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PROSTATE CANCER

ROR-γ drives androgen receptor expression

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Androgen receptor (AR) expression in metastatic castration-resistant prostate cancer (mCRPC) is driven by nuclear receptor ROR-γ, which could be a new therapeutic target in prostate cancer, according to new data published in *Nature Medicine*.

The AR is highly expressed in mCRPC tumours, whether or not the gene is amplified or mutated. Resistance to recently approved therapies such as enzalutamide and abiraterone is likely to occur via intratumoral androgen synthesis and AR splice variants (AR-Vs), such as AR-V7, meaning a need for new therapeutics exists.

Wang and colleagues reasoned that nuclear receptors are attractive therapeutic targets, and that receptors other than AR could have a vital role in prostate cancer progression. Interrogation of gene-expression data sets from benign prostate tissue, primary prostate cancer and mCRPC revealed that *RORC* (which encodes ROR-γ) expression was significantly increased in mCRPC. Immunohistochemical analysis also showed that >50% of prostate tumours overexpressed nuclear ROR-γ and high expression was associated with metastasis. Expression was also observed in AR-positive cell lines, but not in cells derived from nonmalignant prostate epithelium.

In vitro, knockdown of *RORC* using short-interfering RNA (siRNA) inhibited the growth of and induced apoptosis in androgen-sensitive LNCaP and VCaP cells and also and also androgen-insensitive 22Rv1 and C2-4B cells, but not in AR-negative PC-3 cells. Expression of full-length AR (AR-FL) and AR-Vs (including AR-V7) messenger RNA and protein was suppressed by ROR-γ knockdown.

The team then developed a ROR-γ inhibitor named XY018 that potently inhibited activity in HEK293T cells, and examined the effects of this compound, along with other ROR-γ antagonists, on prostate cancer cell lines. All the compounds tested inhibited cell growth to a greater extent than enzalutamide, reduced colony formation, and increased apoptosis. Gene-set enrichment analysis using the AR activity signature revealed that ROR-γ antagonists disrupted AR target gene programmes and inhibited the expression of genes that are preferentially upregulated by AR-V7. Dose-dependent inhibition of AR-FL and AR-V mRNA and protein expression was also observed, whereas ROR-γ overexpression increased AR expression in LNCaP cells. Treatment of C4-2B cells with the inhibitors SR2211 or XY018 reduced AR binding to its target loci and genome-wide abundance of histone 3 lysine 27 acetylation. Transcriptional activation-linked histone marks at the *KLK3* promoter and recruitment of RNA polymerase II to target promoters were also significantly reduced.

In vivo, ROR-γ antagonists inhibited the growth of xenografts generated from AR-positive tumour models, including those derived from enzalutamide-resistant 22Rv1 and VCaP cells, reduced the expression of AR and AR target genes and induced tumour-cell apoptosis. ROR-γ antagonists were well tolerated by the mice, with no overt toxicity observed.

Hongwu Chen, corresponding author on the paper, told *Nature Reviews Urology*, "The finding that ROR-γ acts truly upstream of AR means that targeting it will likely circumvent many of the resistance problems associated with agents that target AR protein signalling. Indeed, we found that enzalutamide-resistant tumours are still sensitive to ROR-γ antagonists, suggesting that targeting ROR-γ could benefit patients with mCRPC." He concluded, "One of the major and immediate efforts will be to translate these findings to the clinic. With the favourable profiles of the antagonists observed in preclinical models, we hope that the translation journey won't take too long."

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