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ANTICANCER DRUGS

Stapled peptide reactivates p53

The tumour suppressor protein p53 is commonly inactivated in human cancers, and restoring p53 activity is a major anticancer therapeutic strategy. One promising approach entails targeting the interaction of p53 with its major negative regulators — MDM2 and MDMX — which are overexpressed in some tumours. Now, writing in *PNAS*, Sawyer and colleagues demonstrate that the stapled α-helical peptide ATSP-7041 — which is a specific dual inhibitor of MDM2 and MDMX — effectively induces p53-dependent apoptosis and inhibits cell proliferation in mouse models of human tumours.

MDM2 and MDMX bind directly to p53, performing multifaceted, non-redundant roles in modulating p53 protein activity. Although their relative contributions to the regulation of p53 are not completely understood, evidence suggests that MDM2 and MDMX most effectively inhibit p53 when in a complex. There are several classes of small-molecule MDM2 antagonists currently under clinical investigation, but all are practically inactive against MDMX. Therefore, Sawyer and colleagues set out to identify a dual MDM2 and MDMX inhibitor.

The authors developed a stable hydrocarbon-stapled α-helical peptide therapeutic, based on the biologically active α-helical conformation found in the p53 protein. Although a stapled p53 α-helical peptide (SAH-p53-8) that exhibits high affinity towards MDM2 and MDMX has been developed previously, it was unsuitable for drug development.

First, they optimized stapled peptides to improve their biological and biophysical properties; this resulted in a potent lead, ATSP-7041, which bound with the same high-affinity conformation to both MDM2 and MDMX. Analysis of the crystal structure of ATSP-7041 bound to MDMX revealed that the peptide bound in the p53 binding site of MDMX, as expected, using three key positions — Phe19, Trp2 and Leu26 — with additional interactions between Tyr22 and the staple moiety itself.

In vitro, ATSP-7041 efficiently penetrated the cell membrane and substantially reduced levels of both MDM2 and MDMX bound to p53. The inhibitory effect of ATSP-7041 was prolonged for up to 48 hours, compared to just 4 hours for the investigational small-molecule MDM2 inhibitor RG7112. In osteosarcoma (SJSA-1) and breast cancer (MCF-7) human cell lines, which overproduce MDM2 or MDMX, respectively, ATSP-7041 dose-dependently increased p53 protein levels and elevated expression of the p53 transcriptional target p21. Importantly, these effects translated into reactivation of the cellular functions of p53, with ATSP-7041 causing effective cell-cycle arrest and the induction of apoptosis. Notably, in MCF-7 cells the effects of ATSP-7041 were more pronounced than those achieved with the small-molecule MDM2-specific inhibitor nutlin 3a, reflecting the added MDMX-inhibitory activity of the dual antagonist.

In vivo, intravenous administration of ATSP-7041 resulted in significant tumour growth inhibition (TGI) in human cancer xenograft models harbouring wild-type p53 and elevated levels of MDM2 or MDMX proteins, achieving TGI of 61% and up to 87% in the SJSA-1 and the MCF-7 models, respectively. By contrast, the selective MDM2 small-molecule inhibitor RG7112 inhibited tumour growth by up to 74% in the MCF-7 model. Importantly, ATSP-7041 exhibited favourable pharmacokinetic and tissue distribution properties in mice, rats and monkeys.

Together, these results demonstrate that stapled peptides may offer a new modality for p53-activating cancer therapy. A next-generation dual-targeting stapled peptide is scheduled to enter clinical trials in 2014 for the treatment of both solid and haematological cancers.

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ORIGINAL RESEARCH PAPER Chang, Y. S. *et al.* Stapled α-helical peptide drug development: a potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy. *Proc. Natl Acad. Sci. USA* **110**, E3445–E3454 (2013)