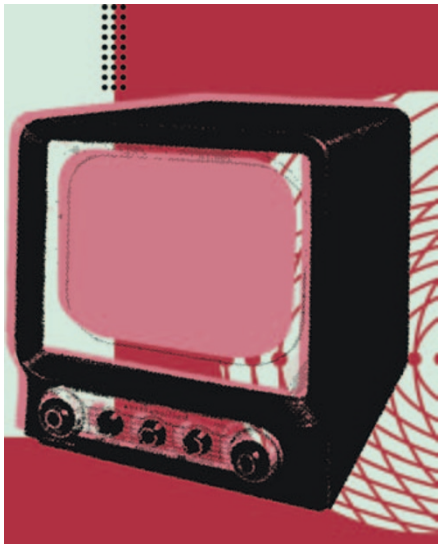
CANCER GENETICS

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-β 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

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convey growth signals from the cell surface to the nucleus, and their overexpression or aberrant activation is an important cause of cancer. Although activating mutations of RAS are frequent in human cancers, many tumours still retain wild-type copies of the gene, prompting Agami and colleagues to look for genes for which inhibition activates RAS.

They identified the homeodomain gene paired-like homeodomain transcription factor 1 (*PITX1*), which is frequently downregulated in bladder and prostate tumours. Although it is not yet known whether *PITX1* is mutated or deleted in human cancers, the authors present some intriguing evidence that loss of PITX1 can lead to RAS activation and a transformed phenotype. They found that PITX1 transcriptionally activates *RASAL1*, a RAS-GAP gene that belongs to a family of genes that suppress RAS activity.

Although *REST* and *PITX1* are exciting candidates, further studies will be needed before their roles in cancer can be confirmed. However, these studies show how powerful screens using RNAi libraries can be for identifying potential tumour-suppressor genes.

Jenny Bangham

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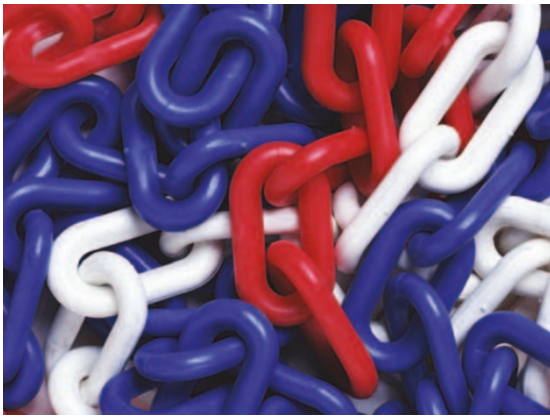
CHROMATIN DYNAMICS

Repressive links



The regulation of gene transcription relies on the concerted efforts of several proteins, including those that modify the structure of the chromatin. Jan-Herman Dannenberg, Gregory David, Ron DePinho and colleagues looked at the transcriptional networks regulated by the co-repressor mSIN3A in both normal and neoplastic cells using combined genetic, biochemical and computational approaches.

mSIN3A interacts with histone deacetylases (HDACs) and numerous transcription factors to regulate diverse signalling pathways and biological processes. To further investigate the function of this protein, Dannenberg and co-authors engineered mice that harboured a conditional *mSin3A*-knockout allele with embedded Lox-P sites ($mSin3A^{L}$) that delete the gene *in vivo* on crossing to mice expressing Cre recombinase, or *in vitro* on exposure to Cre-encoding retroviruses.

mSin3A-null mice are embryonic lethal, indicating a crucial function for mSIN3A in normal development. As early lethality hampered in-depth dissection of mSIN3A function, additional studies were conducted in $mSin3A^{L/L}$ mouse embryonic fibroblasts (MEFs) that were depleted of mSIN3A following exposure to the Cre-encoding retrovirus. Loss of mSIN3A initiates unscheduled DNA synthesis, triggering an S-phase checkpoint that results in profound growth arrest at G2/M and increased apoptosis — data that might explain the lethal phenotype observed in the null embryos.

As mSIN3A–HDAC has been implicated in regulatory modifications of the p53 tumour-suppressor protein, the investigators assessed the impact of combined deletion of mSIN3A and p53 on these cellular phenotypes. Working with MEFs as well as lymphomas and sarcomas derived from $Trp53^{-/-}$ mice carrying conditional *mSin3A* alleles, loss of p53 was shown to not be enough to overcome mSIN3A-mediated growth arrest or apoptosis. The authors also showed that loss of both p53 and RB tumour suppressors failed to alter the lethal outcome.

So what genes trigger the growth arrest in mSIN3A-deficient cells? To address this question, Dannenberg and colleagues conducted a time course to map the changes in gene expression after mSIN3A deletion. They showed that genes involved in numerous crucial processes were altered, including DNA replication, cell-cycle regulation, apoptosis and mitochondrial metabolism. Computational analysis of the mSIN3A transcriptome, using a knowledge-based database, identified several well-known genes as nodal points, including the *Myc–Mad, E2f* and *Trp53* transcriptional networks.

Loss of mSIN3A expression also changed the expression of many components of the mSIN3A–HDAC complex, consistent with the ability of mSIN3A to regulate its own transcription and that of other members of this complex. In addition, mSIN3A showed novel links to DNA-repair and peroxisome-proliferator-activated-receptor networks. These findings were verified using *in silico* promoter analyses and complemented by chromatin-immunoprecipitation assays that also revealed links to the signal transducer and activator of transcription (STAT) network, and the nucleosome remodelling factor FALZ. However, the authors additionally note that not all MYC, E2F and p53 target genes are upregulated on loss of mSIN3A, indicating that mSIN3A might only regulate a subset of these targets and/or that mSIN3A regulates these genes depending on the physiological conditions or the tissue type.

This integrated approach has produced novel insights into the diverse functions of mSIN3A and has confirmed the importance of mSIN3A in cancer-related pathways and processes. No doubt this approach will be useful to address the function of other components of multi-protein transcriptional complexes.

Nicola McCarthy

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