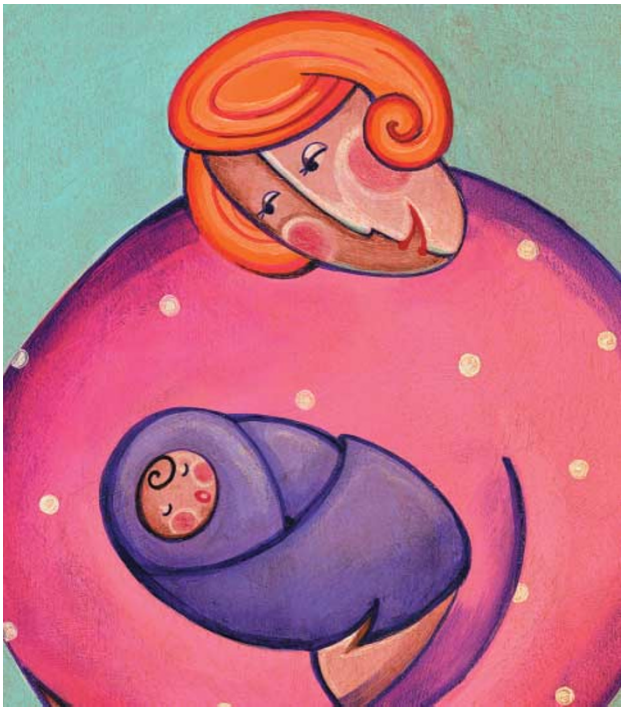
MOUSE MODELS

Inducing dormancy



The *MYC* oncogene is commonly activated in hepatocellular carcinoma. In a letter to *Nature*, Dean Felsher and colleagues describe how they have used a conditional transgenic mouse model to study expression of MYC in liver cells and show that MYC inactivation induces tumour regression and dormancy.

To establish a suitable model, the authors crossed a mouse in which the liver activator protein (LAP) promoter drives expression of the tetracycline transactivating protein (tTA) in liver cells with a mouse in which MYC is under control of the tetracycline response element (which is bound by tTA) — producing the LAP-tTA/tet-off-MYC mouse. Only mice expressing both the *Lap* and *Myc* transgenes and not treated with doxycycline — which also regulates tTA — expressed MYC and developed hepatocellular carcinomas.

As liver tumours are usually refractory to therapy, the authors expected that inactivation of MYC in the mice with established liver tumours would be ineffective in causing tumour regression. However, this was not the case; 50 transgenic mice moribund with liver tumours showed rapid and sustained tumour regression when treated with doxycycline to inactivate MYC.

So, what mechanism induces this regression? Within a few days of treatment with doxycycline, the tumour cells had lost their high mitotic index and markers of high proliferation, and showed increased apoptosis. In addition, expression of the immature differentiation marker α-fetoprotein decreased and expression of the hepatocyte marker carcinoembryonic antigen increased — the cells had differentiated into normal liver cells. When tumour cells were transplanted into the skin of severe combined immunodeficient (SCID) mice and MYC was inactivated, tumours quickly regressed and normal liver cells resembling hepatic lobules were observed. Interestingly, reactivation of MYC expression led to tumour regrowth with identical histology and genomic signatures to the original tumour, and the authors therefore conclude that the tumour cells remain dormant and retain the capacity to regain neoplastic features.

DRUG RESISTANCE

Sidelined

A stem-cell population — called the side population (SP) — has been identified previously in normal bone marrow that has the capacity to export lipophilic dyes. Charlotte Hirschmann-Jax *et al.* wondered whether a similar SP might be present in tumours, with the capacity to pump out lipophilic anticancer drugs, and so contribute to early relapse of disease. They identified such a subpopulation in tumour cells taken from 23 patients with neuroblastoma who had relapsed after therapy.

