ONCOGENES

Receptor relay

c-SRC is a common oncogenic partner of the epidermal growth-factor receptor (EGFR), as both c-SRC and EGFR are overexpressed in human malignancies such as breast cancer. However, the mode of oncogenic cooperation between these two proteins has been unclear. Now, Yosef Yarden and colleagues report that c-CBL — a ubiquitin ligase that is known to be a regulator of EGFR endocytosis — is the 'middle leg' in the relay of oncogenic signalling between c-SRC and EGFR.

To investigate whether c-SRC affects the expression of EGFR, the receptor was co-expressed with different forms of c-SRC in receptor-negative Chinese hamster ovary (CHO) cells. Both wild-type c-SRC and an active-mutant form resulted in increased levels of expression of EGFR, in contrast to a kinase-defective mutant, which led to decreased EGFR expression levels. As the active form of c-SRC was shown to have no effect on receptor synthesis (mRNA levels) or maturation, the authors propose that c-SRC stabilizes the mature, cell-surface form of EGFR.

c-CBL ubiquitylates EGFR, which results in its endocytosis and degradation and, therefore, receptor desensitization in response to growth-factor signalling. Could effects on c-CBL be responsible for the change in expression of EGFR in response to c-SRC? Ectopic expression of c-CBL increased the removal of EGFR from the surface of SYF cells (which lack SRC, YES and FYN), but this receptor endocytosis was inhibited by the co-expression of an active-mutant c-SRC. The authors found that c-SRC prevents the c-CBL–EGFR interaction (which is required for receptor ubiquitylation), specifically downregulates the expression of c-CBL and recruits c-CBL to vesicles.

So, how does c-SRC decrease the level of expression of c-CBL? Expression of active-mutant c-SRC leads to polyubiquitylation of c-CBL, which targets this protein for degradation by the proteasome. This process does not occur for a RING-finger

TUMORIGENESIS

Back in time

A number of developmental signalling pathways have been shown to be reactivated during tumour formation — the Hedgehog (HH) pathway seems to be the latest member of this growing list. HH signalling mediates pattern formation during embryogenesis, and has recently been shown to regulate epithelial–mesenchymal interactions during lung development. In *Nature*, Watkins *et al.* now report that HH signalling also promotes lung tumour development.

Sonic Hedgehog (SHH) — a secreted ligand for the HH receptor patched (PTCH) — is a signalling switch expressed by a variety of differentiation subpopulations of cells throughout the embryo. Loss of Shh function results in severe lung defects in mice. Unlike skin and colon, the adult airway epithelium only proliferates in response to injury. In a search for factors that activate airway epithelial-cell proliferation after injury, Watkins *et al.* observed increased expression of both Shh and its transcriptional effector Gli1 in an adult mouse model of acute airway repair. This was surprising, as this pathway had been previously only associated with embryonic lung epithelial cells, where it signals adjacent lung mesenchyme to regulate branching morphogenesis.

Watkins *et al.* next looked to see if SHH was upregulated in lung tumours. They examined different tumour types, and found that 5 of 10 human small-cell lung carcinoma (SCLC) samples expressed SHH and GLI1. Only 9 of 40 non-SCLC (NSCLC) tumour samples expressed SHH, however, and only 4 of these also expressed GLI1. These findings indicate that the HH signalling pathway is reactivated in lung cancer cells — predominantly in SCLC.

But is ligand-driven HH pathway activation required for SCLC formation? Antibody inhibition of SHH prevented the growth of cultured SCLC cells. Furthermore, treatment of nine SCLC cell lines that expressed both SHH and GLI1 with cyclopamine — an alkaloid inhibitor of the HH pathway — induced both growth arrest and apoptosis. Cyclopamine had no effect on growth of NSCLC cells, and a closely related compound that does not inhibit HH signalling had no effect on SCLC cells. Cyclopamine also inhibited growth of three different SHH- and GLI1-expressing SCLC xenografts in nude mice, but not of NSCLC or colon cancer xenografts.

Activation of HH signalling has been previously associated with medulloblastoma. The HH pathway regulates cerebellar progenitor differentiation, and in this brain tumour it is believed to allow malignant cells to maintain progenitor-like fates. Similarly, SCLC might represent a malignancy that arises from an airway epithelial progenitor and has maintained its HH signalling capabilities, as these cells continue to express SHH and lack *PTCH* mutations. Drugs designed to inhibit HH signalling could therefore have therapeutic effects in patients with SCLC.

Kristine Novak

References and links

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FURTHER READING

Berman, D. M. *et al.* Medulloblastoma growth inhibition by Hedgehog pathway blockade. *Science* **297**, 1559–1561 (2002)

WEB SITE

Stephen Baylin's lab: http://www.hopkinsmedicine.org/graduateprograms/cmm/baylin.html

mutant of c-CBL (lacking ubiquitin-ligase activity), which indicates that c-SRC activates a self-ubiquitylating function of c-CBL. However, the RING-finger mutant is still susceptible to the degradative effects of c-SRC, so it is probable that alternative, ubiquitin-independent mechanisms for the degradation of c-CBL are also used.

