A role of increased transcription and high translation efficiency in upregulated androgen receptor splice variant expression in castration-resistant prostate cancer

Background

Upregulated expression of androgen receptor splice variants (AR-Vs), especially the most frequently expressed variant, AR-V7, is associated with resistance of prostate cancer to androgen deprivation therapy (ADT). At the RNA level, AR-V7 upregulation is generally coupled with increased expression of the full-length AR (AR-FL); consequently, AR-V7 mRNA and also the collective abundance of AR-V mRNAs constitute a minority of the AR population. However, Western blot analyses have shown that the relative abundance of the AR-V proteins is much higher in a sizable proportion of castration-resistant prostate cancers (CRPCs).

Methods

To address the mechanism underlying this discrepancy, we first analyzed RNA-seq data from ~350 CRPC samples for correlation between canonical and alternative AR splicing. We then knocked down AR-FL expression via shRNA or a PROTAC degrader and CRISPR-deleted the AR-binding site in the AR gene in preclinical models to determine the involvement of a negative autoregulatory circuit, in which androgen-bound AR-FL represses the transcription of the AR gene, in AR-V induction by ADT. We also measured the mRNA and protein stabilities of AR-FL and AR-Vs in response to ADT by mRNA and protein stability assays and pulse-chase analysis. Lastly, we examined the translation efficiency of AR-FL and AR-V mRNAs by nascent protein synthesis assays and polysome profiling analysis.

Results

Our analysis of clinical samples revealed a strong positive correlation between all canonical and alternative AR splicing, indicating that increased alternative splicing is not at the expense of canonical splicing. Instead, ADT disrupts the AR negative autoregulatory circuit to induce coordinated increase of AR-FL and AR-V mRNAs. At the protein level, however, ADT induces AR-FL degradation without affecting AR-V stability, and AR-V7 is translated at a faster rate than AR-FL irrespective of androgen levels.

Conclusions

Thus, ADT-induced AR-gene transcription and AR-FL protein decay, together with efficient AR-V7 translation, provide the first explanation for the discrepancy between the relative AR-V mRNA and protein abundances in many CRPCs. This mechanism highlights the inevitability of AR-V induction after ADT.