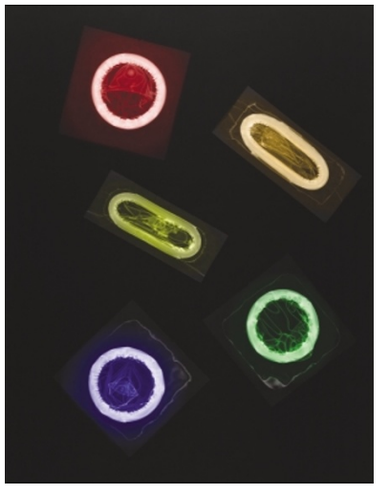
DIAGNOSTICS

One size fits all



Microarrays are a powerful tool for making pairwise comparisons between tumour types, allowing us to categorize tumours that cannot be distinguished histologically. But imagine being able to categorize any tumour by plugging its gene-expression data into a universal database. This is the vision of Todd Golub and colleagues, and a paper in *Proceedings of the National Academy of Sciences* makes the first steps towards realizing that vision.

Using commercially available oligonucleotide arrays, the authors set about classifying tumours from 14 different classes using two approaches. The first method — unsupervised learning or clustering — organizes samples on the basis of similar gene-expression patterns without any knowledge of the tumour type. This could distinguish haematopoietic or central nervous system tumours, but couldn't tell epithelial tumours apart. The second approach — supervised learning — 'trains' an algorithm to distinguish between different tumour types so that it can recognize blinded samples. This method is excellent at making pairwise distinctions, but how would it cope with 14 possibilities at once? The trick was to break the problem down into numerous pseudo-pairwise comparisons by running the data for each sample through 14 classifier algorithms that compare a specific tumour type — for example, breast cancer — with all of the other types. Each classifier can then either accept or reject the sample, depending on whether the tumour's expression pattern resembles that of the classifier. The classifier also generates a 'confidence value' that quantifies how similar the data set from the sample is to its trained breast cancer signature.

Training this classifier using 144 primary tumour samples of known class allowed it to classify 78% of the samples correctly, and for half of the mistakes, the second or third most confident predication was correct. Increasing the number of tumours in the training set might improve this score. The classifier was then let loose on 54 test samples, with similar results. Interestingly, six of eight metastatic samples were correctly classified, indicating that metastatic tumours retain a gene-expression pattern similar to that of the

GENOMIC INSTABILITY

Let's stick together

Defects in components of the mismatch-repair and nucleotide-excision-repair pathways are known to induce genomic instability and, hence, cancer; so might impairment of another repair pathway also lead to tumorigenesis? Reporting in the December issue of *Molecular Cell*, Sharpless, Ferguson *et al.* show that heterozygosity of *Lig4* — a DNA ligase that, during repair of double-strand breaks by non-homologous end joining, sticks the DNA ends together — can promote tumour formation in mice.

As *Lig4*-null mice are embryonic lethal, the effect of losing one copy of *Lig4* (haploinsufficiency) was investigated in mice that were tumour prone because they had lost the *Cdkn2a* locus, which encodes overlapping transcripts of the Ink4a and Arf tumour suppressors. Cells derived from these mice were transfected with oncogenic mutants of *Myc* and *Ras* to determine whether *Lig4* affected the transformation rate. $Cdkn2a^{-/-}/Lig4^{+/-}$ cells developed more than twice the number of transformed foci than did $Cdkn2a^{-/-}/Lig4^{+/+}$ cells, but re-expression of *Lig4* in the heterozygous cells did not reduce the number of foci formed. This indicates that decreased Lig4 activity results in oncogenic mutations that cooperate with *Myc* and *Ras* to transform cells, rather than Lig4 actively suppressing transformation. But is this due to a deficiency in DNA repair? Following treatment with ionizing radiation, which induces double-strand breaks, 50% of $Cdkn2a^{-/-}/Lig4^{+/-}$ mice, but no $Cdkn2a^{-/-}/Lig4^{+/+}$ mice, died within 14 days. So, *Lig4* haploinsufficiency increases sensitivity to DNA damage, presumably because of an inability to repair double-strand breaks.

So how does *Lig4* status affect the tumour-prone phenotype of $Cdkn2a^{-/-}$ mice? Mice that are also heterozygous for *Lig4* develop tumours more rapidly and have a shorter disease-free survival than $Lig4^{+/+}$ mice. The tumour spectrum is also dramatically altered: $Cdkn2a^{-/-}/Lig4^{+/+}$ mice develop mostly lymphomas, but $Cdkn2a^{-/-}/Lig4^{+/+}$ also get soft-tissue sarcomas.

So how does *Lig4* heterozygosity promote formation of sarcomas? Using spectral karyotypic analysis, the authors showed that tumours derived from $Cdkn2a^{-/-}/Lig4^{+/-}$ mice frequently possessed translocations — six of ten compared with one of nine from $Cdkn2a^{-/-}/Lig4^{+/+}$ mice. These translocations were clonal, indicating that they were involved in oncogenesis, and a number mapped near to known tumour suppressors and oncogenes. But are translocations the sole mechanism, or could unrepaired double-strand breaks also lead to gene amplifications and deletions? Comparative genomic hybridization revealed that, again, mice heterozygous for *Lig4* had significantly more of these mutation types. In fact, several tumours analysed were amplified in the region containing the *Mdm2* oncogene.

A similar pattern of increased amplifications and deletions, coupled with unbalanced translocations, has been seen in mice with telomeric dysfunction, and it has been suggested by the Jasin and Wahl labs that DNA breaks could be the substrate for both classes of lesion. These data, together, now indicate that either an increase in the formation of DNA breaks, or a decrease in their repair, can promote genomic instability that is associated with carcinomas of adult humans.

