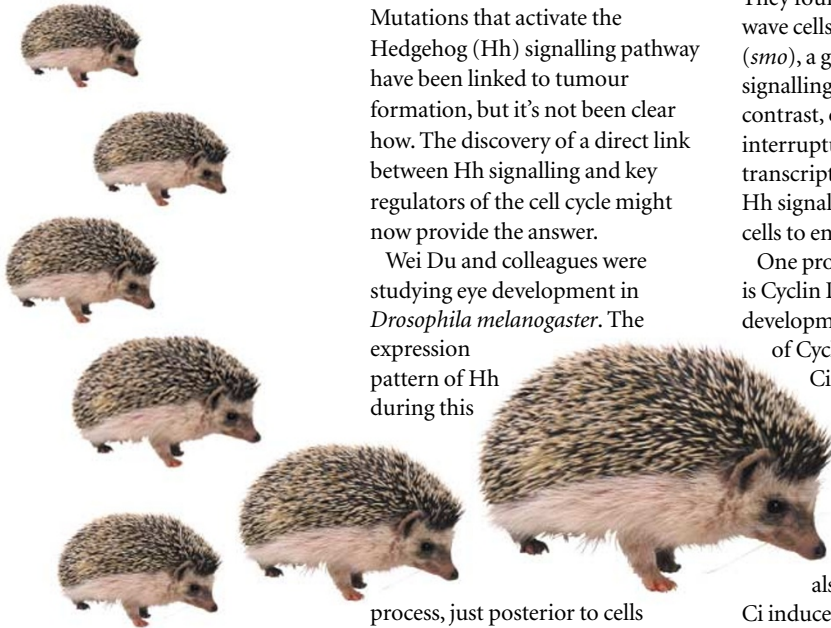DEVELOPMENT

Hedgehog proliferation

Mutations that activate the Hedgehog (Hh) signalling pathway have been linked to tumour formation, but it's not been clear how. The discovery of a direct link between Hh signalling and key regulators of the cell cycle might now provide the answer.

Wei Du and colleagues were studying eye development in *Drosophila melanogaster*. The expression pattern of Hh during this process, just posterior to cells entering S phase, indicated that reception of the Hh signal might be needed for entry to S phase. To test this, the authors looked at what would happen if Hh signalling was blocked during eye development. They found that second mitotic wave cells with mutated *smoothened* (*smo*), a gene that is required for Hh signalling, do not enter S phase. By contrast, overexpression of Cubitus interruptus (Ci) — the transcription factor that mediates Hh signalling — drove G1-arrested cells to enter S phase.

One protein that promotes S phase is Cyclin D. During eye development, the highest expression of Cyclin D overlaps with that of Ci — so could Ci promote the expression of Cyclin D? Support for this idea came from the observation that levels of Cyclin D are reduced in *smo*-mutant clones, and also that overexpression of Ci induces high levels of Cyclin D mRNA and protein.

As well as promoting entry into S phase, Cyclin D induces cell growth. Du and co-workers therefore wondered whether Hh might also regulate growth, so they studied the effects of overexpressing either Ci or Patched (Ptch; an inhibitor of Hh signalling) in clones of undifferentiated wing-disc cells. Whereas Ptch overexpression clones were considerably smaller than controls, Ci overexpression clones were much larger, which indicates that Hh signalling not only promotes S phase, but that it also regulates cell growth.

Cyclin E also promotes S phase, and reduced or increased levels of this protein could be detected with loss of Smo or overexpression of Ci, respectively. The authors then looked at how Hh signalling might induce the transcription of Cyclin E. They identified several sequences in the Cyclin E promoter with homology to the consensus Ci-binding site, and used chromatin immunoprecipitation to show that

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EPITHELIAL–MESENCHYMAL TRANSITION

A deadly combination

In some cell lines, an epithelial–mesenchymal transition (EMT) arises as the result of a joint effort between Hras and transforming growth factor-β (Tgf-β). How relevant this is to the multistage nature of *in vivo* tumour progression, though, is a burning question.

