PROSTATE CANCER

AR targeting—it all makes sense

Antisense oligonucleotides (ASOs) targeting the full-length androgen receptor (AR-FL) and androgen receptor variants (ARVs) are a rational third-line approach for treating castration-resistant prostate cancer (CRPC) that is also resistant to androgen-receptor (AR)-pathway inhibitors, new research has shown. Yamamoto *et al.* designed three ASOs to target exon-1, intron-1 or exon-8 in androgen receptor pre-mRNA (termed AR-ASOs) to investigate the functional roles of AR-FL and ARVs in enzalutamide-resistant CRPC. These AR-ASOs selectively knock down AR-FL alone (exon-8 AR-ASO) or AR-FL and ARVs simultaneously (exon-1 and intron-1 AR-ASOs).

Investigations in parental and enzalutamide-resistant LNCaP cells revealed that exon-1 and intron-1 AR-ASOs decreased expression of both AR-FL and AR-V7—which is one of the most studied ARVs and is associated with cancer recurrence and poor survival. The exon-8 AR-ASO had no effect on AR-V7 expression but reduced expression of AR-FL. ARV and AR-FL expression was similarly affected in 22Rv1 cells, which have high levels of ARVs and low androgen receptor activity relative to LNCaP cells.

The differential knockdown of AR-FL and AR-V7 observed in prostate cancer cell lines was used to evaluate their relative contributions to the overall androgen-receptor-regulated transcriptome. The results showed that the expression of *PSA*, *FKBP5*, *TMPRSS2* and *NKX3.1* was suppressed by all AR-ASOs in enzalutamide-resistant LNCaP cells, indicating that AR-FL is the driver of androgen receptor transcriptional activity in this model. By contrast, in 22Rv-1 cells, exon-1 and intron-1 AR-ASOs suppressed AR-dependent gene expression to a greater degree than the exon-8 AR-ASO.

Exon-1 and exon-8 AR-ASOs were also used to evaluate the effect of AR-FL and AR-V7 knockdown on cell growth and apoptosis. In enzalutamide-resistant LNCaP cells, both AR-ASOs similarly inhibited cell growth and induced apoptosis; however, in 22Rv-1 cells, the exon-1 AR-ASO suppressed cell growth to a greater extent than the exon-8 AR-ASO. When AR-V7 was specifically silenced in both cell types (using small interfering RNA) differences in PSA response and apoptosis were observed. Knockdown of AR-V7 in enzalutamide-resistant LNCaP cells had no effect on PSA protein or mRNA expression and did not induce apoptosis. However, AR-V7 silencing in 22Rv-1 cells resulted in decreased PSA expression at both the mRNA and protein level and also inhibited cell growth and induced apoptosis.

These data, taken together, indicate that the biological consequences of ARVs are specific to cell type, and that the role of AR-FL and AR-V7 in cancer progression to enzalutamide resistance is context-dependent—AR-FL seems to be the driver in enzalutamide-resistant LNCaP cells, whereas in 22Rv-1 cells ARVs might have the dominant role.

The *in vivo* activity of both exon-1 and exon-8 AR-ASOs was analysed using enzalutamide-resistant LNCaP cells and patient-derived CRPC xenografts. Both AR-ASOs significantly reduced mean tumour volume from 2,653 mm³ to 1,168 mm³ for exon-1 and 994 mm³ for exon-8 ($P < 0.01$ and $P < 0.01$, respectively) in enzalutamide-resistant LNCaP cell xenografts. Significant PSA decline was also observed with both treatments ($P < 0.05$ and $P < 0.05$, respectively). As in the cell-line investigations, treatment with exon-1 reduced expression of AR-FL and AR-V7 mRNA, whereas exon-8 treatment only effected AR-FL mRNA expression, compared with controls. Both AR-ASOs also reduced mRNA expression of AR-dependent genes and induced apoptosis. In patient-derived CRPC xenografts, exon-1 AR-ASO treatment significantly reduced tumour volume (from 919 mm³ to 574 mm³) and PSA levels ($P < 0.01$ and $P < 0.01$, respectively) compared with controls.

These results suggest that the AR is a key driver in the progression of prostate cancer to an enzalutamide-resistant state. However, the relative roles of AR-FL and ARVs are context-dependent. Nevertheless, ASO strategies targeting AR-FL and ARVs could provide a rational third-line treatment for enzalutamide-resistant CRPC.

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Original article Yamamoto, Y. *et al.* Generation 2.5 antisense oligonucleotides targeting the androgen receptor and its splice variants suppress enzalutamide resistant prostate cancer cell growth.

Clin. Cancer Res. doi:10.1158/1078-0432.CCR-14-1108