So, a third repair pathway has been found to affect tumorigenesis in mice. It now remains to be determined whether deficiency in non-homologous end joining also promotes tumorigenesis in humans.

Emma Greenwood

References and links

ORIGINAL RESEARCH PAPER Sharpless, N. E. *et al.* Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions. *Mol. Cell* **8**, 1187–1196 (2001)
FURTHER READING Pierce, A. J. & Jasin, M. NHEJ deficency and disease. *Mol. Cell* **8**, 1160–1161 (2001)
WEB SITE
Ron DePinho's lab:
http://www.hms.harvard.edu/dms/bbs/fac/depinho.html

tumour of origin. But the classifier fared less well on poorly differentiated (high-grade) carcinomas, indicating that their gene-expression patterns are fundamentally different from those of well-differentiated tumours from the same tissue. Might this reflect a different cellular origin for these tumours? Perhaps it's time to refine our tumour classification systems to take these differences into account.

Cath Brooksbank

References and links

ORIGINAL RESEARCH PAPER Ramaswamy, S. *et al.* Multiclass cancer diagnosis using tumor gene expression signatures. *Proc. Natl Acad. Sci. USA* **98**, 15149–15154 (2001)

FURTHER READING Su, A. I. *et al*. Molecular classification of human carcinomas by use of gene expression signatures. *Cancer Res*. **61**, 7388–7393 (2001)

WEB SITES

Complete list of tumour marker genes:
http://www-genome.wi.mit.edu/MPR/GCM.html

SVM-FU algorithm software:
http://www.ai.mit.edu/projects/cbcl

APOPTOSIS

Drifting downstream

How many ways are there of blocking p53-mediated apoptosis? Many cancer cells avoid programmed cell death by inactivating p53 itself or one of its upstream regulators, but Fei Su and colleagues, in the January issue of *Genes & Development*, describe an unusual oncogenic signalling pathway that inhibits the pathway downstream of p53.

The story begins with an oncogenic growth factor — called WNT-1-induced secreted protein 1 (WISP1) — that is overexpressed in 30–40% of human colon cancers. WISP-1 belongs to a family of growth factors known as the CCN family, some members of which signal by binding to integrins rather than to classical receptor tyrosine kinases. One of the downstream targets of integrin-mediated signalling is the serine/threonine kinase AKT, a well-known promoter of cell survival. So does WISP-1 activate AKT? To find out, the authors treated cells with medium from cells that had been transfected with either *WISP1* or an empty vector. Western blots detected activated AKT, which is phosphorylated on serine 473, only in extracts from cells treated with the WISP1-conditioned medium. This activation was definitely caused by WISP1 because it was blocked by an antibody to WISP1. One of AKT's targets — glycogen synthase kinase 3β (GSK3β) — was also phosphorylated in response to WISP1-conditioned medium.

So does WISP1 block apoptosis by activating AKT? The authors treated cells with two DNA-damaging agents — etoposide and ultraviolet (UV) light — that activate the stress-mediated apoptotic pathway. Cells treated with WISP1-conditioned medium were protected from apoptosis mediated by these agents. Caspase-3 is an important 'effector' protease in the apoptotic pathway, but its activation by proteolytic processing was abrogated in cells treated with WISP1-conditioned medium. The caspase cascade is activated by the release of cytochrome *c* from mitochondria, which then binds and activates APAF1. Treatment with WISP1-conditioned medium also blocked cytochrome *c* release in response to etoposide or UV light. All these experiments were performed in cells that contain wild-type p53, but, in cells lacking p53, WISP1 was unable to prevent apoptosis induced by etoposide or UV light. There are two main apoptotic pathways: stress-activated apoptosis (such as that mediated by etoposide or UV light) is p53 dependent, whereas receptor-activated apoptosis (mediated by 'death receptors' such as FAS) is independent of p53 and APAF1. WISP1 seems to target the stress-activated pathway as it couldn't block apoptosis mediated by FAS.

So does WISP1 block stress-mediated apoptosis by disabling p53? Surprisingly, the answer to this question is no. p53 seemed to be activated normally in the presence of WISP1: three of p53's target genes — *MDM2*, *CDKN1A* and *BAX* — were upregulated in response to UV light or etoposide.

Is the WISP1-mediated blockade of p53-mediated apoptosis entirely dependent on AKT? This would make sense, as several of AKT's substrates are involved in regulating cytochrome-*c*-mediated apoptosis. Transfection with a kinase-dead mutant of AKT attenuated, but didn't completely block, the protective effect of WISP1, so the search was on for another anti-apoptotic mechanism that might be triggered by WISP1. The BCL-2 family of proteins regulate sensitivity to apoptosis by controlling the exit of cytochrome *c* from mitochondria. So the authors compared the levels of BCL-2 family members in WISP1-expressing and non-expressing cells, and found an increase in a single member of this family — the anti-apoptotic protein BCL-X_L.

So WISP1 seems to work downstream of p53, blocking cytochrome *c* release by activating both AKT and BCL-X_L. The gaps now need to be filled in by paddling back upstream from these two effectors of survival to WISP1's receptor.

Cath Brooksbank

References and links

ORIGINAL RESEARCH PAPER Su, F. *et al.* WISP-1 attenuates p53-mediated apoptosis in response to DNA damage through activation of the Akt kinase. *Genes Dev.* **16**, 46–57 (2002)

WEB SITE

Arnold Levine's lab:
http://www.rockefeller.edu/labheads/levine/levine.html