METASTASIS

Chain-store cancer

Imagine that you are the proud owner of a successful grocery store, having found the perfect recipe for a thriving business. However, before considering expansion, you should be aware that the necessary conditions for a successful chain operation can be surprisingly different to those required for the primary store. Similarly, different properties are required for the metastasis of primary tumours to secondary sites than for primary tumorigenicity. Twelve genes have already been identified as specific suppressors of metastasis. Keller and colleagues now add RAF kinase inhibitor protein (RKIP) to this list as a suppressor of prostate cancer metastasis.

The authors had previously shown that RKIP is expressed at a lower level by the metastatic prostate cancer cell line C4-2B than by the non-metastatic cell line LNCaP from which it is derived. This result was also shown to be true for human prostate cancer samples. RKIP expression was highest for benign samples, lower for primary tumours and absent in metastases.

What is the functional relevance of RKIP expression? To answer this, C4-2B cells were stably transfected with sense *RKIP* to increase their level of expression of RKIP, and LNCaP cells were transfected with antisense *RKIP* to decrease their expression. Transfection did not alter primary tumorigenic properties of the cells, such as *in vitro* proliferation rate or ability to form colonies, but did affect their invasive potential. RKIP expression was found to be inversely associated with *in vitro* invasion.

An *in vivo* model was then used to confirm these findings by implanting cell clones into the prostates of mice. After 10 weeks, there were no differences in the sizes of the primary tumours, but fewer mice injected with RKIP-expressing C4-2B cells had lung metastases than mice injected with control C4-2B cells, and the average number of metastases was lower. For the few *RKIP*-transfected C4-2B cells that formed metastases, these secondary tumours did not express RKIP. RKIP expression was correlated with decreased vascular invasion in the primary tumour, indicating a potential mechanism by which RKIP might suppress metastasis.

TELOMERASE

Pathways to telomerase activation

Telomerase is expressed in most human cancers — it is thought to confer immortality — and can transform primary cells in conjunction with *RAS*, and large and small T antigen. However, neither mutation nor amplification of the gene have been identified in tumours, so a causal role in tumorigenesis has yet to be established. Now, Lin and Elledge have identified several pathways by which telomerase might be regulated, strengthening the link between telomerase and tumorigenesis.

As telomerase is not mutated in cancer, it is likely that upstream regulators of its expression are, and the authors undertook a genetic screen to identify them. They transfected HeLa cells — which express high levels of TERT, the protein component of telomerase — with a green fluorescent protein (GFP) reporter driven by the *TERT* promoter, and then co-infected the cells with a vector that randomly inserts into the genome to activate expression from adjacent genes. Clones in which GFP expression was decreased by at least threefold — thought to express negative regulators of *TERT* — were isolated for further study. Six gene candidates were identified — four transcriptional regulators (menin, MAD1, SIP1 and SIR2), a protein kinase thought to be involved in signalling (RAK1) and a BRCT-domain-containing protein (BRIT1). Interestingly, when overexpressed in telomerase-expressing cancer cell lines, menin, MAD1, SIP1 and RAK1, but not SIR2, were still able to repress *TERT* transcription. It is thought that insertion of the vector in the screen actually created a dominant-negative SIR2, and that it is, in fact, a positive regulator of *TERT*.

To investigate whether the candidate negative regulators could also act at endogenous levels, they were depleted using RNA interference (RNAi) in U2OS cells — which do not normally express *TERT* — to see if *TERT* expression could be activated. All but SIP1 were able to induce expression of *TERT* mRNA, and menin and MAD1 were also able to induce expression of TERT protein. Chromatin immunoprecipitation experiments confirmed that both MAD1 and menin could bind directly to the *TERT* promoter. MAD1 is the inhibitory partner of MYC, and acts by preventing MYC-induced activation of *TERT*.

Depletion of SIP1 did not induce *TERT* expression, but as it is a downstream target in the TGF-β pathway, thought to downregulate *TERT*, the authors investigated whether SIP1 is required for the effect of TGF-β on *TERT* expression, and found that it is at least partially dependent on SIP1.

As menin is a tumour suppressor, its activity could be exerted through telomerase repression. Indeed, depletion of menin in BJ cells — a primary human diploid fibroblast line — by RNAi led to reactivation of *TERT* expression and telomerase activity, and an increase in telomere length. This correlated with an increase in lifespan, as the cells seem to be immortalized. Loss of menin can also replace expression of *TERT* in the transformation of primary cells with *RAS*, and large and small T antigen.

