EPIGENETICS

Less is more

Site-specific DNA hypermethylation is frequently implicated as a cancer-causing mechanism, but what of genome-wide hypomethylation, which also occurs in many human tumours? Whether this is a cause or consequence of cancer has long been debated, but Rudolf Jaenisch and colleagues, reporting in the 18 April issue of *Science*, have now developed a mouse model to address this question.

They generated viable, but small, mice that were compound heterozygous for a hypomorphic allele and a null allele of *Dnmt1* — the DNA methyltransferase that maintains DNA methylation. These mice expressed just 10% of wild-type levels of the protein, and Southern blot analysis following digestion with a methylation-sensitive restriction enzyme revealed that global methylation levels were decreased. Interestingly, 80% of the mice developed aggressive T-cell lymphomas at 4–8 months of age. These were shown to be monoclonal, which indicates that hypomethylation initiates cancer in a single cell that undergoes other events to become a malignant tumour.

So how might hypomethylation induce this lymphomagenesis? The authors proposed three possible mechanisms: induction of endogenous retroviral elements could insertionally activate proto-oncogenes; proto-oncogenes could be activated by epigenetic effects; or genomic instability might be induced.

The first possibility was ruled out as retroviral element activation was not observed. c-Myc had already been found to be over-expressed in most of the hypomethylation-induced T-cell lymphomas, but this locus was not rearranged in any of the 18 tumours tested. This was in contrast to Moloney murine leukaemia virus (MMLV)-induced tumours, in which 3 of 12 tumours had an insertional rearrangement in c-Myc. It was also thought unlikely that the mechanism could be epigenetic, as hypomethylation existed throughout development and c-Myc was expressed at normal levels in the thymuses of 2–4-week-old mice.

However, there was already evidence that hypomethylation affected genomic stability, and this was confirmed by carrying out array-based comparative genome hybridization. When hypomethylation-induced tumours were compared with MMLV-induced tumours, a significant increase in chromosome gains — particularly of chromosome 15, which contains c-*Myc* — was observed. Only 2 of 12 tumours did not have this change, and these also had lower levels of c-Myc.

TUMOUR SUPPRESSORS

Unexpected relations

Transforming growth factor (TGF)-β signalling induces a growth-arrest response in normal cells. Cancer cells, however, frequently lose the ability to undergo TGF-β-mediated growth suppression, even without any genetic defects in the TGF-β signalling pathway. Stefano Piccolo and colleagues performed a screen to identify new modulators of the TGF-β response, and made the surprising discovery that p53 is involved in activation of TGF-β target genes.

TGF-β family members bind to serine/threonine kinase receptors and activate phosphorylation of the SMAD family of transcriptional regulators. SMADs form a DNA binding complex with other cofactors to activate transcription of their target genes, but little is understood about other components that modify this signalling pathway. Piccolo and colleagues performed an unbiased functional screen for activators of TGF-β signalling during embryonic development, and found that one of these was a splice variant of p53 (p53AS). They showed that p53AS activated a subset of TGF-β target genes during frog embryonic development, and that lack of Xp53 caused abnormal development due to impaired TGFβ-induced gene responses. p53AS activated transcription of TGFβ target genes in frog cells, by interacting with SMAD and acting as a sequence-specific transcription factor. So, is p53 involved in TGF-β signalling in human cancer cells?

The authors used small interfering RNAs (siRNAs) to reduce p53 levels in HepG2 cells — a cancer cell line that is highly responsive to TGFβ and that normally expresses p53. They found that reduction of endogenous p53 levels reduced expression of TGF-β target genes, and allowed cells to overcome the growth arrest that is normally imposed by TGF-β signalling. Furthermore, restoring p53 activity in a p53-null cancer cell line, which is normally insensitive to TGF-β signalling, resulted in SMAD-dependent inhibition of cell growth.

But how does p53 mediate the TGF-β transcriptional response? The authors found that p53 associates with SMAD2 and SMAD3 in HepG2 cells, and that this association depends on TGF-β signalling. Furthermore, p53 family members acted synergistically with the SMAD complex to activate transcription from the SMAD-specific promoter, *Mix2*. Several TGF-β target genes were found to be under joint control of p53 and SMAD in mammalian cells.

