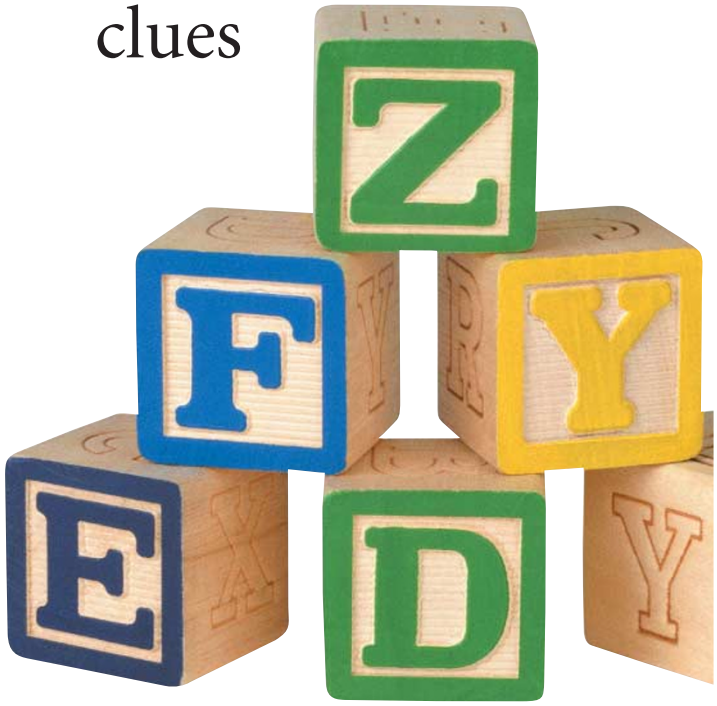
MOUSE MODELS

Reconstruction clues



One of the key challenges to breast cancer research has been to create a realistic model of this disease. Bob Weinberg and colleagues now report a new method for reconstructing human mammary epithelium in mice, which highlights the importance of the stroma in breast tumorigenesis.

The authors hypothesized that establishing human mammary stroma within the mouse mammary fat pad would provide a suitable environment for the human mammary epithelial cells (MECs) to grow. To do this, they injected a mixture of irradiated and non-irradiated mammary stromal fibroblasts into the mammary fat pads of immunocompromised mice — irradiation has been previously shown to activate expression of proteases, matrix proteins and growth factors in fibroblasts. Subsequent injection of a mixture of human breast fibroblasts and human MECs, prepared from histologically normal human reduction mammoplasty tissues, into the humanized stromal fat pads led to development of human epithelial growths with acinar, ductal and lobular structures. Weinberg and colleagues observed that this human breast tissue was fully functional during pregnancy — the cells were histologically normal, and synthesized and secreted lipids into the lumina of the human acini.

When human MEC preparations isolated from patients who had undergone mammoplasty were introduced into the humanized stromal fat pads without addition of primary human mammary fibroblasts, 30% of the samples underwent hyperplastic ductal growth. This indicates that the primary human fibroblasts suppress hyperplasia.

Two growth factors synthesized by mammary stromal cells are overexpressed by human breast cancer cells — hepatocyte growth factor causes hyperproliferation of mammary epithelium, and transforming growth factor-β1 inhibits proliferation of stromal cells and induces neoangiogenesis. The authors observed that when either of these factors was ectopically

THERAPEUTICS

Means to an end

Many chemotherapeutic drugs induce cancer-cell apoptosis, so disruption of this pathway is a primary method by which tumours become drug resistant. For example, a survival signalling pathway mediated by the kinase AKT is activated in many tumour types. Scott Lowe and colleagues show that disruption of Akt signalling with the drug rapamycin reverses tumour chemoresistance in a mouse model of lymphoma, so this drug might be used to increase the efficacy of cytotoxic cancer therapies.

Several signalling pathways regulate the fine balance between whether a cell undergoes apoptosis or survives. Much attention has recently been focused on the phosphatidylinositol 3-kinase (PI3K) pathway, which signals through the kinases AKT and TOR. TOR is inhibited by the drug rapamycin, which is used in the clinic as an immunosuppressant. Lowe and colleagues explored the consequences of using rapamycin to inhibit the Akt signalling pathway in their mouse lymphoma model.

Activation of Akt accelerates tumorigenesis and allows tumours to become refractory to chemotherapeutic agents such as cyclophosphamide and doxorubicin. But when Akt signalling was blocked with rapamycin, tumours became sensitive to the chemotherapeutic agents. Cancer cells rapidly underwent apoptosis and complete remission was achieved in mice. The authors showed that rapamycin acted specifically on the Akt signalling pathway — it had no effect on tumours that developed chemoresistance through upregulation of other anti-apoptotic proteins, such as Bcl2. Rapamycin was also not effective as a single agent.

How does AKT–TOR signalling promote cell survival? The kinase TOR normally regulates translation in response to nutrients and growth factors by phosphorylating key components of the protein-synthesis machinery, such as the ribosomal protein S6 kinase p70S6K and the 4E-BP proteins. Phosphorylated 4E-BP1 then releases the translation initiation factor eIF4E, leading to protein synthesis (see figure). So what happens when eIf4e is constitutively activated in this cancer model? Expression of eIf4e accelerated lymphomagenesis in a manner that was similar to Akt, and these tumours also became resistant to chemotherapy. They were not, however, sensitive to rapamycin — presumably because eIf4e acts downstream of Tor.

