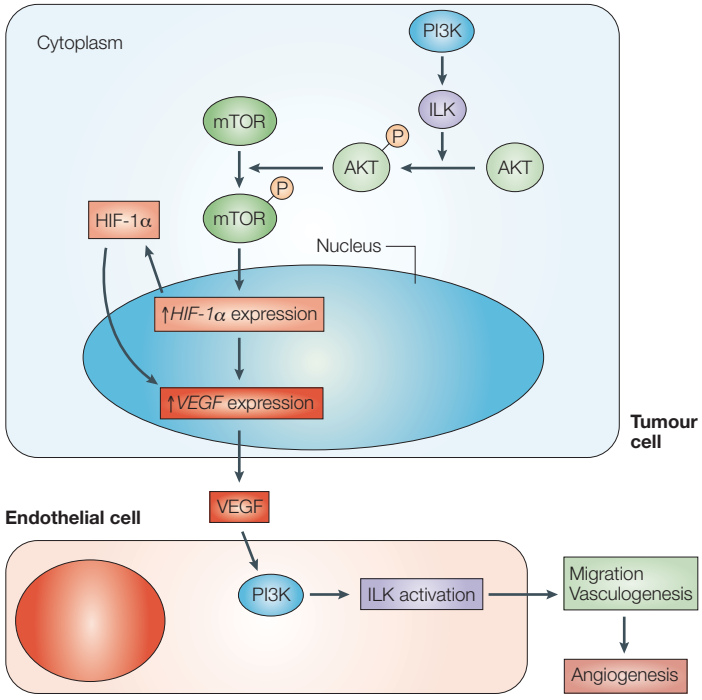
ANGIOGENESIS

Going for the double



Angiogenesis inhibitors have great potential as anticancer therapies, but have so far given disappointing results in clinical trials. Reporting in *Cancer Cell*, Shoukat Dedhar and colleagues reveal that integrin-linked kinase (ILK) has two key roles in tumour angiogenesis, and is therefore a promising new target for anti-angiogenic therapies.

Vascular endothelial growth factor (VEGF) is crucial for tumour angiogenesis, as it is produced by tumour cells and promotes the proliferation and migration of endothelial cells. VEGF expression can be stimulated by the phosphatidylinositol-3-kinase (PI3K) pathway. PI3K stimulates the phosphorylation and activity of AKT, which, in turn, increases the synthesis of transcription factors such as hypoxia-induced factor-1α (HIF-1α), to upregulate VEGF expression.

As ILK is directly upstream of AKT in the PI3K pathway, Dedhar and colleagues investigated whether it is also required for tumour angiogenesis. Overexpression of Ilk in rat intestinal epithelial cells led to increased Vegf expression compared with control cells, and to increases in the levels of phosphorylated Akt and Hif-1α-mediated transcription. Conversely, a small-interfering RNA (siRNA) directed against *Ilk* suppressed Vegf expression. The authors also showed ILK is required for high levels of VEGF expression in the PC3 human prostate cancer cell line, and went on to show that this involves the activation of mTOR, a target of AKT. So, ILK has a key role in the ability of tumour cells to stimulate VEGF expression through its effects on AKT activation (see figure).

To test the potential of ILK inhibition as an antiangiogenic therapy, the authors exposed PC3 cells and DU145 cells — another prostate cancer cell line — to an ILK inhibitor. This suppressed both VEGF and HIF-1α expression in a dose-dependent manner.

As ILK is known to promote cell motility in response to growth factors, Dedhar and colleagues tested whether ILK might also function in endothelial cells in VEGF-mediated migration and vasculogenesis. Human umbilical-vein endothelial

METASTASIS

Migratory cues

Like birds flying south for the winter, cancer cells can migrate from the site of the primary tumour and disseminate around the body. But what signals turn a non-invasive cancer cell into a metastatic cell? Using a unique *in vitro* system that models the early stages of breast cancer, Joan Brugge and colleagues show that ERBB2 and transforming growth factor-β (TGF-β) work together to cause invasion and migration of breast cancer cells.

Under certain culture conditions, MCF10A cells that are engineered to express activated ERBB2 (10A.B2 cells) produce structures that share properties with ductal carcinoma *in situ* (DCIS), an early-stage, non-invasive breast cancer. So, 10A.B2 cells were infected with retroviral vectors expressing breast-cancer-associated cDNAs to identify factors that could promote metastasis. Only TGF-β1 and TGF-β3 increased migration of the activated 10A.B2 cells, indicating that ERBB2 and TGF-β might cooperate to induce migration. Soluble TGF-β had a similar effect, although to a lesser extent, indicating that autocrine TGF-β stimulation is particularly important. Furthermore, this induction of migration seems to be dependent on ERBB2, as TGF-β1 and TGF-β3 had no effect on MCF10A cells expressing the activated epidermal growth factor receptor ERBB1.

