Nature Reviews Clinical Oncology | Published online 31 Aug 2017; doi:10.1038/nrclinonc.2017.142

IMMUNOTHERAPY

CRISPR unveils T-cell-resistance mechanisms in tumours

Many patients with advanced-stage disease have shown dramatic responses to immunotherapy; however, despite some long durations of response, only a subset of patients are cured and many do not respond. Why some cancers resist the immune system is unclear; thus, a better understanding of the mechanisms of immune resistance and tumour escape could help to predict which patients might respond to existing immunotherapies. A study published in *Nature* reports a major effort researchers undertook to identify all the protein-encoding genes that are required in a tumour for T cells to recognize and destroy cancers.

Nicholas Restifo and colleagues developed a two-cell-type CRISPR/Cas9 assay screen, with human T cells as effectors and melanoma cells as targets, to understand how genetic interactions in one cell type can affect complex interactions with another cell type. By systematically eliminating genes in a melanoma cell line they were able to test every gene for its effect on the T-cell response. Using a library of 123,000 single-guide RNAs, the researchers profiled genes whose loss in tumour cells impaired the function of effector $CD8^+$ T cells. The genes found to be enriched were those involved in antigen processing and presentation, as well as responses to cytokines, such as IFNγ. By comparing their candidate genes with the expression profiles of almost 11,500 tumours from The Cancer Genome Atlas (TCGA) database, spanning 36 different tumour types, the researchers identified 19 genes that were upregulated. Importantly, loss of expression of these 19 genes within tumours prevented the presentation of tumour antigens within the tumour microenvironment, which is responsible for driving T-cell infiltration and T-cell mobilization.

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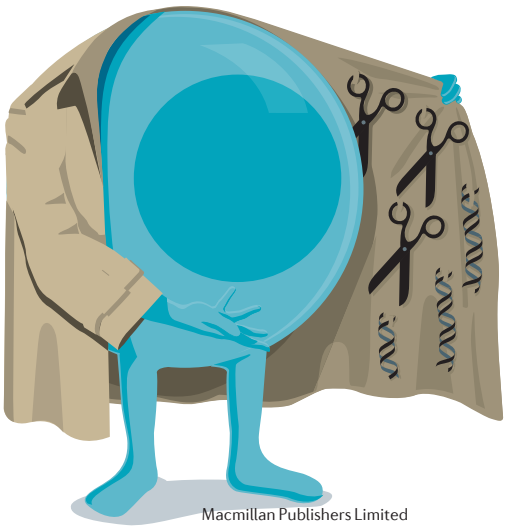

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Next, the researchers focused on one gene of interest, *APLNR*, which encodes a G-protein-coupled apelin receptor and is known to be mutated in several cancers. Loss of function of APLNR, via seven different mutations, was observed in tumours from patients who were refractory to immunotherapy. Moreover, APLNR interacts with JAK1 to augment IFNγ response, increasing the sensitivity of tumour blood vessels to IFNγ.

As Restifo explains: "surprisingly, this screen identified dozens of new genes that potentially influence the susceptibility of tumour cells to T-cell attack. When these genes were 'knocked-out' tumour cells were significantly more likely to survive and continue to multiply after exposure to T cells that we had genetically engineered to recognize tumour-associated antigens." Perhaps not surprisingly, loss-of-function mutations in the novel resistance genes were abundant in patients who failed to respond to immunotherapy.

As Restifo summarizes: "we hope our findings will serve as a blueprint to guide comprehensive studies of what makes some tumours resist T-cell control. Ultimately, novel combination immunotherapies based on individual gene mutations might enable the expansion of curative immunotherapy."

Lisa Hutchinson

ORIGINAL ARTICLE Patel, S. J. *et al.* Identification of essential genes for cancer immunotherapy. *Nature* http://dx.doi.org/ 10.1038/nature23477 (2017)