

TUMOUR IMMUNITY

Plugging the gap



Most of the tumour antigens that have been identified are aberrantly expressed self-molecules that are poorly immunogenic. This realization, together with the fact that normal individuals have T cells that react against self-antigens but are held in check by peripheral-tolerance mechanisms, has resulted in attempts to develop antitumour therapies that stimulate these anti-self T cells and elicit antitumour responses. A new study in the *Journal of Clinical Investigation* shows that stimulating the homeostatic proliferation of a polyclonal population of adoptively transferred T cells — including those that are

specific for tumour self-antigens — in lymphopaenic animals can induce antitumour immunity.

Mice with lymphopaenia (induced by sublethal irradiation) and wild-type mice were challenged with a subcutaneous injection of melanoma cells, and tumour growth was assessed after 52 days. The lymphopaenic mice developed smaller tumours than wild-type mice. This antitumour effect was enhanced in a dose-dependent manner by the adoptive transfer of syngeneic lymph-node T cells before challenge with the tumour cells. *In vitro* testing of the homeostatically expanded T cells showed that they specifically recognized the melanoma tumour cells, but not unrelated colon carcinoma cells.

Next, the authors investigated whether T-cell clonal expansion *per se* was sufficient to induce the antitumour response or whether antigen presentation of tumour antigens was

also important. The inhibition of tumour growth was more efficient in wild-type lymphopaenic mice transfused with T cells and challenged with melanoma than in lymphotoxin- α -deficient mice, which lack lymph nodes. When T cells that cannot travel through lymph nodes (because they do not express β 7-integrin or L-selectin) were transferred, they were much less effective than normal T cells at eliciting the antitumour response. Together, these results show that the proliferating T cells need to be exposed to tumour antigens that are presented in the lymph nodes.

When mice that had rejected the melanoma cells were rechallenged with either melanoma or colon carcinoma cells, they again rejected the melanoma cells, but not the colon carcinoma cells, which indicates that the T-cell homeostatic proliferation had resulted in the development of

ONCOGENES

One small step for mankind...

The first oncogenic transcription factor, v-Jun, was discovered 15 years ago; shortly after this, its cellular counterpart, c-Jun, was also shown to be capable of transforming cultured cells. It has taken until now, however, to take that next great leap — to show that c-JUN is involved in human cancer.

By investigating the DNA-binding activity of the AP1 transcription factor — a dimer that is composed of members of the JUN, FOS and ATF families — in a number of lymphoma cell lines, Stephan Mathas *et al.*, reporting in *The EMBO Journal*, were able to show that AP1 was specifically activated in Hodgkin's lymphoma and anaplastic large-cell-lymphoma cell lines. The AP1 complex largely contained c-JUN, but another family member, JUNB, was also detectable in some of the cell lines; the increase in DNA-binding activity of AP1 correlated with an increase in protein levels of these components. This was confirmed when immunohistochemistry was performed on lymph-node biopsy samples, which were taken from patients with Hodgkin's disease.

AP1 is normally activated in response to extracellular signals, but is this also the case

in Hodgkin's lymphoma? As expected, treating the lymphoma cell lines with phorbol esters activated the mitogen-activated protein kinase (MAPK) pathway and an AP1 complex; however, this was distinct from the one that is constitutively expressed in Hodgkin's disease — it was predominantly composed of c-FOS. c-JUN is also not phosphorylated in unstimulated cells, confirming that MAPK is not involved in its activation.

So, if JUN is activated independently of the normal extracellular signals, what does activate it? The remaining possibilities include increased transcription and decreased protein turnover. Pulse-chase experiments revealed that the half-life was unchanged, ruling out the latter option, but what of the former?

The *c-JUN* promoter is known to be autoregulated by AP1, which could therefore be responsible for the upregulation of c-JUN. To investigate this, a reporter construct containing either the wild-type or a mutated *c-JUN* promoter was transfected into a Hodgkin's lymphoma and a Burkitt's lymphoma cell line. The wild-type promoter was specifically activated in

only the Hodgkin's lymphoma cells, indicating that AP1 is responsible for the activation of c-JUN.

The transcription factor NF- κ B is also constitutively activated in Hodgkin's lymphoma cells, and transfecting these cells with an adenovirus that encoded an NF- κ B repressor resulted in a decrease in the level of JUNB. Interestingly, this did not affect the activity of AP1, indicating that c-JUN is the more important AP1 component in these cells.

So what effect does this increased AP1 activity have on Hodgkin's lymphoma cells? Its inhibition, using A-FOS, results in a decrease in cell division, which corresponds with a reduction in cyclin D2 expression; the proto-oncogene *c-MET* and the lymphocyte homing receptor CCR7 were also downregulated. Interestingly, the cellular response to inhibition of AP1 by A-FOS was different in anaplastic large-cell lymphoma cells — they underwent apoptosis, indicating that AP1 acts as a survival factor in these cells.

So, aberrant expression of JUN is involved in human cancer. This should strengthen the argument for the development of therapeutics that target this transcription factor.

