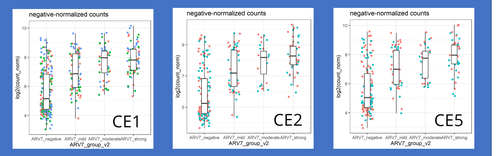


A. Cribiform pattern with stronger AR-V7 in peripheral pattern
B. AR-V7 staining in vasculature of metastasis

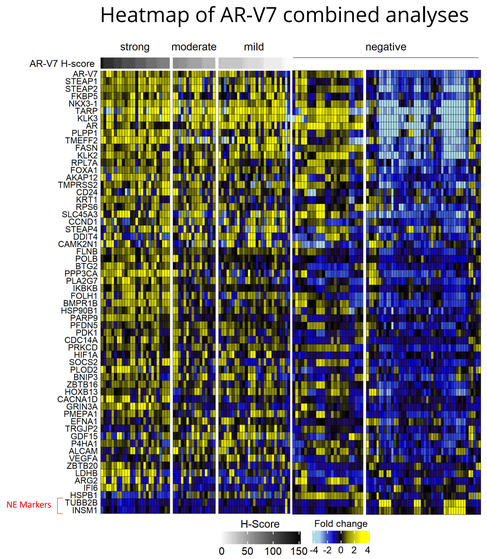
AR-Cryptic Exon 3 DSP counts correlate qualitatively with AR-V7 IHC



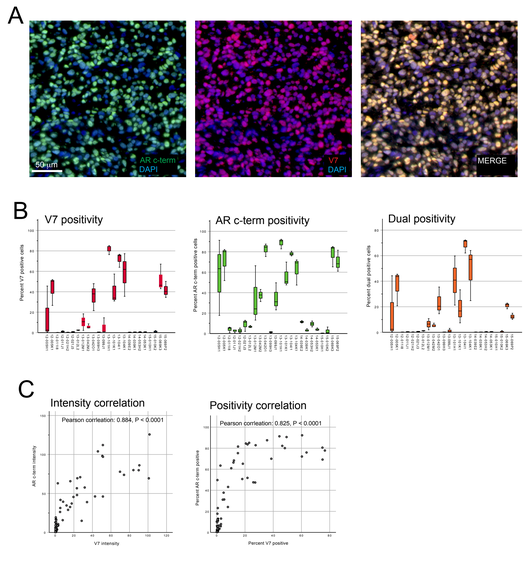
AR-V7 IHC status also correlates with DSP counts for Cryptic Exons 1, 2, 5



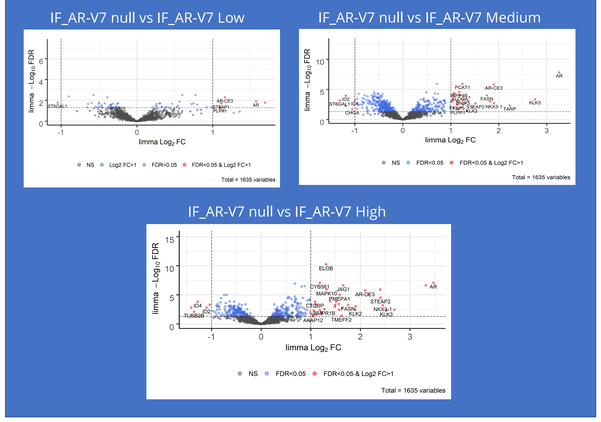
Heatmap of AR-V7 combined analyses



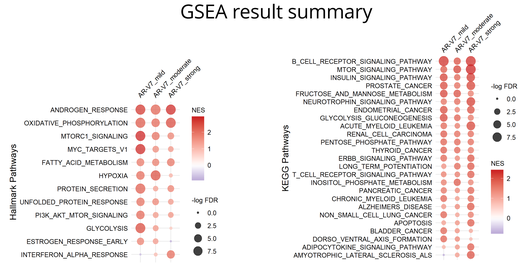
Dual IF staining of AR c-terminus and AR-V7



AR-V7 Immunofluorescence Volcano Plots



GSEA result summary



Hallmark & KEGG pathways with FDR<0.05 – in at least one comparison
All three comparisons to AR-V7_negative; ordered by mean NES

RESULTS

The most differentially expressed genes (FDR<0.05) based on association with AR-V7 staining were known downstream AR regulated genes including KLK2 and 3, FKBP5, NKX3.1, TMPRSS2, FASN, and TARP. Additionally, genes associated with proliferation and stemness, e.g., POLB, KRT1, SOX2, were significantly expressed. Since 93% of patients were on ADT at time of tissue collection and over 80% also had been treated with either abiraterone or enzalutamide, the increase in AR downstream genes would not be expected to occur from ligand activation of AR-FL. We also have previously shown that knock-down of AR-V7 in LNCaP95 cells results in loss of AR binding to AREs. In these metastases, then, activation of AR downstream genes would be a result of AR-V7 nuclear transport of AR-FL through AR-V7/AR-FL heterodimers or transcriptional activation by AR-V7 homodimers. Of further note, AR cryptic exons 1, 2, and 5 were also significantly expressed in AR-V7 positive ROIs ($p < 0.0001$). RNA-seq intron/exon junction reads were used to demonstrate that additional AR-Vs, most commonly AR-V9, were also expressed in tissues positive for AR-V7, suggesting that AR splicing is a common event in CRPC. Finally, expression of NE genes INSM1 and TUBB2 were not expressed in AR-V7 positive ROIs (negatively correlated, $p<0.001$), indicating that AR-V7 and NE phenotypes cannot co-exist in the same cell.

CONCLUSIONS

AR-V7 continues to drive prostate cancer through activation of the AR-cistrome. Its expression is heterogenous in metastases along with NE cells, suggesting that in the presence of AR-V7 and NE markers, therapy needs to be directed at both the N-terminus of AR and NE components.

ACKNOWLEDGEMENTS

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