These data indicate the following mechanism of oncogenic cooperation. Increased expression of c-SRC or expression of a constitutively active form leads to the destruction of c-CBL (through self-ubiquitylation or other means). In turn, reduced levels of c-CBL mean that EGFR ubiquitylation and endocytosis (receptor desensitization) are inhibited and growth-factor signalling is increased, thereby potentiating EGF-induced mitogenesis.

Kirsty Minton

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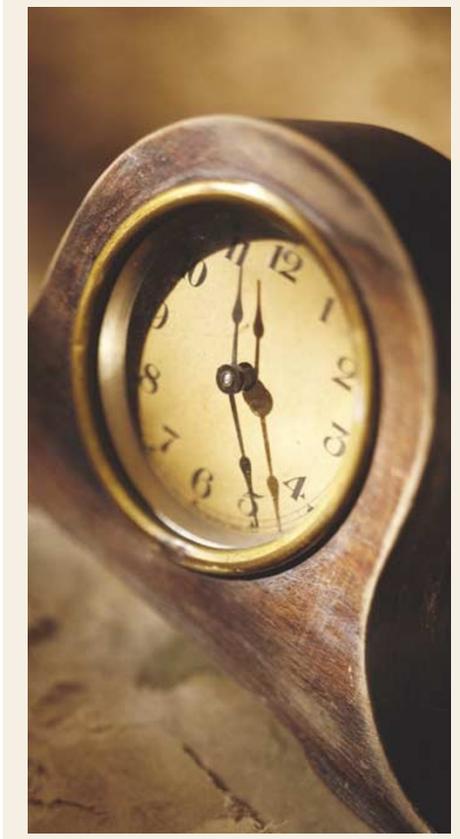

ONCOGENESIS

Winging your way to cancer with wingless

The *WNT* oncogene — first identified as *wingless* in *Drosophila* — is known to have a role in tumour development, so could it be a useful therapeutic target? Lewis Chodosh and colleagues, reporting in the 15 February issue of *Genes & Development*, investigate this using a conditional mouse model of breast cancer and show that loss of Wnt1, even in advanced cancer, can result in tumour regression.

Transgenic mice were generated that expressed Wnt1 in mammary tissue only in the presence of doxycycline. Induction of Wnt1 resulted in expression of *Myc* — a Wnt1 transcriptional target — and an increase in ductal sidebranching by 96 hours. Prolonged exposure to Wnt signalling by continuously administering doxycycline resulted in the development of invasive mammary tumours — mostly adenocarcinomas — in 90% of mice within 1 year; control mice remained tumour-free over this period. Similar to human breast cancers, which frequently metastasize to the lung, 3 of 10 mice with overt Wnt-induced mammary tumours had lung metastases at the time of sacrifice.

The long latency for tumour development indicates that other genetic alterations are required for tumour formation, and this might lessen the therapeutic effect of inhibiting Wnt1. However, removal of doxycycline after mammary tumour formation resulted in complete regression in 94% of cases within approximately 2 weeks. Analysis of gene expression in tumours grafted onto syngeneic mice confirmed that expression of both *Wnt1* and *Myc* was significantly decreased by 36–54 hours after doxycycline withdrawal.

Even more genetic changes must occur for a tumour to acquire the ability to metastasize, but, interestingly, metastatic lesions that had been explanted onto host mice were still sensitive to doxycycline withdrawal and transgene downregulation — all seven that were investigated regressed completely within 2–4 weeks.

So, do any genetic lesions influence this regression? Loss of p53 frequently occurs in human breast cancers and has previously been shown to increase the aggressiveness of Wnt1-induced mammary tumours. Many Wnt-induced tumours that lack p53 were still able to regress fully following doxycycline withdrawal, indicating that p53 itself is not required for tumour regression. However, loss of a single *Trp53* allele did markedly reduce the number of tumours that regress completely — 40% failed to regress to a non-palpable state (compared with 6% of tumours in *Trp53* wild-type mice) and rapidly resumed growth despite the continued absence of *Wnt1* transgene expression. This might have been due to the spontaneous loss of heterozygosity for *Trp53*, and resulting chromosomal instability that was observed by FACS analysis in tumours arising in $Trp53^{+/-}$ mice.

For Wnt1 inhibition to be an effective cancer treatment, it must be able to induce long-term regression. Almost a third of mammary tumours recurred within a year, and both the extent and rate of recurrence was accelerated in $Trp53^{+/-}$ mice, a group in which almost 80% of tumours had recurred by 30 weeks.

So, p53 loss increases tumour recurrence in animals whose tumours have fully regressed, and so decreases disease-free survival. Wnt inactivation exerts a selection pressure for loss of p53 and this, in turn, could impair the effectiveness of drugs that target this pathway. Nevertheless, this report does show that developing inhibitors of Wnt could be an effective anticancer strategy that is worth pursuing.

Emma Greenwood

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WEB SITE

Lewis Chodosh's lab:
http://www.uphs.upenn.edu/abramson/chodosh.html