ANDROGEN RECEPTOR

How splicing confers treatment resistance in prostate cancer

A splice variant of the androgen receptor that drives prostate cancer resistance translocates into the nucleus using a different mechanism from the full-length receptor and exhibits distinct molecular properties once inside.

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Prostate cancer is the second leading cause of cancer-related deaths among men and claims over 350,000 lives worldwide each year. Most prostate cancers are driven by male hormones called androgens, which bind to and activate the androgen receptor (AR). Once an androgenic ligand, such as testosterone, binds to the androgen receptor, the receptor-ligand complex travels from the cytoplasm of the cell into its nucleus. Within the nucleus, the activated androgen receptor interacts with androgen-responsive elements in DNA to induce gene transcription.

While most prostate cancers initially shrink following treatment, resistance invariably emerges and the cancer relapses. This leads to the development of castration-resistant prostate cancer (CRPC), which can grow and spread to different parts of the body despite low levels of androgens. Multiple studies have shown that one of the most common molecular alterations seen in CRPC is the re-activation of signaling through the androgen receptor. This has led to the development of androgen receptor pathway inhibitors (ARPIs) that either block the part of the AR that binds to the ligand (thus preventing the receptor from becoming active) or interfere with the synthesis of androgens (Chen et al., 2004; Robinson et al., 2015; Visakorpi et al., 1995; Watson et al., 2015).

Unfortunately, many CRPCs develop resistance even to these drugs. It is thought that one of the mechanisms through which this resistance develops may be the production of splice variants of the androgen receptor. A splice variant is a version of a protein that results from ‘alternative splicing’ of the mRNA before it is translated into a protein. When an mRNA molecule is transcribed from the genome, it has introns, which are non-coding regions, and exons, which are coding regions. If this splicing process is modified, due to a mutation, this can lead to different

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regions being spliced in or out of the mRNA. Consequently, different versions of the protein, or splice variants, are translated. In the case of the androgen receptor, splice variants that preserve the DNA-binding domain of the protein but lack the ligand-binding domain allow the protein to continue driving androgen receptor signaling even under low-androgen conditions (Figure 1; Dehm et al., 2008; Watson et al., 2015; Westaby et al., 2022).

One important mechanism of resistance to ARPs is the production of different androgen receptor splice variants. The most well-studied, AR-V7, is a truncated receptor that results when exons four to eight of the full-length mRNA sequence are missing. AR-V7 lacks the ligand binding domain of the full-length version, allowing it to signal in the absence of an androgenic ligand (Figure 1). The truncated receptor is both a biomarker of CRPC and a possible contributor to ARPI resistance (Antonarakis et al., 2014; Armstrong et al., 2019; Luo et al., 2018; Sharp et al., 2019; Westaby et al., 2022).

Interestingly, AR-V7 also lacks the hinge domain that the full-length receptor requires to translocate from the cytoplasm to the nucleus (Figure 1; Thadani-Mulero et al., 2014), leading to the question of how AR-V7 moves into the nucleus and exerts its transcriptional activity. Now, in eLife, Paraskevi Giannakakou and colleagues from Weill Cornell Medical College – including Seaho Kim, CheukMan Cherie Au, and Mohd Azrin Bin Jamalruddin as joint first authors – report how AR-V7 enters the nucleus (Kim et al., 2022).

Using microscopy to image live cells over time, Kim et al. demonstrated that, similar to the full-length receptor, AR-V7 is imported into the nucleus relatively quickly. However, AR-V7 does not rely on microtubules or importin – two protein complexes involved in the nuclear transport of the full-length version. Additionally, Kim et al. implicate the zinc finger (D-box) domain of AR-V7 in the nuclear import of the truncated receptor (which is not the case for the full-length receptor).

Fluorescence recovery after photobleaching, combined with other advanced microscopy techniques, demonstrated that AR-V7 is constantly moving within the nucleus, and does not stay in contact with the same region of DNA for long periods of time. This contrasts with the full-length counterpart (and other nuclear hormone receptors), which stay on the same region of DNA for prolonged durations, and as such, exhibit comparatively less movement within the nucleus.

These findings point to a ‘hit-and-run’ transcription model for AR-V7, in which it transiently binds to DNA sequences and recruits secondary transcription factors that keep the target gene active, even after it unbinds. While hit-and-run transcription has been typically associated with proteins that repress transcription, it has been proposed that AR-V7 may have repressive activity.
in CRPC (Cato et al., 2019). Intriguingly, Kim et al. found that AR-V7 also promotes the nuclear translocation of full-length androgen receptor without its ligand, although the exact mechanism remains unclear. It is also unknown how this may impact AR signaling. This information may be clinically relevant, as it is common for patients with CRPC to co-express the full-length receptor and spliced variant AR-V7 (Watson et al., 2010).

Taken together, these findings shed light on important distinctions between spliced AR-V7 and full-length androgen receptor, although several open questions remain. Further studies will be needed to identify which proteins transport AR-V7 into the nucleus. Additionally, it will be important to determine the link between the hit-and-run activity of AR-V7 and the function of this spliced receptor. Finally, the extent to which the mechanisms elucidated by Kim et al. apply to other spliced variants of the androgen receptor merits further investigation. More broadly, a thorough understanding of how the full-length androgen receptor is mechanistically distinct from its splice variants may provide opportunities to selectively block the variants from signaling in advanced prostate cancer.

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Androgen Receptor | How splicing confers treatment resistance in prostate cancer

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