So, at least three pathways — TGF-β, MAD1 and menin — all of which are involved in human cancer, regulate expression of telomerase, and this finding explains how telomerase could be activated in tumours without amplification or mutation in telomerase genes.

Emma Greenwood

References and links

ORIGINAL RESEARCH PAPER Lin, S.-Y. & Elledge, S. J. Multiple tumor suppressor pathways negatively regulate telomerase. *Cell* **113**, 881–889 (2003)

WEB SITE

Stephen Elledge's lab:
http://www.hhmi.org/research/investigators/elledge.html

Finally, the authors looked at the molecular mechanism of RKIP action. RKIP is an inhibitor of RAF-mediated signalling, so its loss of expression in metastases might lead to increased RAF signalling. This was confirmed by the observed negative correlation between the level of RKIP expression and the degree of phosphorylation (activation) of the RAF targets MEK and ERK. Inhibiting the kinase activity of MEK with the inhibitor PD-098059 decreased the invasive ability of C4-2B cells, indicating that MEK might have an important role in the regulation of metastasis through RKIP.

Just as over-ambitious expansion can be the end of many businesses, most patients with prostate cancer die from metastases rather than from the primary tumour, so this work could have important therapeutic implications. It also provides the most complete description of a metastasis suppressor so far, which might extend to other forms of cancer.

Kirsty Minton

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ONCOGENES

Who's doing the dirty work for MYC?

MYC, a basic helix–loop–helix leucine-zipper transcription factor, has to be one of the most famous oncoproteins. Among its targets are a wide range of genes that coordinate cell-cycle progression and cell growth, which sensitize the cell to apoptotic stimuli. But MYC might also promote tumorigenesis by activating expression of genes that are involved in cellular immortalization or escape from senescence. Gandori and colleagues now show that the human Werner syndrome gene, *WRN*, is a direct target of MYC and that MYC-driven *WRN* upregulation prevents cellular senescence and consequently leads to tumorigenesis.

Werner syndrome — a rare disorder associated with premature ageing, genomic instability and increased risk of malignancy — is caused by a loss-of-function mutation in *WRN*, which encodes a RecQ helicase implicated in mitotic recombination and repair. Also, *WRN* overexpression is seen in immortalized cell lines and might actually aid sustained proliferation. So, Gandori *et al*. wondered whether *WRN* could be a direct target of MYC.

The first clue came from expression studies — in different cell types and under different conditions, expression of the two genes was coordinated (if one went up, so did the other). Importantly, there was a short time lag between the induction of c-*MYC* and *WRN* transcription. The next clue came when Gandori and colleagues found several non-canonical MYC–MAX (MYC binds DNA as a heterodimer) binding sites in the *WRN* promoter, and *in vitro* and *in vivo* experiments showed that purified MYC–MAX complex binds to these sites.

So, MYC directly regulates *WRN* expression, but does overexpressing MYC affect the proliferation and survival of cells that lack WRN? To address this question, Gandori *et al*. compared c-*MYC* overexpression in normal human fibroblasts with those from Werner syndrome patients. The result was clear — c-*MYC* overexpression induced senescence in 30–70% of $WRN^{-/-}$ fibroblasts, whereas only ~1% of wild-type fibroblasts underwent senescence, indicating that WRN counteracts senescence induced by high levels of MYC. Taking a closer look at the cell cycle, the expression of cell-cycle regulatory proteins and apoptotic response in these two cell lines showed that, whereas all cell types had characteristics of MYC oncogenic stimulation, cellular events that accompanied cell-cycle arrest and senescence response were limited to $WRN^{-/-}$ cells.

The final evidence nailing WRN as the culprit came when the authors used RNA interference to remove it from normal fibroblasts in which c-*MYC* was overexpressed. Fibroblasts in which WRN had been knocked out proliferated poorly, and high levels of MYC not only failed to rescue this phenotype, but also exacerbated it.

So, Gandori *et al*. propose a model in which MYC overexpression promotes cell growth and proliferation, as well as genomic instability, which is counteracted by WRN — its role in DNA recombination and repair ensures cell survival (not a desirable outcome if non-WRN-dependent effects of MYC induce uncontrolled proliferation). So, in the absence of WRN, genetic instability increases and cell survival is compromised. As well as adding to our knowledge of genome maintenance and cell proliferation, these findings have important implications for cancer studies. Without WRN, cells senesce more readily, so it is little wonder that Werner syndrome patients rarely suffer from certain types of cancer that are associated with MYC overexpression. Importantly, this study also points to a new opportunity for the treatment of human tumours that arise as a result of MYC overexpression.

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