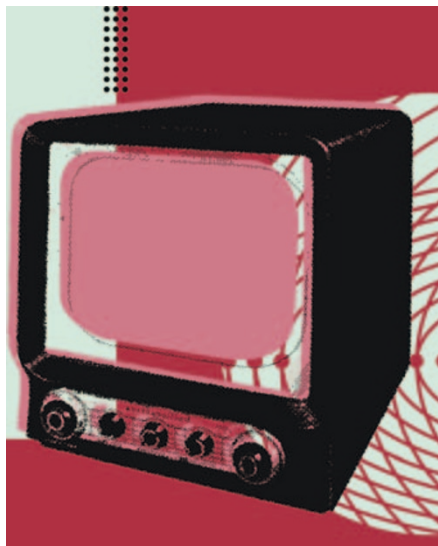


## CANCER GENETICS

## A screen success

Screening human cell lines for genes involved in cancer can be tricky — such genes are often recessive, and the mechanisms through which they are inactivated can be complex. RNAi screens provide a potentially powerful way to



identify genes that are involved in cancer by investigating the effects of reduced gene expression on specific cellular phenotypes. Now, two independent groups have verified the importance of RNAi-based screening in human cell lines by using this approach to identify novel tumour-suppressor candidates.

Both groups produced libraries of retroviral vectors that contained short hairpin RNAs (shRNAs); each construct was complementary to a single human gene, and 8,000 or so genes were represented in total. The researchers transduced these vectors *en masse* into engineered human cell lines that were poised for transformation, with the rationale being that knocking down a candidate tumour-suppressor would be enough to tip these cells over into the transformed phenotype.

Because most cancers arise from epithelial tissues, Steve Elledge and colleagues chose an immortalized mammary epithelial cell line for their screen. For normal growth *in vitro*, these cells need support from the extracellular matrix, but following transformation they become 'anchorage-independent' and can simply be screened for proliferative ability on soft agar. Elledge's group also took the opportunity to carry out the first usage test of 'genetic barcodes' in mammalian cells. By adding a unique 60-nucleotide barcode to each shRNA vector, they could successfully identify

the shRNA responsible for the knockdown using microarrays.

Reassuringly, several of the genes that Elledge and colleagues picked up are known tumour suppressors, such as transforming growth factor- $\beta$  receptor II (TGFBR2) and the phosphatase and tensin homologue (PTEN). They also found a new candidate tumour-suppressor gene — RE1-silencing transcription factor (*REST*). Like *TGFBR2*, *REST* resides in a locus that often suffers loss of heterozygosity, a frequent chromosomal characteristic of human tumour suppressors.

*REST* is often downregulated in prostate and small-cell lung cancers, and is frequently deleted in colorectal tumours. The authors present evidence that *REST* downregulation augments phosphatidylinositol-3-kinase signalling, an important pathway in cancer progression. Furthermore, the fact that *REST* is responsible for repressing neuronal genes in non-neuronal tissues provides another clue to its potential function in cancer. Several human tumours aberrantly express neuron-specific genes, and the authors reasoned that this might have an important role in cancer, with *REST* potentially having a part.

Reuvan Agami's group introduced their shRNA vectors into an immortalized fibroblast cell line that required only the deregulation of RAS for transformation. RAS proteins

## SMALL MOLECULE INHIBITORS

## Stabilizing influence

Although in many tumours the loss of the tumour suppressor p53 is caused by a mutation in the gene, inactivation of wild-type p53 can also occur through failure to inactivate the ubiquitin ligase (E3) HDM2, which targets p53 for proteasomal degradation. Allan Weissman, Karen Vousden and colleagues have identified compounds that inhibit HDM2 activity, and thereby stabilize and activate wild-type p53 — these data indicate that ubiquitin ligases are viable targets for drug discovery.

High-throughput screening of small-molecule libraries identified a family of 7-nitro-5-deazaflavin compounds (HLI98s) that strongly inhibited HDM2-mediated autoubiquitylation *in vitro*. HLI98 compounds specifically inhibited the RING-finger domain of HDM2, and not the regions that interact with p53. When primary human fibroblasts were treated

with HLI98 compounds, both p53 and HDM2 levels increased. Ubiquitylated p53 was not detected, which is consistent with the compounds inhibiting ubiquitylation instead of proteasome function.

HLI98 compounds showed some selectivity for HDM2 compared with other RING-finger E3s in cell lines. The compounds did not stabilize p53 in the absence of MDM2 (the mouse homologue of HDM2) in mouse embryonic fibroblasts (MEFs), indicating that they do not inhibit other E3s that target p53 for degradation, such as PIRH2 and COP1. In addition, they did not stabilize another protein, p21, which is regulated by E3 ligases other than MDM2.

So does stabilization of p53 by the HLI98 compounds also activate p53? The authors showed that the p53 that accumulated following treatment with the HLI98 compounds was transcriptionally active and induced transcription of the

p53 target genes *CDKN1A* (encoding p21) and *PUMA*. An important part of the tumour-suppressor role of p53 is to induce apoptosis, and the ability of HLI98-stabilized p53 to induce apoptosis was shown by activation of caspases and an increase in cell death in treated MEFs. However, HLI98 treatment also caused p53-independent cell-cycle arrest and apoptosis, reflecting off-target activities of these compounds, such as actions against E2 enzymes of the ubiquitin system or other E3 enzymes.

These data show proof of principle for inhibitors of ubiquitin ligases and provide an alternative to inhibiting HDM2-p53 interaction as a way of reactivating p53 in tumours.

Ezzie Hutchinson

## References and links

**ORIGINAL RESEARCH PAPER** Yang, Y. *et al.* Small molecule inhibitors of HDM2 ubiquitin ligase activity stabilize and activate p53 in cells. *Cancer Cell* **7**, 547–559 (2005)

**FURTHER READING** Chene, P. Inhibiting the p53-MDM2 interaction. *Nature Rev. Cancer* **3**, 102–109 (2003)

Allan Weissman's lab:

<http://ccr.cancer.gov/staff/staff.asp?Name=weissman>

Karen Vousden's lab:

<http://www.cancerschool.gla.ac.uk/reports/dr-karen-vousden-20-03-02.html>