So, Allan Balmain's group studied whether changes in the levels of Hras and Tgf-β affect tumour progression, using a series of well-characterized tumour cell lines that arise from initiated cells that carry activating mutations in the *Hras1* gene. And, as they now report in *Nature Cell Biology*, Smad2 (a downstream target of Tgf-β signalling) and Hras surpass discrete thresholds during progression from early-stage papillomas, through squamous carcinomas, to late-stage undifferentiated spindle-cell tumours.

First, the authors studied the molecular changes that occur when squamous carcinomas are converted into spindle-cell tumours. Tgf-β-mediated transcriptional activity was very high in the spindle cells, and phosphorylated Smad2 accumulated in the nucleus, which indicated that the Tgf-β pathway was activated in these cells. Furthermore, in primary material from spindle-cell tumours, but not from differentiated tumours or squamous carcinomas, Smad2 was phosphorylated and predominantly localized in the cytoplasm.

Although Smad2 alone induced changes in the migration of squamous carcinoma cells, only in the presence of increased levels of mutated Hras did changes in cell shape and the expression of genes such as α-smooth-muscle actin (a mesenchymal marker) occur, resulting in EMT.

The authors then investigated whether, once this stage has been reached, Tgf-β signalling by Smad2 is still necessary for tumour progression. Expression of a dominant-negative form of Smad2 showed that this is indeed the case; spindle cells that expressed this construct reverted to a more epithelial phenotype and took on many features of epithelial gene expression. Notably, surface expression of αvβ3 integrin was lost, and this was coincident with the loss of collagen-matrix invasion. *In vivo*, this correlated with an inability to form tumours. By contrast, parental spindle cells or spindle cells that express a dominant-active form of Smad2 formed tumours, and those formed by dominant-active Smad2 were particularly invasive. Expression of dominant-active Smad2 also promoted extravasation into the target tissue, and a subsequent increase in lung metastases.

As the ability of a tumour to metastasize is the main determinant of whether or not patients with cancer die of their disease, these findings that different thresholds of Hras and Tgf-β activity — intermediate levels of Smad2 co-operating with Hras to induce EMT and invasiveness, and even higher levels of Smad2 being required for metastasis — are crucial for metastasis offer the opportunity for the design of small-molecule inhibitors to prevent the spread of tumours.

Katrin Bussell, Associate Editor, Nature Reviews Molecular Cell Biology

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WEB SITE
Allan Balmain's lab: http://cc.ucsf.edu/balmain/

Ci indeed binds these sites *in vivo*. Hh signalling therefore seems to promote S phase by direct induction of Cyclin E expression, as well as Cyclin D.

This study shows a direct link between Hh signalling and cell growth (through Cyclin D) and proliferation (through both Cyclin D and Cyclin E). And, as the authors conclude, "constitutive Hh signalling, which promotes deregulated expression of G1–S cyclins that have been associated with diverse forms of human cancer, would promote both cell proliferation and growth in tumours".
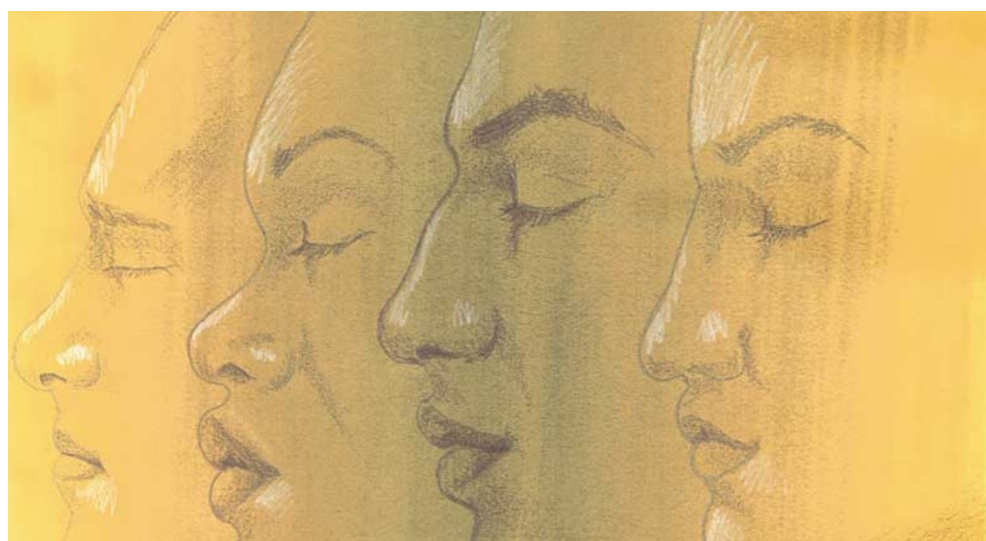
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ONCOGENESIS