Various assays confirmed that TGF-β1 and TGF-β3 expression induced migration and invasion of activated 10A.B2 cells. As the extracellular signal-regulated kinase ERK is implicated in migration and is activated by ERBB2 and TGF-β, the authors investigated ERK activity in the TGF-β-expressing 10A.B2 cells. Phosphorylation of both ERK and the ERK kinase MEK was increased in these cells compared with controls, indicating that TGF-β and ERBB2 increase ERK activation. This was investigated further by expressing TGF-β and activated MEK in the absence of activated ERBB2; migration and invasion were substantially increased, compared with controls expressing activated MEK alone. So, MEK activation is insufficient to induce migration and invasion, but it can substitute for ERBB2 in the presence of TGF-β. MEK inhibitors reduced migration of TGF-β-expressing 10A.B2 cells by 85% and invasion by 65%, confirming that ERK activation is required for the synergism between TGF-β and ERBB2.

How might ERBB2 and TGF-β induce this migration? Cultured medium from activated TGF-β-expressing 10A.B2 cells induced migration of MCF10A cells, but mixing cultured medium from cells expressing either TGF-β or activated ERBB2 alone did not enhance migration, indicating that TGF-β and ERBB2 must cooperate to produce soluble migratory factors. ERBB1-specific antibodies reduced the stimulating activity of the cultured medium by 50–60%, indicating that other pathways contribute to migratory activity.

As about 80% of DCIS lesions express ERBB2, this work indicates that TGF-β expression could represent one step in the process that turns a non-invasive tumour into metastatic breast cancer.

Emma Croager

References and links

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WEB SITE

Joan Brugge's lab:
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cells (HUVECs) that were exposed to VEGF showed increased ILK activity. This was dependent on PI3K, as this effect was blocked by the addition of a PI3K inhibitor. Furthermore, inhibition of ILK function suppressed the migration and proliferation of HUVECs in response to VEGF. The authors also showed that ILK inhibitors block VEGF-stimulated angiogenesis in two standard assays.

A mouse xenograft model was used to test the effects of ILK inhibitors *in vivo*. Nude mice were injected with PC3 cells, and animals bearing well-established tumours were treated with an ILK inhibitor. Treated mice showed a reduced density of tumour-associated blood vessels and a decrease in tumour mass, as compared with untreated mice, and did not show any obvious side-effects. These results indicate that ILK, with its dual role in tumour angiogenesis, might prove to be a useful target for anticancer therapies.

Louisa Flintoft

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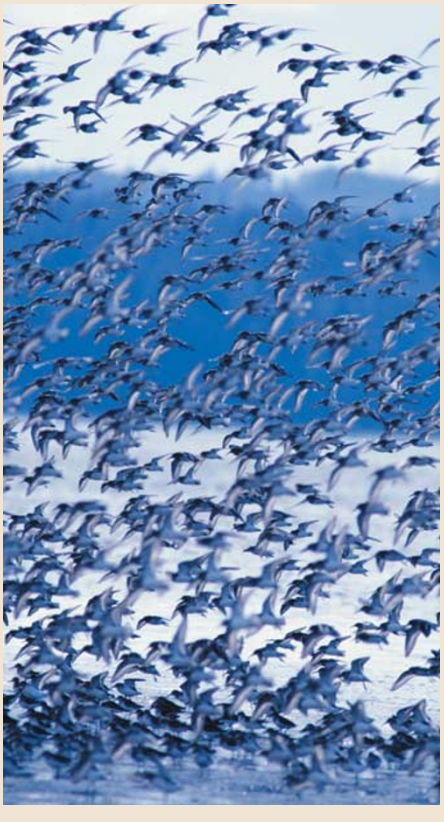