Emma Greenwood

References and links

ORIGINAL RESEARCH PAPER Mathas, S. *et al.* Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF- κ B. *EMBO J.* **21**, 4104–4113 (2002)

specific long-term antitumour immunity.

Could homeostatic proliferation be useful for the treatment of established tumours? Lymphopaenic mice were challenged with melanoma cells, and when the tumours reached a specific size, the mice were transfused with lymph-node cells. Tumour growth was markedly inhibited in treated mice compared with controls.

These data indicate that, in the clinic, it might be beneficial to begin tumour immunotherapy soon after the completion of chemotherapy to induce effective homeostatic T-cell proliferation.

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ANGIOGENESIS

Endostatin and Wnt signalling

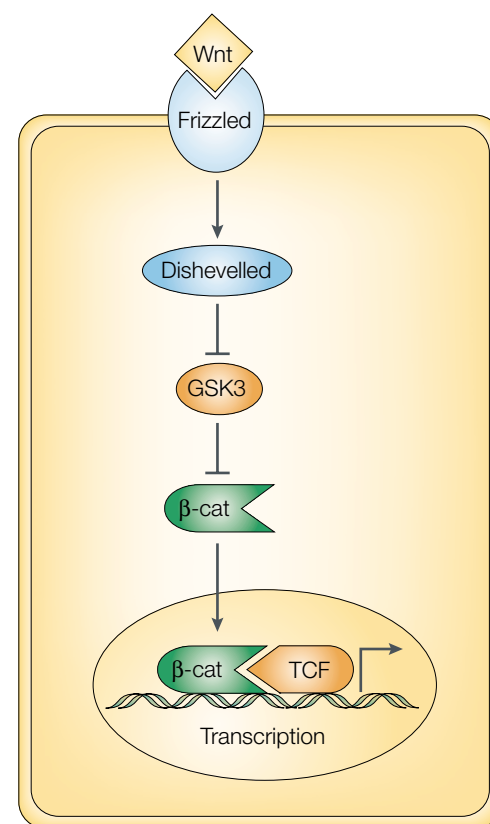
Although *Xenopus* is more often used as a model for embryogenesis than for cancer research, it has recently provided insight into the mechanisms of endostatin signalling, as Hanai and colleagues report in the 5 August issue of *The Journal of Cell Biology*. Endostatin is an anti-angiogenic factor, and recombinant endostatin, which inhibits tumour growth in animal models, is now in clinical trials.

Hanai *et al.* found that injection of high concentrations of endostatin RNA into *Xenopus* blastomeres caused developmental abnormalities in cement-gland and eye formation. Injection of inactivated mutated endostatin, however, had no effect on development. The defects seen are characteristic of those observed when key components of the Wnt signalling pathway are manipulated — this pathway is important for regulation of cell proliferation, differentiation, motility and morphogenesis. From these observations, the authors hypothesized that endostatin might have a role in Wnt signalling.

When Wnt binds to Frizzled, cytoplasmic Dishevelled (Dsh) proteins are activated. These proteins then antagonize glycogen synthase kinase (GSK3), leading to stabilization of β -catenin — an essential mediator of Wnt signalling. β -catenin translocates to the nucleus and binds to members of the T-cell-specific factor (TCF) transcription-factor family, stimulating transcription of target genes that encode such proteins as c-Myc and cyclin D.

So, does endostatin modulate the Wnt pathway? Experiments by Hanai *et al.* showed that endostatin inhibits β -catenin activity — injection of β -catenin into *Xenopus* led to the development of double-headed embryos, and co-injection of endostatin inhibited this abnormal phenotype. Inhibition of the Wnt pathway was confirmed in human umbilical-vein endothelial cells (HUVECs) that were transfected with β -catenin and endostatin.

Although Hanai and co-workers now knew that endostatin directly inhibited the Wnt/ β -catenin pathway, they wanted to find out how and where in the pathway endostatin acts. They therefore tested the ability of endostatin to block signal transduction at different levels, in both *Xenopus* embryos and endothelial cells. Co-injection of endostatin with a TCF construct that lacked the β -catenin binding site — and so constitutively activates transcription independently of β -catenin — failed to inhibit axis duplication, but axis duplication induced by



Dsh overexpression was inhibited by endostatin expression. Furthermore, endostatin, but not mutant endostatin, decreased the levels of a form of β -catenin that is insensitive to upstream antagonists of Wnt signalling. The data therefore indicate that endostatin modulates the Wnt pathway by regulating β -catenin stability via a novel GSK3-independent mechanism.

In addition, the authors showed that endostatin inhibition of endothelial-cell migration and entry into the S phase of the cell cycle — properties of endostatin that have been reported by these authors and other groups to be linked with its anti-angiogenesis activity — is due to endostatin modulation of the Wnt pathway. The constitutively activated form of TCF could rescue the inhibitory effects of endostatin on angiogenesis in HUVECs.

This paper provides insight into a possible function of the Wnt/ β -catenin pathway in angiogenesis and shows that endostatin has a role in inhibiting this pathway. Understanding the mechanism of action of endostatin should facilitate the development of selective and effective endostatin therapies for the treatment of cancer.

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WEB SITE

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signal transduction pathways in development: Wnts and their receptors

