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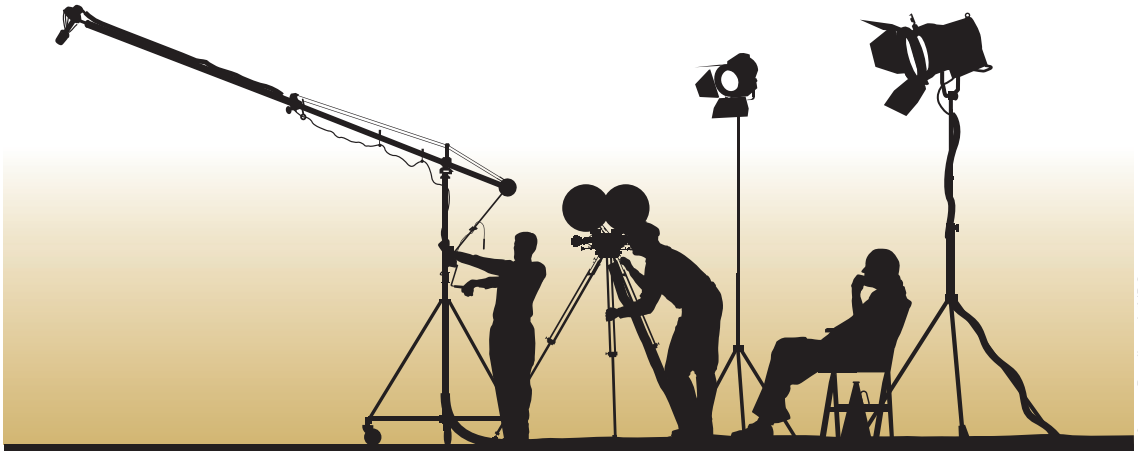

Simon Bradbrook/NPG

CANCER

Live screening of immunotherapy targets

Therapeutic targeting of inhibitory receptors on T cells can boost immune responses to tumours and improve patient survival but, for reasons that remain unclear, such responses are only seen in a subset of individuals. Studies to identify the processes that govern immunosuppression of tumours have typically been conducted *in vitro* and have therefore not addressed the complex interaction among tumour cells, immune cells and the tumour microenvironment. Now, a study published in *Nature* has outlined an approach for the *in vivo* discovery of immunotherapy targets in melanoma using RNA interference technology, which could pave the way to modification of immune responses in diverse cancer subtypes.

In the current study, Zhou *et al.* focused on the response of cytotoxic ($CD8^+$) T cells to B16 melanoma, an aggressive tumour, in mice. The researchers constructed two thematic libraries of short hairpin RNA (shRNA) molecules — one targeting genes associated with T cell anergy or exhaustion, the other aimed at genes encoding kinases and phosphatases. $CD8^+$ T cells, each infected with an shRNA from these libraries, were injected into tumour-bearing mice. The T cells were engineered to express the receptor OT-1, and tumour cells expressed the cognate antigen ovalbumin, ensuring homing of the T cells to the tumour target site.

> “the screen highlighted *Ppp2r2d* … as being upregulated in T cells from tumours”

The rationale behind the approach was that shRNA molecules that were complementary to mRNA transcripts of upregulated, immunosuppressive genes in T cells would block the translation of these genes and, by definition, become enriched in tumours by enabling proliferation of these derepressed T cells. Seven days after injection, T cells were purified from tumour tissue as well as from the spleen (to act as a control tissue). Deep sequencing of the shRNAs was then performed to identify the upregulated genes responsible for T cell suppression in the tumour microenvironment.

Zhou *et al.* found 43 genes that were enriched in T cells from the tumour tissue, including several that were known to be inhibitors of T cell function, suggesting that the approach was valid. In addition to established targets, the screen highlighted *Ppp2r2d* (protein phosphatase 2 regulatory subunit B delta) as being upregulated in T cells from tumours. This gene encodes a regulatory subunit of the family of PP2A phosphatases that discourages T cell mitosis and regulates apoptosis. Biochemical assays revealed that shRNA-mediated knockdown of *Ppp2r2d* was associated with increased T cell proliferation and enhanced cytokine production by T cells.

Importantly, when the researchers injected melanoma-targeted T cells containing *Ppp2r2d*-specific shRNA into tumour-bearing mice, they observed significantly enhanced antitumour immunity in the form of increased melanoma cell death, reduced tumour burden and prolonged survival compared with mice treated with T cells containing *lacZ* control shRNA. The approach was also effective when *Ppp2r2d* knockdown was performed in $CD4^+$ T cells, which suggests that broad immunomodulatory effects can be achieved by targeting this gene.

Together, the findings of this study not only highlight the potential therapeutic value of targeting *PPP2R2D* in T cells to treat melanoma but also provide a valuable approach to identify new targets for cancer immunotherapy.

Katie Kingwell

ORIGINAL RESEARCH PAPER Zhou, P. *et al.* *In vivo* discovery of immunotherapy targets in the tumour microenvironment. *Nature* **506**, 52–57 (2014)