ONCOGENES

Determining fate

The OCT-3/4 transcription factor is known to determine cell fate — it is required for embryonal stem-cell self renewal and its altered expression results in different cell fates. However, Sharon Gidekel and colleagues now report in *Cancer Cell* that it can also determine the oncogenic potential of germ-cell tumours and could be a useful therapeutic target.

As tumours are thought to be maintained by tumour stem cells, the role of *OCT-3/4* in maintaining the stem-cell fate of germ cells led the authors to investigate whether it might also be expressed in germ-cell tumours. In the adult male, the expression of *OCT-3/4* is restricted to type A spermatogonia, but it was also found to be expressed in all 45 germ-cell tumours that were tested. The expression was particularly high in the pre-malignant cells, so it might contribute to germ-cell neoplasia from an early stage. It was not expressed in any of 182 non-germ-cell tumours.

But what effect does it actually have on tumorigenicity? The authors used several lines of engineered embryonic stem (ES) cells that expressed from 0–150% of wild-type *OCT-3/4*. These were injected subcutaneously into mice and the incidence of tumour formation was determined. Mice that were injected with wild-type cells developed tumours at an incidence of 83%, but this decreased to only 4.3% if *OCT-3/4* was not expressed. Upregulating *OCT-3/4* also increased the proportion of tumours with primitive neural and malignant-appearing tissues, further confirming that it influences cell fate.

Another question was whether OCT-3/4 is required to maintain the malignant phenotype. In one engineered ES cell line, the only copy of OCT-3/4 was under the tetracycline promoter, so could be controlled by doxycycline. When OCT-3/4 expression was switched off after tumours were established, the tumours regressed significantly — in one-third of tumours, the

THERAPEUTICS

Heading for a cure

For many types of brain tumours there is no effective treatment, but promising work by Rosalind A. Segal and colleagues has found that a small molecular antagonist of the chemokine receptor Cxcr4 causes a big headache for brain tumours in mice. As this antagonist already has an encouraging safety profile in humans, they believe it could be considered for immediate evaluation in clinical trials.

Chemokine stromal-cell-derived factor 1α (CXCL12) and its receptor CXCR4 have a crucial role in brain development and are expressed in adult glioblastoma multiforme (GBM), so Segal and colleagues wondered if other types of brain tumours also expressed these proteins. Immunohistochemical staining of human brain tumours identified CXCR4 expression in 9/10 paediatric medulloblastomas and 3/5 anaplastic astrocytomas examined. In addition, published gene-array data from paediatric brain tumours showed that *CXCR4* expression was increased in tumours of glial and neuronal origin. CXCL12 expression in the medulloblastomas was similar to that in GBM, indicating that the CXCR4–CXCL12 interaction might contribute to tumour formation.

CXCR4–CXCL12 signalling is known to cause chemotaxis, increase proliferation and decrease apoptosis, and when Daoy medulloblastoma or U87 GBM cells were stimulated with CXCL12, all of these effects were observed. But addition of AMD 3100 — a small-molecule inhibitor of CXCR4 — to the cultures reduced chemotaxis and proliferation, and blocked serum-free survival. The next step was to determine whether AMD 3100 produced a similar effect *in vivo*. First, they established intracranial xenografts of Daoy and U87 cells that were engineered to express luciferase — which allowed non-invasive imaging of the tumours — then osmotic pumps containing AMD 3100 or PBS were implanted into tumour-bearing mice. Tumour burden was substantially diminished in the AMD-3100-treated animals. Twice-daily subcutaneous injection of AMD 3100 to tumour-bearing animals also reduced tumour growth with no evidence of toxicity in the treated animals.

Interestingly, the antitumour effects of AMD 3100 were different for different tumour types. AMD 3100 increased apoptosis in the GBM tumours, but had no effect on proliferation, whereas apoptosis was increased and proliferation reduced in medulloblastomas. AMD 3100 decreased phosphorylation of ERK1/ERK2 and AKT, downstream effectors of CXCL12, in tumour cells from both animal models, confirming that CXCR4 signalling is impaired.

These are promising results, as the safety of AMD 3100 has already been established in human clinical trials and trials to evaluate its effect on malignant brain tumours could be rapidly established. If successful, CXCR4 antagonists might be useful for treating other types of malignancies that express CXCR4.

Emma Croager

References and links

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WEB SITE

Rosalind Segal's lab:
http://research.dfci.harvard.edu/segallab/

malignant component disappeared completely — so OCT-3/4 does seem to be required for tumour maintenance, which makes it an interesting therapeutic target.

To confirm its function as an oncogene, the authors next transfected Swiss 3T3 fibroblasts with *OCT-3/4* or *RAS*. Interestingly, *OCT-3/4* transfected cells had a similar ability to grow in the absence of anchorage — a hallmark of cancer cells — as *RAS*-transfected cells, and they formed more invasive tumours than *RAS*-transfected cells when injected into immunodeficient mice.