Hirschmann-Jax *et al.* first identifed a SP in primary neuroblastoma tumour cells using the fluorescent dye Hoechst 3342 to separate out the population of cells with high efflux capability. SP cells account for ~0.03% of mononuclear cells in normal human bone marrow — the proportion of viable cells classified as SP cells in the neuroblastoma samples was 1.9% (ranging from 0.8–51%).

So, did these cells have any definitive markers and how did they behave? The authors found that the neuronal marker ganglioside (GD2) and the stem-cell growth-factor receptor KIT were overexpressed. This indicated that the cells have an early neural-crest progenitor cell phenotype — before they become neuroblasts or mature into differentiated progeny. Consistent with this finding, the authors observed that the SP subpopulation had a high proliferation rate and self-renewal capacity.

Next, they sorted SP and non-SP cells from the tumour samples and quantified the expression of the ABC-transportor protein (ABCG2). All SP fractions expressed higher levels of ABCG2 than non-SP cells, but levels of other ABC transporters, ABCA3 and MDR1, did not differ.

So, do the neuroblastoma SP cells show high efflux of lipophilic antineoplastic drugs? The authors incubated cells from five neuroblastoma patients with Hoechst dye and the naturally fluorescent drug mitoxantrone. Increased efflux of mitoxantrone with Hoechst dye was shown. Treatment with increasing concentrations of mitoxantrone led to an increase in the proportion of SP cells present, indicating selection of this population. Non-SP cells treated with mitoxantrone formed no colonies, whereas the number of colonies formed by the SP cells was unchanged following treatment.

The authors concede that because the patients from whom the samples were taken were in relapse, there might already have been selection for the SP population — the percentage of SP cells in newly diagnosed neuroblastomas might be much lower and needs investigating. Examination of various solid tumour cell lines in this study indicated that the SP might also be a more general feature of malignant disease and might therefore be an important target for anticancer treatment.

Ezzie Hutchinson

References and links

ORIGINAL RESEARCH PAPER Hirschmann-Jax, C. *et al.* A distinct 'side population' of cells with high drug efflux capacity in human tumor cells. *Proc. Natl Acad. Sci.* USA 28 Sept 2004 (doi:10.1073/pnas.0400067101)

WEB SITE

Malcolm K. Brenner's lab:
http://www.bcm.edu/genetherapy/faculty/brenner.html

If this hypothesis is true, some tumour cells must persist after MYC inactivation, so the authors crossed the LAP-tTA/tet-off-MYC mice with mice transgenic for luciferase, so that the liver tumour cells transplanted into SCID mice could be tracked by measuring light emitted by luciferase activity. Eight months after MYC inactivation, luciferase activity was still detectable in tumour cells, whereas normal liver cells were undetectable 5 days after MYC inactivation.

Serial transplantation of liver tumours with inactivated MYC only rarely led to relapse — the relapsed tumours had compensatory increases in L-MYC and N-MYC. The authors conclude that targeted inactivation of MYC might be an effective treatment for some liver cancers.

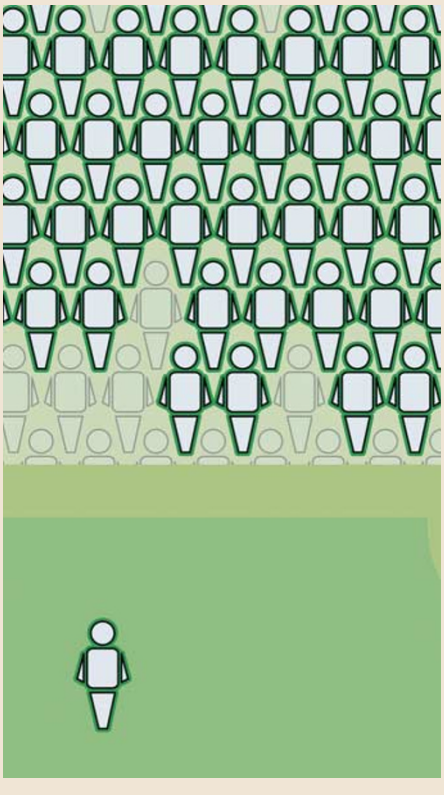
Ezzie Hutchinson

References and links

ORIGINAL RESEARCH PAPER Shachaf, C. M. *et al.* MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. *Nature* 10 Oct 2004 (doi:10.1038/nature03043)