The ability of p53 to cooperate with SMAD to control gene expression reveals a new mechanism of regulating TGF-β signalling. p53 activity is disrupted in many tumour types, which might underlie their inability to undergo TGF-β-mediated growth inhibition. Further experiments are required to determine how p53/SMAD gene targets block proliferation, and whether other p53 family members, such as p63 and p73, can also modulate TGF-β signalling.

Emma Croager

References and links

ORIGINAL RESEARCH PAPER Cordenonsi, M. *et al.* Links between tumor suppressors: p53 is required for TGF-β gene responses by cooperating with Smads. *Cell* **113**, 301–314 (2003)

FURTHER READING Massague, J. How cells read TGF-β signals. *Nature Rev. Mol. Cell Biol.* **1**, 169–178 (2000)

WEB SITE

Stefano Piccolo's lab: http://www.bio.unipd.it/piccolo/

So, it seems that hypomethylation can cause tumorigenesis through genomic instability. A report by the same group, also in *Science*, provides further support for this, as they show that hypomethylation promotes cancer in tumour-prone mice — due to heterozygosity of the tumour suppressors *Nf1* and *Trp53* — because it increases the rate of loss of heterozygosity. Perhaps we should reconsider the use of demethylating agents to treat cancer in light of these results.

Emma Greenwood

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WEB SITE

Rudolf Jaenisch's lab:
http://web.mit.edu/biology/www/facultyareas/facresearch/jaenisch.shtml

METASTASIS

Love thy neighbour

If such a thing were to exist, the 'Ten Commandments' for a happy, healthy cellular community would surely include this one. Normal cells recognize, adhere to and respond to their neighbours to maintain appropriate tissue functions. By contrast, some tumour cells fail to obey this command — often as a result of the disruption of cell–cell adhesion molecules — and begin to invade surrounding tissue. One such adhesion molecule is E-cadherin, alterations of which are known to be involved in the metastasis of epithelial tumours such as breast cancer. Now, in a study published in *Cell*, Wade and colleagues have been able to tie these observations in with the poor clinical prognosis of patients with oestrogen receptor (ER)$^-$, compared with ER$^+$, breast tumours. They show that transcriptional regulation by MTA3 (metastasis-associated gene 3) provides a functional link between loss of expression of ER and loss of expression of E-cadherin.

First, the authors used co-precipitation experiments to show that MTA3 (similar to its better-known relatives MTA1 and MTA2) is associated with Mi-2, MBD3 and HDAC1, and is therefore likely to be a component of the Mi-2/NuRD histone deacetylase complex. Consistent with the known ability of the Mi-2/NuRD complex to act as a transcriptional repressor by chromatin modification, MTA3 was shown to repress transcription of a luciferase reporter gene.

So, what are the physiological upstream and downstream targets of the MTA3 pathway? In a comparison of mRNA and protein levels between ER$^+$ and ER$^-$ breast cancer cell lines, there was shown to be a correlation between levels of MTA3 and expression of ER. Steroid depletion from the culture medium of ER$^+$ cell lines decreased the level of expression of MTA3, whereas overexpression of ER had the opposite effect, showing that ER signalling regulates MTA3 levels. In terms of downstream targets, the *Snail* gene promoter was identified as a binding target for MTA3 protein. Transcription from a *Snail*-promoter–luciferase reporter was repressed by co-transfection with MTA3, in a manner that was sensitive to histone-deacetylase inhibitors. The fact that Snail has previously been described as a transcriptional repressor of E-cadherin completes the link.

According to these results, ER$^-$ breast tumours would have decreased levels of MTA3, leading to increased levels of Snail and decreased levels of E-cadherin, thereby favouring tumour metastasis.

This chain of events was confirmed by monitoring expression levels of MTA3, Snail and E-cadherin in the ER$^+$ cell line T47D, which was grown in medium depleted of steroids for 7 days and then exposed to oestradiol. Importantly, the *in vitro* results have been borne out by analysis of clinical samples of breast carcinoma, in which the level of MTA3 expression strongly correlated with ER status. Microarray data from 115 women with breast cancer showed a positive correlation between ER and E-cadherin expression.

In addition to explaining why ER$^-$ breast tumours might have a poor clinical prognosis owing to their invasive potential, this study also has implications for the use of the selective oestrogen-receptor modulators (SERMs) that are commonly used to treat ER$^+$ tumours. It will be important to determine that SERMs do not affect the ability of the ER to upregulate *MTA3* transcription, as this could lead to decreased levels of E-cadherin and increased metastatic potential of the tumour.

Kirsty Minton

References and links

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