MODEL ORGANISMS

Overgrow without scribble

The *Drosophila* model system is increasingly being used to uncover new areas of investigation for cancer researchers, and a recent report in *The EMBO Journal* proves no exception. Anthony Brumby and Helena Richardson show that loss of the tumour suppressor scribble, which is involved in cell polarity, induces an overgrowth phenotype, and suggest that loss of such proteins could also contribute to tumour formation in humans.

Loss of *scrib* in the larval eye imaginal disc — using FLP/FRT-mediated recombination — resulted in cells losing their columnar shape and monolayered morphology, to become more rounded and multilayered. They also lost differentiation markers, and expressed increased levels of cyclin E, which induces proliferation. This correlated with increased labelling with the S-phase marker bromodeoxyuridine, to confirm the increased proliferation.

However, as larval development progresses, the *scrib*$^-$ tissue is lost through apoptosis. This seems to be mediated through the c-Jun amino-terminal kinase (Jnk) stress response, as inhibiting Jnk activity using a dominant-negative mutant prevented this apoptosis and increased the size of the *scrib*$^-$ clones. Interestingly, this response was not a cell-intrinsic response — removing the surrounding wild-type tissue resulted in massive overproliferation, presumably because apoptosis was prevented.

The authors next investigated whether other oncogenic mutations could overcome the suppressive effect of Jnk pathway activation on the *scrib*$^-$ phenotype. Whereas activated forms of Ras and Notch cooperated with *scrib*$^-$ to induce overgrowth — the mutant tissue proliferated in three dimensions and did not differentiate — activation of the Wingless, Hedgehog and Decapentaplegic pathways had somewhat different effects. Although they showed some differentiation defects and the *scrib*$^-$ clones were generally increased in size, they did not have the same overgrowth phenotype as that observed with activated Ras and Notch.

So, which Ras effector was important for the overgrowth response? Neither overexpression of PI3K, nor its effectors, could mimic the effect of activated Ras, but an activated *Raf* allele could induce overproliferation of *scrib*$^-$ clones. The mitogen-activated protein kinase (MAPK) pathway therefore mediates this cooperation, but it, in turn, can influence processes such as survival, growth, proliferation and differentiation. Surprisingly, recapitulating proliferation by expressing cyclin E, and apoptosis inhibition by expressing the caspase inhibitor p35, could still not mimic the overgrowth phenotype that is caused by activated Ras. Other effectors of Ras could therefore also be important.

Although *scrib* homologues have not yet been shown to be tumour suppressors in humans, this research does indicate that proteins that dictate cell polarity could be important in tumorigenesis and should be investigated further.

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References and links

ORIGINAL RESEARCH PAPER Brumby, A. M. & Richardson, H. E. *scribble* mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in *Drosophila*. *EMBO J.* **22**, 5769–5779 (2003)

THERAPEUTIC STRATEGIES

Less is best

Metronomic dosing — long-term, low-dose, frequent administration of chemotherapeutic drugs — reduces the toxic side effects of traditional chemotherapy. Rather than directly killing cancer cells, it prevents blood-vessel formation by blocking endothelial-cell growth. Why the endothelial cells of new blood vessels are specifically targeted by this dosing strategy is a mystery that Guido Bocci, Robert Kerbel and colleagues are on the way to solving — the angiogenesis inhibitor thrombospondin-1 (TSP1) thinks less is best.

Bocci *et al.* began by analysing gene-expression profiles of microvascular endothelial cells and found that long-term exposure to the antitumour agent BAL-9504 increased *TSP1* expression. Further *in vitro* investigations confirmed this observation, showing that *TSP1* expression increased in the drug-treated cells, which also secreted the protein into the culture medium. The effects of the metronomic chemotherapy — blocked proliferation and reduced cell survival — were partially reversed by TSP1-neutralizing antibodies, indicating that TSP1 regulates the metronomic dosing response *in vitro*. But does it have a similar effect *in vivo*?

They administered a previously well-characterized, low-dose, daily cyclophosphamide treatment to wild-type and *Tsp1*-null mice and assessed *in vivo* angiogenesis. After 7 days, the cyclophosphamide treatment had significantly reduced neovascularization in the wild-type mice, but not in the *Tsp1*-null mice, indicating that Tsp1 is required to mediate the anti-angiogenic effects of the metronomic dosing regimen *in vivo*. To establish whether lack of Tsp1 also affected tumour growth, fast-growing tumour cells were injected into wild-type and *Tsp1*-null mice. The mice were initially treated with the maximum-tolerated dose of cyclophosphamide, which slowed growth of the tumours in both mice strains. This was followed by low-dose cyclophosphamide treatment, which reduced tumour growth in only wild-type mice. So, although lack of Tsp1 does not affect the response to the maximum-tolerated dose of cyclophosphamide, it does prevent the effects of metronomic chemotherapy.

As soluble, circulating TSP1 was observed after *in vitro* metronomic dosing, it might be a useful surrogate marker for monitoring the clinical outcome of metronomic chemotherapy treatments. Human-tumour-bearing immunodeficient mice were treated with various metronomic regimens and after 20 days antitumour effects were seen in all treated animals. This antitumour response correlated with a 2–6-fold increase in the Tsp1/tumour volume ratio, indicating that increased Tsp1 coincides with a decrease in tumour size.

This work provides an initial insight into the mechanisms that regulate the antitumour and anti-angiogenic effects of metronomic therapies. TSP1 might also be a useful clinical tool for monitoring how patients are responding to this treatment strategy.

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References and links

ORIGINAL RESEARCH PAPER Bocci, G. *et al.* Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. *Proc. Natl Acad. Sci. USA* **100**, 12917–12922 (2003)

WEB SITE

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