METASTASIS

Developing a sense of direction

Metastasizing cancer cells seem to know exactly where they are going. But cancer cells don't normally express the chemokine receptors that are required for homing to target organs, so how do they develop this sense of direction? According to a report in *Nature* by Wilhelm Krek and colleagues, expression of the CXCR4 chemokine receptor is negatively regulated by the von Hippel–Lindau (VHL) tumour suppressor protein under normoxic conditions, and is induced by hypoxia-inducible factor (HIF) during hypoxic conditions.

The authors were comparing gene-expression profiles of VHL-deficient A498 renal carcinoma cells (RCCs) with A498 cells that were engineered to express a haemagglutinin-tagged VHL, and noticed that *CXCR4* mRNA expression was strongly suppressed in the VHL-expressing cells. However, they went on to show that a mutant form of VHL — which prevents VHL from degrading HIF1α — could not suppress *CXCR4* expression, indicating that *CXCR4* is directly regulated by HIF. *CXCR4* mRNA accumulation in hypoxic renal epithelial cells occurs at a similar rate to *GLUT3* — a known target of HIF — providing further evidence that *CXCR4* is a hypoxia-inducible gene. So, there seems to be a link between loss of VHL function, hypoxia and overexpression of *CXCR4*.

They analysed the *CXCR4* promoter and intron, and found four potential hypoxia-responsive elements. Luciferase reporter assays that analysed this region showed a twofold increase in luciferase expression in response to hypoxia in VHL-positive human embryonic kidney cells. Co-transfection with wild-type HIF1α enhanced reporter activity by tenfold and deletion analysis revealed an upsteam hypoxia-response element that was crucial for this HIF1α-inducible reporter activity. Electrophoretic mobility-shift assays showed that HIF2α binds to this element, confirming that the *CXCR4* promoter is targeted by HIF.

So, does expression of *CXCR4* have an effect on cell migration? In transwell assays, increasing concentrations of stromal-derived factor 1α (SDF1α) — the chemokine that is specific for CXCR4 — caused directed migration of A498 cells, and restoration of wild-type VHL abrogated this response. SDF1α stimulation activates rapid accumulation of LIM kinase-1 and extracellular-signal-related kinases (ERKs) that control cell movement and proliferation. But, is inactivation of VHL and upregulation of CXCR4 occurring *in vivo*?

MOUSE MODELS

Different strokes

Lung cancer can come in one of two forms — small cell and non small cell — that are initiated by different sets of genetic lesions and that have very different phenotypes. Mouse models have previously proven useful in identifying the changes that are required to induce non-small-cell lung cancer, and Anton Berns and colleagues have used the same approach to investigate the changes that cause small-cell lung cancer (SCLC). Interestingly, the changes are very different: instead of an activating mutation in *Kras*, both the *Trp53* and *Rb* tumour-suppressor genes must be inactivated.

The authors investigated the role of Rb and p53 because they are frequently mutated in human SCLC. They generated mice with conditional alleles of the two genes, which could be inactivated in lung epithelial cells by administering Ad-Cre by either intratracheal injection or intubation. The lungs of these mice were examined after 2–5 months and were found to contain areas of neoplasia in which both the *Rb* and *Trp53* alleles had been lost. These cells expressed two neuroendocrine (NE) cell markers — synaptophysin and the neural cell adhesion molecule 1 (Ncam1) — which indicates that they resemble SCLC, as SCLC is derived from cells that share the NE phenotype.

Mice that were not examined at this early stage were sacrificed when they became moribund. Three strains were investigated — $Trp53^{f/f};Rb^{f/f}$ (in which all alleles were conditional), $Trp53^{+/f};Rb^{f/f}$ and $Trp53^{f/f};Rb^{+/f}$ (in which one of the tumour suppressors retains a wild-type allele) — which differed in several respects. The $Trp53^{f/f};Rb^{f/f}$ mice had the shortest median tumour-free survival time of 210 days, with the $Trp53^{+/f};Rb^{f/f}$ and $Trp53^{f/f};Rb^{+/f}$ mice remaining tumour free until 364 and 575 days, respectively. Tumours from the $Trp53^{f/f};Rb^{f/f}$ mice frequently metastasized to bone, brain, adrenal glands, ovaries and liver, whereas tumours from $Trp53^{f/f};Rb^{+/f}$ mice rarely did and from $Trp53^{+/f};Rb^{f/f}$ mice never did.

The primary tumour types that developed were also somewhat different. The $Trp53^{f/f};Rb^{f/f}$ mice developed mostly SCLC — histological analysis confirmed their resemblance to human SCLC — but the other strains also developed some pulmonary adenocarcinomas. The longer survival time of the $Trp53^{+/f};Rb^{f/f}$ and $Trp53^{f/f};Rb^{+/f}$ mice is probably related to the delay before the remaining wild-type allele is lost, as Southern blotting of the loci confirmed that loss of heterozygosity had occurred in all SCLC cases. Interestingly, this was not the case for pulmonary adenocarcinomas; PCR analysis of microdissected tissue from $Trp53^{f/f};Rb^{+/f}$ mice showed that loss of Rb was not required for development of the adenocarcinomas.