The many faces of MYC

The oncogene c-*MYC* is known to stimulate both cell life and cell death — two opposing processes that battle for supremacy in c-MYC-induced tumours. But now, two groups have reported in *Molecular Cell* that c-MYC can also induce the accumulation of reactive oxygen species (ROS), with very different results.

Omid Vafa *et al.* were interested in the finding that expression of c-*MYC* could induce chromosomal abnormalities. This could be a direct effect, or an indirect effect of c-MYC's ability to drive cells into S phase prematurely, and their aim was to distinguish between these possibilities.

They developed an *in situ* TUNEL-based assay to allow them to visualize damaged DNA, and used this to confirm that c-MYC activation — achieved using a tamoxifen-inducible system — in normal human fibroblasts did indeed cause DNA damage. Cell-cycle analysis showed that only 1% of cells had entered S phase 8–9 hours after c-MYC induction, but that most cells had an average of 23 TUNEL foci by 4 hours, which increased to ~70/cell after 8–9 hours.

So, c-*MYC* expression can cause DNA damage independently of cell cycling; could the mechanism be a product of c-MYC's apoptotic programme? This possibility was ruled out because apoptotic markers — such as cytochrome *c* release — were not seen, and addition of a caspase inhibitor did not affect the number of TUNEL foci.

Instead, c-MYC seems to induce accumulation of the metabolic intermediate ROS — which can damage DNA directly or by activating topoisomerases — 3–4 hours after c-MYC activation. Treating cells with antioxidants prevents ROS accumulation, and hence DNA damage. c-*MYC*-expressing cells also show decreased viability, as cells arrest in a senescence-like state, but this is also mitigated by antioxidants.

Interestingly, although c-MYC induces growth arrest as a result of DNA damage, it also seems to partly overcome the p53-induced growth arrest. Cells that are treated with γ-irradiation normally block in G1 — only 1.2% had entered S phase after 24 hours — but c-MYC activation resulted in 11.5% entering S phase at the same time point.

So, c-MYC induces accumulation of ROS — which damages DNA — and also impairs the arrest response, which could further increase genomic instability to provide a growth advantage for cancer cells.

However, Hirokazu Tanaka *et al.* obtained different results. They also showed that expression of c-*MYC* — in NIH-3T3 and Saos-2 cell lines following serum deprivation — induced ROS, but that instead of causing DNA damage and growth arrest, it induced apoptosis. The mechanism behind the accumulation of ROS seems to be that c-MYC induces E2F1, which inhibits the transcription factor NF-κB, thereby preventing it from transcriptionally activating the antioxidant MnSOD — hence, the net effect is an increase in ROS. But how can the discrepancy between the two effects of accumulated ROS be explained? The most obvious explanation is related to the different cell types that are used. Saos-2 cells, for example, do not have p53, which could alter the response, and Omid Vafa *et al.* showed that rat cells expressing c-*Myc* underwent apoptosis, whereas normal human fibroblasts did not.

The important issue that now remains to be determined is whether c-MYC-induced ROS accumulation occurs in human cells *in vivo* to promote tumorigenesis.

Emma Greenwood

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