So, *OCT-3/4* seems to act as a dose-dependent oncogene and determines the specific cell fate of arising tumours. What is left to be determined is the mechanism by which this transcription factor acts.

Emma Greenwood

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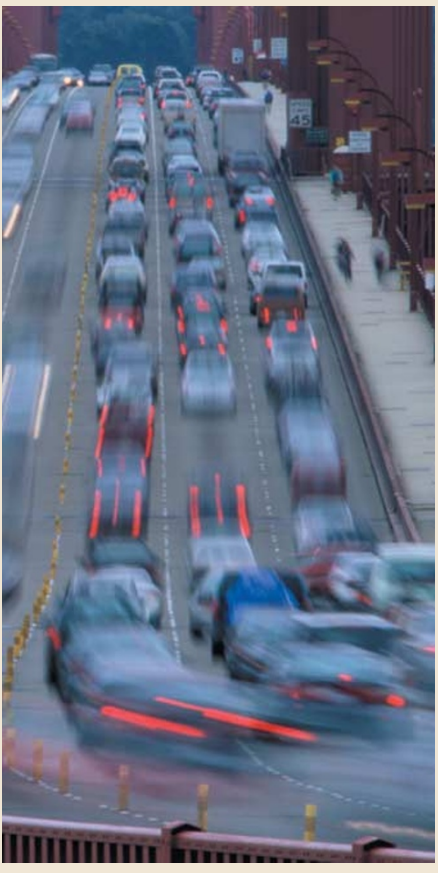

BREAST CANCER

EMSY forges a link

One of the recent puzzles in cancer research has been why the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* do not seem to be involved in sporadic cancer. Expression of *BRCA1* is occasionally lost because of promoter hypermethylation, but the same has not been found for *BRCA2*. The discovery of the BRCA2-interacting protein EMSY, by Tony Kouzarides and colleagues, might now allow part of this puzzle to be solved. It is thought to provide the link between *BRCA2* and sporadic cancer.

EMSY was identified in a yeast two-hybrid screen with the amino terminus of BRCA2 and the interaction was confirmed using purified proteins. The activities of BRCA2 include transcriptional activation, DNA repair and chromatin remodelling, and EMSY is implicated in all of these. It can repress the ability of BRCA2 to activate transcription of a reporter construct; it localizes to sites of DNA damage after γ-irradiation; and it interacts with chromatin-remodelling proteins.

The potential involvement in chromatin remodelling was discovered after *EMSY* was cloned. The sequence was novel, but contained an 80-amino-acid domain — named EMSY N-terminal domain (ENT) — that was found in nine *Arabidopsis* proteins. These proteins also contained a new Royal-family domain, designated Agenet, that can recognize lysine-methylated histones, which explains the link with chromatin remodelling.

As EMSY does not possess such a domain, might it, instead, interact with other proteins that do contain Royal-family domains? In two-hybrid screens with the N terminus of EMSY, over 80% of the interacting clones contained a Royal-family domain. The authors showed that two of these, HP1β and BS69, could also interact *in vivo*. So, it is likely that one of the functions of EMSY is in chromatin regulation.

But what about the connection with cancer? *EMSY* maps to chromosome 11q13.4-5, which is frequently amplified in sporadic breast cancer. Four different amplicons are found within this region and the authors used fluorescence *in situ* hybridization (FISH) to show that *EMSY* was amplified in 5/28 (18%) breast cancer cell lines and in 1/5 samples from newly diagnosed patients. The degree of amplification correlated with the expression level of *EMSY* and the authors showed that *EMSY* could be amplified independently from other genes in the region.

To investigate the significance of this amplification, the authors next looked at how it affects prognosis by comparing expression in tissue samples from patients with sporadic breast cancer with their outcome. *EMSY* was shown to be amplified in 70/551 (13%) cases and the median disease-specific survival for node-negative breast cancer was 6.4 years with the amplification, but 14 years without. So, *EMSY* amplification correlates with a poorer prognosis, specifically for node-negative breast cancer.

As *BRCA2* mutation increases susceptibility to ovarian cancer, as well as to breast cancer, the authors investigated whether *EMSY* was amplified in sporadic ovarian cancer. They found amplification in 17% of high-grade carcinomas, but none in low-grade tumours.

So, the BRCA2 pathway might be involved in sporadic breast and ovarian cancer after all. Although this is yet to be confirmed, the fact that EMSY and BRCA2 have overlapping functions and cause the same pathologies is encouraging. As EMSY inhibits the transcriptional activation function of BRCA2, it is certainly possible that *BRCA2* deletion and *EMSY* amplification have similar effects.

Emma Greenwood

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ORIGINAL RESEARCH PAPER Hughes-Davies, L. *et al. EMSY* links the BRCA2 pathway to sporadic breast and ovarian cancer. *Cell* **115**, 523–535 (2003)

WEB SITE

Tony Kouzarides' lab:
http://www.welc.cam.ac.uk/groups/kouzarides.html