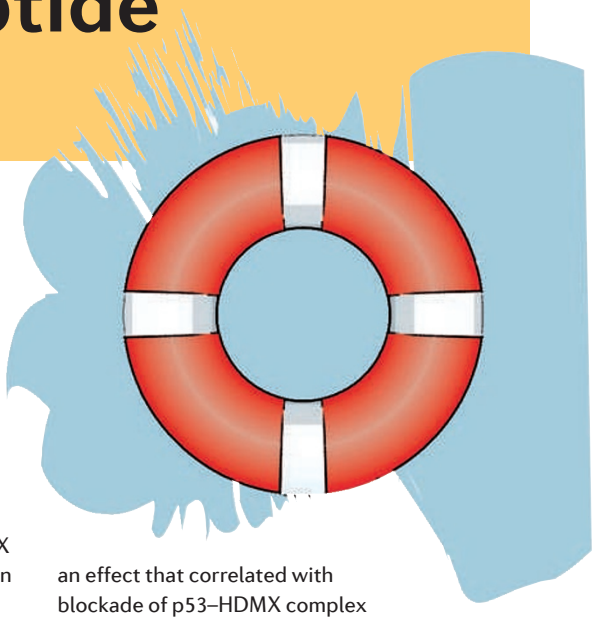
ANTICANCER DRUGS

Stapled peptide rescues p53



Loss of the tumour-suppressing activity of the p53 pathway occurs in many cancers, so targeting the negative regulators — HDM2 (also known as MDM2) and HDMX (also known as MDM4), which are overexpressed in some tumours — has been pursued as an anticancer strategy. Now, Walensky and colleagues explore the therapeutic potential of a 'stapled' p53-based peptide that prevents p53–HDMX binding, enabling activation of the p53 response and tumour growth suppression. A strategy for determining which cancers HDMX and/or HDM2 inhibition may be most effective in is also revealed.

Previously, peptides and small molecule HDM2 inhibitors (such as nutlin 3) have been developed that block p53–HDM2 binding, triggering cell cycle arrest and apoptosis in cancer models. However, overexpression of HDMX, which binds to and sequesters the HDM2 inhibitor-mediated elevated p53, renders some cancer cells resistant to this approach. Selectively targeting HDMX has therefore become an attractive alternative strategy. Here, Walensky and colleagues investigate the ability of peptides based on the transactivation domain of p53 to inhibit HDMX activity and exert tumour suppressing activity. These peptides are 'stapled' by installation of a hydrocarbon linkage to restore the α-helical structure, confer protease resistance and promote cellular uptake.

One such stabilized α-helix (SAH) of p53, SAH-p53-8, was found to have a 25-fold greater binding preference for HDMX over HDM2. This peptide demonstrated dose-dependent cytotoxicity in cancer cell lines overexpressing HDM2, HDMX or both proteins. Strikingly, this stapled peptide was most effective in cells expressing high HDMX and p53 levels, which were most resistant to nutlin 3. Mechanistic studies confirmed that SAH-p53-8 acted by blocking HDMX-mediated p53 sequestration, thereby restoring the p53 pathway.

Next, a series of synergy experiments in various cancer cell lines revealed in which cellular context HDMX and/or HDM2 inhibition would be most effective. In MCF7 cells that develop resistance to nutlin 3 owing to high HDMX levels, SAH-p53-8 restored nutlin 3 activity. Moreover, combining the two agents enhanced cytotoxicity, an effect that correlated with blockade of p53–HDMX complex formation. By contrast, in JEG3 cells, nutlin 3 did not synergize with SAH-p53-8 as endogenous p53 levels are already elevated in these cells. And in SJSA1 cells, which exhibit low HDMX and p53 expression levels but high HDM2 expression, SAH-p53-8 provided no added benefit over nutlin 3. However, enforcement of HDMX expression in SJSA1 cells engendered nutlin 3 resistance and restored the synergistic benefit of co-treatment.

Together, these *in vitro* studies indicate that targeting HDMX will be most effective in tumours in which p53 levels are endogenously or pharmacologically elevated. Moreover, the presence of the p53–HDMX complex may represent a potential biomarker to predict therapeutic efficacy.

Finally, to further demonstrate the therapeutic potential of SAH-p53-8, the authors investigated the *in vivo* actions of this stapled peptide, using a JEG3 mouse xenograft model — an HDMX-expressing and HDM2 inhibitor-resistant cancer. Four days of intravenous SAH-p53-8 treatment reduced tumour burden by 37–46% compared to control mice and nutlin 3 mice. Importantly, p53 pathway reactivation was confirmed and there were no signs of toxicity.

This study provides further confirmation that the p53 antagonists, HDM2 and HDMX, are viable anticancer targets. Clinical validation of this approach is needed.

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ORIGINAL RESEARCH PAPER Bernal, F. *et al.* A stapled p53 helix overcomes HDMX-mediated suppression of p53. *Cancer Cell* **18**, 411–422 (2010)