Clinical trials of rapamycin and its analogues, in combination with other chemotherapeutic agents, are underway. This study reminds us, however, that the tumour genotype should be analysed before patients are given this chemosensitizer — rapamycin would not have an effect on tumours that evade apoptosis through upregulation of BCL2, eIF4E or other pro-survival proteins.

Kristine Novak

References and links

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FURTHER READING McCormick, F. Survival pathways meet their end. *Nature* **428**, 267–269 (2004)

WEB SITE

Scott Lowe's lab:
http://www.cshl.org/public/SCIENCE/lowe.html

expressed in the humanized stromal fat pads, some of the injected human MEC cells that were isolated from mammoplasty samples developed into invasive and poorly differentiated carcinomas. Surprisingly, if MECs taken from the same patient but from different regions of the mammoplasty tissue were used, no carcinoma was formed.

The authors suggest that the reduction mammoplasty MECs must have included cells that were already abnormal when removed from the donor mammary gland (although this was undetectable by histology) and that these cells then thrived in the altered stromal environment. This model provides a new way of studying human breast cancer pathogenesis.

Ezzie Hutchinson

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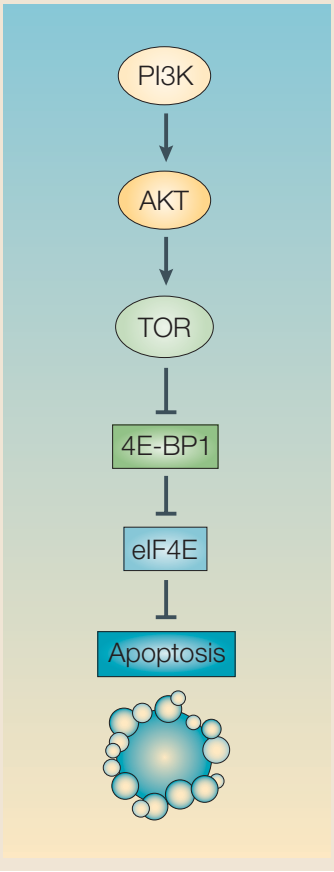
Robert Weinberg's lab:
http://web.wi.mit.edu/weinberg/pub/



TARGETED THERAPIES

Bullseye

Developing inhibitors that specifically act on a single protein can be a tricky business, but researchers have hit the bullseye in their attempt to target the insulin-like growth factor receptor-1 (IGF1R). In *Cancer Cell*, Hofmann and colleagues and Kung and colleagues report that IGF1R inhibitors are selective and have significant antitumour activity.

Insulin-like growth factors and their receptors have been implicated in several processes involved in cancer — proliferation, survival, metastasis and angiogenesis. However, there have been concerns about targeting IGF1R to treat cancer because of its widespread expression in normal tissues and the potential for cross-inhibition of the highly related insulin receptor. Hofmann and colleagues performed a high-throughput screen with their compound archive and identified small molecules of the pyrrolo[2,3-*d*]pyrimidine class that selectively inhibited IGF1R. They optimized the potency and selectivity of this class — which they assessed using *in vitro* kinase and cellular assays — and then characterized the most effective, NVP-AEW541. Of particular importance was that this inhibitor was 27-fold more potent against IGF1R than against the insulin receptor, at the cellular level.

They first showed that NVP-AEW541 inhibited the IGF1-mediated survival of MCF-7 cells and prevented them from growing in the absence of anchorage. It also inhibited the proliferation of NWT-21 cells — this was shown to be through inhibiting the IGF1R kinase and downstream signalling, as the pathway component AKT was no longer phosphorylated and was therefore inactive as a survival protein. In an *in vivo* model, in which NWT-21 cells were grown subcutaneously in nude mice, oral administration of NVP-AEW541 was able to inhibit tumour growth. So, NVP-AEW541 seems to be a potent and specific anticancer agent that is worth further investigation.

Kung and colleagues started by showing that IGF1R was specifically expressed in haematological and solid cancers, in both cell lines and primary cultures. They then investigated the effects of inhibiting the receptor using several strategies: a neutralizing antibody, a competitive peptide antagonist and a small-molecule inhibitor, NVP-ADW742, which is similar to NVP-AEW541. All three strategies similarly suppressed the proliferation of serum-stimulated tumour cells, but multiple-myeloma (MM) cells were particularly sensitive. The authors therefore focused on MM, developing an orthotopic xenograft mouse model. NVP-ADW742 was able to significantly suppress tumour growth and prolong the survival of the mice.

Global expression profiling revealed that inhibition of IGF1R resulted in a highly pleiotropic response, including decreased expression of genes involved in cell-cycle progression, proliferation and survival. This could explain why the inhibitors are effective on a range of tumour types, including those that are resistant to other treatment. Indeed, NVP-ADW742 could sensitize tumour cells to other anticancer agents, such as doxorubicin and melphalan, both *in vitro* and *in vivo*.

So, IGF1R inhibitors have been developed that are potent, selective and effective anticancer agents in preclinical models. Pursuing these further should be a useful approach for treating cancer.

Emma Greenwood

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