Nature Reviews Cancer | AOP, published online 10 May 2013; doi:10.1038/nrc3532

THERAPEUTIC RESISTANCE

Two steps ahead

Simon Bradbrook/NPG

The androgen receptor (AR) antagonist enzalutamide was approved in 2012 by the US Food and Drug Administration for the treatment of castration-resistant prostate cancer (CRPC). Although promising clinical results have been observed with enzalutamide, it is expected that many tumours will develop resistance to this agent. Yang Shen, Charles Sawyers and colleagues have conducted a screen using a combination of *in vitro*, *in vivo* and *in silico* methods to prospectively identify resistance mutations in AR that are selected for during enzalutamide treatment.

Using a mixed population of wild-type LNCaP prostate cancer cells and LNCaP cells expressing a known resistance mutant (W741C) to another AR antagonist, bicalutamide, and sorting for cells that reactivated AR transcription (assessed by an AR-regulated enhanced green fluorescent protein (EGFP) reporter), the authors showed that the resistance mutant is selected for following several rounds of bicalutamide treatment. Following this proof-of-concept study, they carried out a screen for mutants of AR that could reactivate AR-dependent transcription following enzalutamide treatment using a randomly generated library of AR mutants.

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From this, they identified the AR mutant F876L, which was enriched in two of three replicates.

To validate the activity of AR F876L in the presence of enzalutamide, the authors expressed the mutant in LNCaP cells and found that it allows proliferation *in vitro* and xenograft tumour growth in mice during enzalutamide treatment. AR F876L nuclear localization and target gene expression were enhanced during enzalutamide treatment, suggesting that this mutation converts enzalutamide from an AR antagonist to an AR agonist, a mechanism of resistance that has previously been reported for other AR antagonists. Furthermore, they showed that the F876L mutation, as well as an F876I mutation, is spontaneously selected for in mice bearing tumours with wild-type AR following long-term enzalutamide treatment, highlighting the importance of this residue.

Using structural modelling to investigate the basis of AR F876L resistance to enzalutamide, the authors found that, in the presence of the drug, this mutation allows the repositioning of a helix in AR (helix 12) to an agonist-like conformation that permits coactivator recruitment. They then used this information to synthesize compounds that might restore the position of helix 12 into the antagonist conformation, and identified DR103, which was shown to inhibit the growth of prostate cancer cell lines expressing both wild-type and F876L-mutant AR and did not affect the growth of an AR-null cell line, suggesting that this or a similar inhibitor might be useful clinically.

Although the existence of an F876L AR mutation or other mutations affecting the positioning of helix 12 in patients with CRPC that are treated with enzalutamide awaits confirmation, the general strategy outlined here might be useful for prospectively identifying resistance mutants to other targeted inhibitors.

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ORIGINAL RESEARCH PAPER Balbas, M. D. *et al.* Overcoming mutation-based resistance to antiandrogens with rational drug design. *eLife* 9 Apr 2013 (doi:10.7554/eLife.00499)