High-resolution survey of the AR variant expression landscape in metastatic castration-resistant prostate cancer

Yezi Zhu[a], Pei Zhao[a], Angelo M. Demarzo [b], Emmanuel S. Antonarakis[c], Jun Luo[a]

[a] Department of Urology, The James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b] Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[c] Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Background: Detection of constitutively active AR splice variants (AR-Vs) in patients with metastatic castration-resistant prostate cancer (mCRPC) indicates emergence of therapeutic resistance to agents targeting the androgen receptor signaling pathway. Among the AR-Vs, androgen receptor variant-7 (AR-V7) is the most well-studied AR-Vs with prognostic values in the settings of resistance to novel hormonal therapies. The detection of other AR-Vs has also been reported. However, no systemic study of the overall burden of AR-Vs has been conducted due to lack of validated assays. We have previously developed a novel junction-specific RNA in situ hybridization (RISH) assay that enabled highly specific detection of cytoplasmic AR-FL and AR-V7 mRNA. In this study, we conducted a survey of the AR-V expression landscape using this novel method in clinical mCRPC specimens. **Methods**: We designed RISH probes targeting distinct splice junctions of total AR, AR-FL, AR-V7, AR-V9, AR-V3, AR^{v567es} and AR exon 3 duplication (as detected in CWR22Rv1 cells). All the probes were validated using prostate cancer cell lines with known AR-V profiles prior to testing in 23 metastatic biopsies collected from both castration-sensitive prostate cancer (CSPC, n=2) and mCRPC (n=21) patients. Quantitative RISH scores for each AR-V were analyzed. **Results**: All biopsies had positive (greater than zero) AR-FL RISH score (median 1.68, range 0.09-15.31). AR-V7 and AR-V9 are the most highly expressed AR-Vs analyzed. AR-V7 was detected in 15/23 (median 0.14, range 0.04-0.47) samples. AR-V9 was detected in 13/23 (median 0.12, range 0.02-0.43) samples, all of which also co-expressed AR-V7 and AR-FL. The AR exon 3 duplication, AR-V3, and AR^{v567es} were detected in 3/23, 6/23, and 4/23 samples, respectively, with all RISH scores below 0.10, indicating lower expression frequency and intensity compared to AR-V7 and AR-V9. The overall AR-V burden is mainly driven by AR-V7 and AR-V9. **Conclusions**: Using a specific and quantifiable RISH method, we confirm the relative importance of AR-V7 and AR-V9. The high-resolution AR-V expression landscape also confirms the co-existence of AR-FL and various AR-Vs, and lack of sub clones that may express a predominant AR-V mRNA other than AR-V7 and the often co-existing AR-V9. Given that AR-V7 and AR-V9 are the most abundant AR-Vs in CRPC biopsies, and that AR-V7 is associated with AR-V9 in most cases, it is unlikely that detection of AR-Vs other than AR-V7 would add additional value in clinical biomarker development. Further characterization of the clinical significance of additional AR-Vs derived from AR gene rearrangements may be evaluated with this novel method.