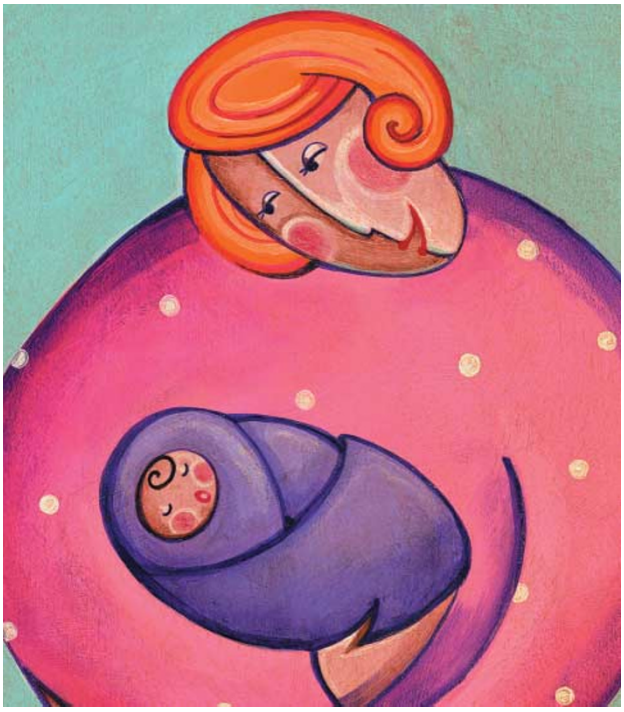
MOUSE MODELS

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.

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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

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