Although this approach cannot address issues such as the possible mutagenic effects of vector integration into a functional gene, it could apply to any properties governed by capsid structure and could help to overcome a variety of problems associated with gene delivery. A directed-evolution approach might lead to improvements in the specificity and efficiency of targeting particular cell types, which are influenced by interactions with cell surface receptors. Additionally, this strategy could help to reduce vector antigenicity. Furthermore, this approach can add to our understanding of the molecular basis of vector properties.

Katherine Whalley

ORIGINAL RESEARCH PAPER Maheshri, N. *et al.* Directed evolution of adeno-associated virus yields enhanced gene delivery vectors. *Nature Biotechnol.* doi:10.1038/nbt1182 (2006)
FURTHER READING Neylon, C. Chemical and biochemical strategies for the randomization of protein encoding DNA sequences: library construction methods for directed evolution. *Nucleic Acids Res.* **32**, 1448–1459 (2004).

PROTEIN–PROTEIN INTERACTIONS

Muscling in on p53

Modulating the activity of proteins by perturbing their interactions with coactivator and corepressor proteins is an attractive therapeutic approach, but effectively targeting the dynamic and complex interactions between proteins can be challenging (see Further Reading). Now, researchers in Ming-Ming Zhou's lab at Mount Sinai School of Medicine, New York, have developed a series of small-molecule inhibitors that prevent the association of p53 with its coactivator, the histone acetyltransferase p300/Creb-binding protein (CBP), and were able to downregulate the p53-mediated response to DNA damage.

DNA damage causes a sequence of regulatory events that culminates in lysine acetylation of p53, which enables it to bind and activate its target genes. Recent reports have suggested that rather than affecting the DNA-binding activity of p53 directly, lysine acetylation promotes the recruitment of p53 coactivators. Little is known, however, about the effects of individual lysine residues on p53 activity because lysine acetylation (AcK) sites are clustered together, making it difficult to study the effects of modification at specific sites. On the basis of previous knowledge that the p53 residue K382 is a binding site for the bromodomain (BRD) of p300/CBP, Zhou and colleagues set out to identify small-molecule binders of this domain that could disrupt the interaction between p300/CBP and p53 as a means to study the functional consequences of lysine acetylation at this site.

The authors first built a focused library of 200 drug-like compounds based on structural knowledge of BRD, and used NMR to screen for BRD-specific ligands. They identified 14 compounds, of which 13 showed selective binding for the BRD of p300/CBP compared with BRDs from other transcriptional proteins. Structural analysis revealed that the aromatic ring of almost all of the compounds bound to the hydrophobic AcK-binding site, and that most of the residues predicted to interact with the ligand were within the specific loops (ZA and BC) that form the hydrophobic pocket of the AcK-binding site.

The three-dimensional structure of the p300/CBP BRD bound to one of the compounds, MS7972, also showed a network of interactions between the ligand and CBP residues within the AcK-binding site, suggesting that MS7972 could block the interaction of the CBP BRD with its protein-binding partners.

The authors studied the compounds in a competition assay in which the BRD ligand competes with a biotinylated p53–AcK382 peptide for streptavidin-immobilized, glutathione-labelled CBP BRD. Incubation with 50–100 μM of the most potent compounds, MS7972 and MS2126, completely blocked the interaction between CBP BRD and p53–AcK382.

These two compounds were next evaluated in an assay of p53 transcriptional activation in human bone osteosarcoma epithelial cells. In this assay doxorubicin is added to cause DNA damage; the upregulation of p53 protein and associated p53-target genes such as p21 is then measured under different conditions. Treatment of resting cells with 200 μM of MS7926 or MS2126 before addition of doxorubicin dramatically decreased the doxorubicin-induced increase in cellular p53 and caused a decrease in p53-mediated p21 activation. Moreover, at different time points after addition of doxorubicin there was a loss of acetylation at K382.

Although further investigation is needed to confirm that these two compounds modulate p53 by blocking the interaction between CBP BRD and p53–AcK382, this study demonstrates a novel method for identifying small molecules that target protein–protein interactions that could be useful for analogous pathways involved in disease.

Joanna Owens

ORIGINAL RESEARCH PAPER Sachchidanand *et al.* Target structure-based discovery of small molecules that block human p53 and CREB binding protein association. *Chem. Biol.* **13**, 81–90 (2006)
FURTHER READING Arkin, M. R. & Wells, J. A. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. *Nature Rev. Drug Discov.* **3**, 301–317 (2004)
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