WEB SITE

Dean Felsher's lab:
http://med.stanford.edu/labs/dean_felsher/



TUMORIGENESIS

Divining forks for developing tumours

In the past 20 years, there has been little progress in identifying improved chemotherapy regimens for children with metastatic rhabdomyosarcoma. Of the two subtypes, alveolar tumours have a worse prognosis than embryonal tumours. Much of the cell and molecular biology of alveolar rhabdomyosarcoma is unknown, apart from the presence of the paired box gene 3 (*PAX3*):forkhead (*FKHR*) t(2;13) translocation in most tumours. So Keller, Capecchi and colleagues have generated a new alveolar rhabdomyosarcoma mouse model to further investigate this rare tumour of skeletal muscle, and they present their results in two papers published in *Genes and Development*.

Keller and co-workers have knocked in a version of the *Pax3:Fkhr* fusion gene — believed to result in a gain-of-function mutation affecting muscle development — and at the same time generated the corresponding inactivation of one allele of *Pax3* and one allele of *Fkhr*, carefully re-creating the genetic situation in the human disease. Importantly, the authors have targeted this mutation to be expressed only in terminally differentiating skeletal muscle. Their paper addressing *Pax3:Fkhr* expression in early development shows, through extensive use of different conditional mouse models, that the *Pax3:Fkhr* mutation in early precursor, embryonal and postnatal muscle stem cells seems unlikely to give rise to tumours, because of the presence of significant muscle defects during embryogenesis and the complete absence of tumour development in mice expressing *Pax3:Fkhr* in postnatal muscle stem cells.

The authors followed the development and growth of 228 *Pax3:Fkhr* mice over 29 months and only one animal developed a rhabdomyosarcoma during this time. So, to investigate what might increase the tumour incidence, the authors first examined if loss of the functional allele of *Fkhr* enhanced the tumorigenic process (as loss of *Pax3* is known not to predispose to tumour formation), but no increase in tumour incidence was seen. Human alveolar rhabdomyosarcomas often have mutations in *TP53* or *CDKN2A* (which encodes INK4A and ARF), so *Pax3:Fkhr* mice were crossed with mice that had muscle-specific loss of one allele of either gene. Again, no increase in tumour incidence was seen, so the authors started to breed homozygotes for each gene. Their results show that rhabdomyosarcomas arise more frequently in these animals only in the absence of functional p53 pathways and mostly require *Pax3:Fkhr* homozygosity. Importantly, these mouse alveolar rhabdomyosarcomas were largely immuno-histologically similar to those found in humans.

The finding that rhabdomyosarcomas in mice most often arise from *Pax3:Fkhr* homozygotes and not heterozygotes differs from the human disease and might reflect that either the *Pax3:Fkhr* fusion gene is not completely identical to that occurring in humans, or might simply be a species-specific difference. However, these results do explain one of the controversies surrounding rhabdomyosarcoma development: that these tumours most likely arise from terminally differentiating skeletal muscle cells that seem to re-express early myogenic markers, and not from early muscle stem cells. Further characterization of this mouse model will hopefully provide novel molecular targets for the effective treatment of childhood alveolar rhabdomyosarcoma.

Nicola McCarthy

References and links

ORIGINAL RESEARCH PAPERS Keller, C. *et al.* Alveolar rhabdomyosarcomas in conditional *Pax3:Fkhr* mice: cooperativity of Ink4a/Arf and Trp53 loss of function. *Genes Dev*. 15 Oct 2004 (doi:10.1101/gad.1244004) | Keller, C. *et al. Pax3:Fkhr* interferes with embryonic *Pax3* and *Pax7* function: implications for alveolar rhabdomyosarcoma cell of origin. *Genes Dev*. 1 Nov 2004 (doi:10.1101/gad.1243904)