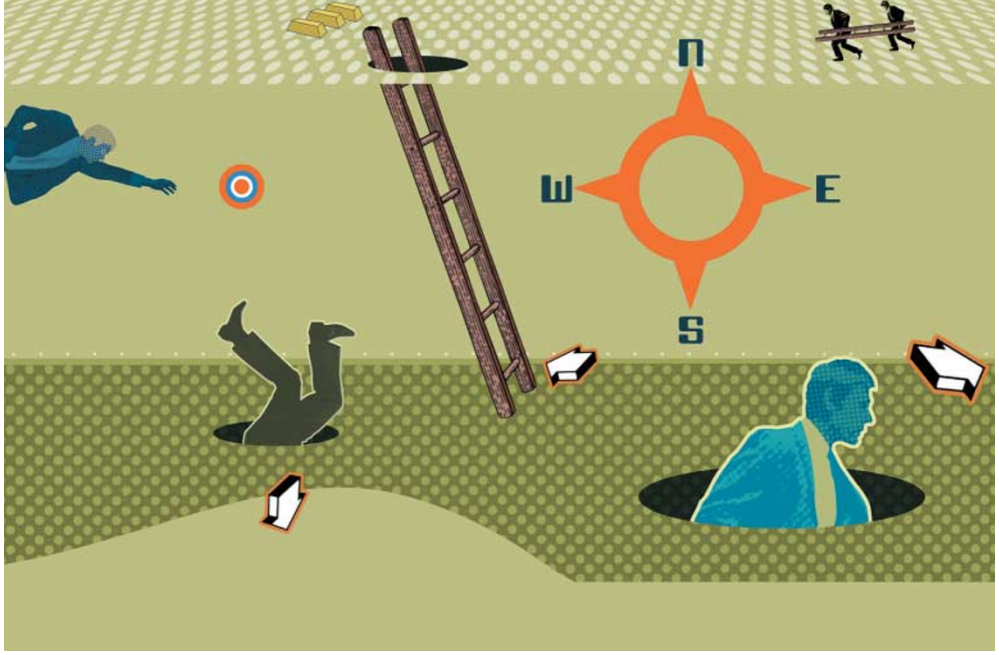

TUMOUR SUPPRESSORS

A topsy-turvy world

Tumour suppressors normally inhibit cell proliferation, but one seems to act in the opposite manner. Chun-Ming Chen and Richard Behringer report in the February issue of *Genes & Development* that it is the loss of OVCA1 that causes proliferation defects — only when its loss is combined with that of p53 is its tumour-suppressive activity unleashed.

The genes that cause ovarian cancer are largely unknown, so the identification of a region of chromosome 17 that is frequently lost was an important find. The *OVCA1* gene has since been shown to reside at that location, but it has yet to be shown to act as a tumour suppressor. The authors had previously identified the mouse orthologue, and continued their studies to understand the function of Ovca1.

They generated knockout mice, but these either died during development or shortly after birth. The embryos showed developmental defects and were generally smaller. Transplanting the ovaries into kidney capsules of wild-type mice allowed their development to be followed over time, and although smaller, development seemed normal and there was no evidence of carcinomas.

The growth defect could be caused by a decrease in proliferation or an increase in apoptosis, so *Ovca1*-null mouse embryonic fibroblasts (MEFs) were established to answer this question. The MEFs grew poorly, but there was no sub-G1 population, which would be indicative of apoptosis. Cell-cycle analysis revealed that fewer cells were in S phase, and this corresponded with a decrease in phosphorylated Rb, which would explain the inability of the cells to enter S phase.

But this is an unusual property for loss of a tumour suppressor, so might elimination of a checkpoint allow the cells to recover from this proliferation defect? The authors found that $Ovca1^{-/-}Trp53^{+/-}$ MEFs also had a reduced S-phase population, but $Ovca1^{-/-}Trp53^{-/-}$ MEFs grew normally. Loss of p53 could not rescue the developmental defects though — the mice still died soon after birth.

The next important question was whether Ovca1 actually did act as a tumour suppressor *in vivo*. Almost 60% of $Ovca1^{+/-}$ mice developed a range of tumours by two years, with an average latency of 92 weeks. This was shortened to 52 weeks in $Ovca1^{+/-}Trp53^{+/-}$ mice, and the incidence was increased to 72%. Importantly, the loss of one *Ovca1* allele increases the tumour incidence when compared with $Trp53^{+/-}$ mice, and although the tumour incidence in $Ovca1^{+/-}Trp53^{-/-}$ and $Trp53^{-/-}$ mice is the same, more mice have numerous tumours when they have lost one *Ovca1* allele. The tumour spectrum is also somewhat different in the *Ovca1* heterozygous mice.

So, despite its role as a positive regulator of cell-cycle progression, it seems that OVCA1 is, indeed, a tumour suppressor. Further work is needed to understand exactly how it functions in normal cells, and how its loss accelerates tumour progression when p53 is also lost.

Emma Greenwood

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FURTHER READING Jensen, M. R. & Helin, K. OVCA1: emerging as a *bona fide* tumor suppressor. *Genes Dev.* **18**, 245–248 (2004)

WEB SITE

Richard Behringer's lab:
http://www3.mdanderson.org/~genedev/behringer.html