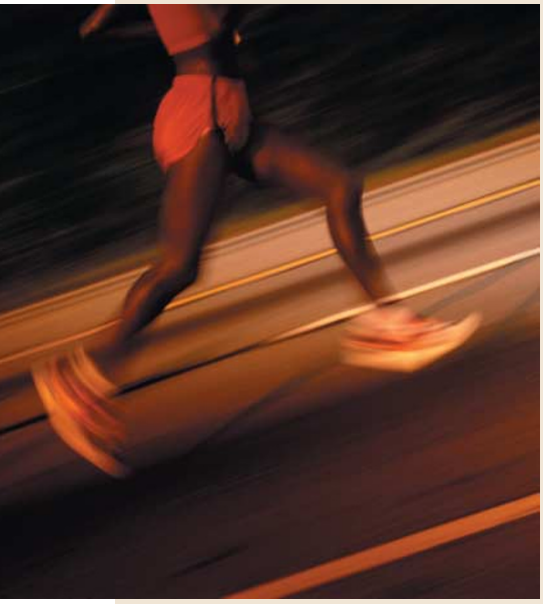
ONCOGENESIS

Staying active



Mutations in *BRAF* are common in human melanomas and stimulate tumour-cell proliferation by overactivating the RAS–ERK pathway. Reporting in *Cell*, Richard Marais, David Barford and colleagues show that even BRAF mutants with decreased kinase activities can have oncogenic effects, through their interaction with another member of the RAF family.

BRAF initiates downstream signalling by phosphorylating MEK, which, in turn, activates ERK. Most cancer-associated *BRAF* mutations affect residues in the kinase domain, indicating that increased catalytic activity is responsible for oncogenesis. To confirm this, the authors determined the abilities of 22 mutant forms of BRAF — all of which are expressed in human cancers — to activate ERK. All but one of these stimulated ERK activation in the absence of upstream signals and, as expected, most had basal kinase activities that exceeded that of wild-type BRAF. Intriguingly, however, three of the mutants had decreased kinase activities, but were still able to activate ERK.

So, how can kinase-impaired BRAF mutants stimulate downstream signalling? Wild-type BRAF is known to form complexes with CRAF — a related protein that is also able to activate ERK. Marais, Barford and colleagues therefore investigated whether BRAF mutants can exert their oncogenic effects through CRAF, independently of kinase activity. All three of the kinase-impaired mutants that are able to activate ERK were shown to form complexes with CRAF and induce its activation. Reducing CRAF levels by RNA interference suppressed the ability of these BRAF mutants to activate ERK, confirming that their oncogenic activities are mediated through interactions with CRAF.

How these mutants activate CRAF is unclear. They all contain altered residues in their kinase domains, which might lead to a conformational change in this region. The authors suggest that such a change could be transmitted to the CRAF kinase domain, leading to its activation, but further work will be needed to confirm this.

Louisa Flintoft

References and links

ORIGINAL RESEARCH PAPER Wan, P. T. C. *et al.* Mechanism of activation of the RAS–ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* **116**, 855–867 (2004)

TUMOUR IMMUNOLOGY

Preventing escape

One important aspect of tumour development is the ability of cancer cells to escape detection and destruction by the host immune system. Gabriel Rabinovich and colleagues provide evidence that mouse melanoma cells express a T-cell inhibitor called galectin-1 (Gal1), which could be a useful therapeutic target.

Gal1 — a carbohydrate-binding protein with regulatory functions — is expressed by many different tumour types. Increased expression of Gal1 has been correlated with tumour aggressiveness and metastasis. The authors studied its function by transfecting B16 mouse melanoma cells — which express high levels of Gal1 — with *Gal1* antisense cDNA to establish knockdown clones. These clones expressed either low or intermediate levels of Gal1. Conditioned medium from wild-type B16 cells induces high levels of apoptosis when it is applied to T cells *in vitro*, whereas conditioned medium from the Gal1 knockdown clones induced only low levels of T-cell apoptosis.

In mice, tumour growth from clones that expressed intermediate levels of Gal1 was substantially delayed, compared with wild-type melanoma cells. Furthermore, cells that expressed low levels of Gal1 were rejected. As all clones grew at the same rate in immunodeficient mice, components of the immune system must be responsible for these different responses. The authors went on to show that tumour rejection induced by Gal1 blockade required intact $CD4^+$ and $CD8^+$ T-cell responses.

In previous studies the authors had shown that Gal1 specifically suppresses the T-helper 1 (T_H1) immune response, so they looked to see if this response occurred in mice injected with the Gal1-knockdown melanoma cells. Tumour-draining lymph nodes were removed from these mice and *ex vivo* stimulation of their immune cells resulted in increased levels of the T_H1 cytokines interleukin-2 and interferon-γ, compared with controls. So, blocking Gal1 production by melanoma cells restores the ability of the host immune system to initiate a T_H1 response. The Gal1-knockdown melanomas were also observed to be infiltrated with mononuclear cells, and T cells from these mice were less susceptible to apoptosis.

These mice eventually rejected the Gal1-knockdown cells. Furthermore, when they were rechallenged with wild-type B16 melanoma cells, tumour growth was delayed, compared with naive mice challenged with B16 cells. So, inhibition of Gal1 expression not only enhances T_H1 tumour-specific immune responses, it also provides resistance to subsequent challenge with Gal1-expressing tumour cells. This work provides an important new link between Gal1 and tumour progression, and identifies an important mechanism that contributes to immune privilege of tumours.

Emma Croager

References and links

ORIGINAL RESEARCH PAPER Rubinstein, N. *et al.* Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection: A potential mechanism of tumor-immune privilege. *Cancer Cell* **5**, 241–251 (2004)