As well as NE markers, the achaete scute complex homologue-like 1 (ASCL1) transcription factor is frequently expressed in human SCLC, and it is thought to determine the onset of NE differentiation in lung epithelia and lung cancers with NE features. Indeed, the mouse SCLCs and their liver metastases generally stained positive for Ascl1 and the NE markers synaptophysin and Cgrp.

So, this mouse model closely resembles human SCLC, and shows that inactivation of both p53 and Rb are required for the initiation of SCLC. The applications of the

They studied expression of *CXCR4* and the HIF target genes carbonic anhydrase (*CA9*) and *GLUT1* in RCCs, and mRNA levels of all three were significantly higher in clear-cell RCC than in papillary RCC or normal renal tissue. *CXCR4* expression was investigated in a wide range of RCCs using renal cancer tissue microarrays. Although there was no correlation between high level *CXCR4* expression and tumour stage and/or differentiation grade, there was a strong correlation between *CXCR4* expression and poor survival.

These results provide a mechanism of how RCCs acquire the ability to metastasize. The absence of oxygen — characteristic of highly aggressive tumours — induces expression of *CXCR4*, allowing metastatic RCCs to find their way to distant sites. In addition, tumour cells could be primed to spread early in tumorigenesis by acquiring mutations in *VHL*.

Emma Croager

References and links

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FURTHER READING Bernards, R. Cues for migration. *Nature* **425**, 247–248 (2003)

WEB SITE

Wilhelm Krek's lab:
http://www.verw.ethz.ch/cgi-win/whoShow.exe/ws7?ID=1678&lang=engl

model are yet to be determined, but should include identification of contributing genetic lesions and the testing of treatment strategies.

Emma Greenwood

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WEB SITE

Anton Berns's lab:
http://www.nki.nl/nkidep/h5/berns_main.htm

TUMORIGENESIS

Complex catastrophe

Mutations in the CDC42 GTPase that cause it to stay in the active GTP-bound form are oncogenic, and injection of this form into nude mice results in tumour formation. However, the mechanism for this is unknown, as it does not seem to be related to the known ability of CDC42 to regulate the actin cytoskeleton. Now, Richard Cerione and colleagues report in *Cell* that active CDC42 sequesters the c-CBL ubiquitin ligase into a complex with COOL1, so that it is unable to ubiquitylate and downregulate the epidermal growth factor receptor (EGFR).

COOL1 was originally placed in the CDC42 pathway because of its identification following two-hybrid experiments with the CDC42 target PAK. Further two-hybrid and immunoprecipitation experiments showed that COOL1 could, in turn, bind to CBLB. The authors continued with these analyses and found that COOL1 could also bind c-CBL, and that c-CBL and COOL1 could bind CDC42, but only mutated, constitutively active forms. EGF can activate CDC42, and the authors found that addition of EGF stimulated formation of a complex between these three proteins.

Formation of this complex seems to be essential for the transforming ability of CDC42, as removal of 13 amino acids that constitute the RHO-insert region diminished the ability of CDC42 to interact with COOL1 and CDC42-induced colony formation in soft agar — a reliable indicator of malignant transformation. COOL1 mutants that are unable to bind to CDC42, and c-CBL mutants that are unable to bind to COOL1, also failed to induce colony formation in soft agar or growth of fibroblasts in low serum.

So, how does this complex initiate transformation? c-CBL is a ubiquitin ligase that can downregulate EGFR, so perhaps its interaction with CDC42 alters this ability. Indeed, expression of activated CDC42 inhibits c-CBL from ubiquitylating EGFR, and prevents the EGF-dependent phosphorylation of c-CBL that stimulates its ubiquitin-ligase activity. Western blotting confirmed that whereas the EGFR is normally downregulated within 5–45 minutes after addition of EGF, this is not the case when activated CDC42 is also expressed — EGFR could still be detected, albeit at a lower level, after 6 hours.

CDC42 therefore seems to promote transformation by sequestering c-CBL and preventing it from degrading EGFR. This results in the maintenance of the EGF signalling pathway that operates through the mitogen-activated protein kinase (MAPK) cascade. Inhibition of this pathway with inhibitors of either EGFR or MAPK kinase (MEK) prevents cells with activated CDC42 from growing in low serum, and they lose their transformed morphology.

EGFRs are frequently upregulated in breast cancer and glioblastomas; although gene amplification is one mechanism by which this might occur, oncogenic mutations that result in activated CDC42 might well constitute another.

Emma Greenwood

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WEB SITE

Richard Cerione's lab:
http://web.vet.cornell.edu/public/research/gradEd/cerione.html