TUMORIGENESIS

Changing signals

The transforming properties of RAS have been apparent since its discovery 30 years ago. But why does oncogenic RAS cause cell-cycle arrest when

introduced into many types of normal cells? Normal cells seem to contain safety mechanisms that inhibit cell-cycle progression when RAS is activated. So, for RAS to have a positive effect on proliferation, additional genetic changes must occur to overcome cell-cycle checkpoint controls and to contribute to the development of tumours. In the April issue of *Molecular and Cellular Biology*, Alison Lloyd and colleagues show that the p53 and RB pathways have distinct roles in this process.

The authors used primary rat Schwann cells — these proliferate indefinitely, but retain the cell-cycle controls that are normally lost during the immortalization process — to investigate which genetic changes cooperate with oncogenic Ras and contribute to a transformed phenotype. They first investigated the contribution of p53, by expressing oncogenic Ras and either a dominant-negative mutant of p53

(Ras/dnp53) or SV40 large T antigen (Ras/LT), which inhibits the p53 and Rb pathways. Both Ras/dnp53 and Ras/LT cells were able to proliferate in the absence of mitogens, but only Ras/LT cells had a high proliferative capacity in soft agar and within monolayers. So, perhaps both the RB and p53 pathways must be inhibited for RAS to overcome anchorage dependence and contact inhibition.

Deletion analysis of LT showed that the amino terminus, which inhibits the Rb pathway, co-operates with Ras and dnp53 to allow growth in soft agar. Cells expressing dnp53 and activated Ras sometimes spontaneously acquire anchorage independence, with expression of the cyclin-dependent kinase inhibitor Ink4a being lost in 5 out of 10 cases due to promoter methylation. As INK4A lies upstream of the RB family in the tumour-suppressor pathway, its loss would be expected

IMMUNOTHERAPY

Don't forget the handbrake

Mirror, signal, manoeuvre...and it's all systems go. You might think that all you need to induce tumour regression are the conditions to stimulate an immune response, but tumour immunologists are discovering that you won't get far unless you remember to release the handbrake.

Tumour cells express antigens that are recognized by the host immune system, but they are generally poorly immunogenic. The most obvious explanation for this is that antigen presentation is inefficient. Various vaccination strategies have been tested to improve antigen presentation, such as engineering autologous tumour cells to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF), which induces dendritic-cell (DC) maturation to produce professional antigen-presenting cells.

In Phase I clinical trials of this strategy, although an antitumour response is elicited in the short term, patients go on to develop progressive disease. Cytotoxic T lymphocyte antigen 4 (CTLA4) might be one of the brakes that holds the immune system back, and transient antibody-

mediated blockade of CTLA4 in combination with vaccination in animal models can increase tumour rejection.

However, once the brakes have been removed, it's difficult to stop the action, and mice treated with CTLA4-blocking antibodies develop fatal lymphoproliferation. So, Allison, Dranoff and colleagues set out to test the safety of this combination therapy in humans.

A single intravenous dose of the CTLA4-blocking antibody MDX-CTLA4 was tested in seven patients with metastatic melanoma and two patients with ovarian carcinoma, all of whom had previously been involved in various Phase I vaccination trials.

In the two patients with ovarian carcinoma and three of the patients with melanoma — who had been previously vaccinated with GM-CSF-secreting tumour cells — there was evidence of enhanced tumour necrosis. There was no evidence of tumour destruction in the other four patients with melanoma, who had been vaccinated with melanoma antigen-derived peptides or DCs expressing tumour

differentiation antigens. This indicates that the effectiveness of CTLA4 blockade depends on the specific nature of the immune response and that CTLA4 might not be involved in inhibiting all antitumour responses.

The patients developed low titres of autoantibodies, but there was no clinical evidence of autoimmune disease. One patient developed an acute hypersensitivity reaction to the antibody, but this could be easily controlled with antihistamines. The patients with melanoma developed a skin rash, but not vitiligo (depigmentation), which shows that although tolerance to melanocyte differentiation antigens was affected, the response was mild and melanocytes were not destroyed.

This study is the first to show the clinical safety and efficacy of vaccination and CTLA4 blockade, even when the two are separated temporally. This approach could be extended to other types of cancer, and CTLA4 blockade might also synergize with other cancer treatments that stimulate the immune system, such as radiation and chemotherapy.

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References and links

ORIGINAL RESEARCH PAPER Hodi, F. S. *et al.* Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc. Natl Acad. Sci. USA* **100**, 4712–4717 (2003)