Tools of the trade

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Multiomics-guided cellular immunotherapies

Cellular immunotherapy with T cells engineered to target specific tumour antigens has shown remarkable efficacy, with frequent complete remissions in haematological cancers. However, these 'living drugs' have had very limited success in solid tumours. The failure of engineered T cells to eradicate solid tumours stems, at least in part, from the inability of T cells to overcome cancer-mediated immune suppression, leading to their exhaustion in the hostile tumour microenvironment (TME).

To overcome this limitation, we developed a pooled genome-scale overexpression approach using a lentiviral library of barcoded human open reading frames to identify positive regulators of T cell function. Top-ranked candidates were selected for a more in-depth study of their T cell-supportive effects. To understand these mechanisms at scale, we developed Overexpression-compatible Cellular Indexing of Transcriptomes and Epitopes by Sequencing (OverCITE-seq), which builds on single-cell multiomics technologies CITE-seq, used for surface protein quantification and ECCITE-seq that captures CRISPR guide RNAs.

Owing to the direct capture of the lentiviral transcripts, OverCITE-seq enabled the detection and confident assignment of overexpressed genes for the majority of single T cells in the assay. Given OverCITE-seq compatibility with other single-cell modalities, such as RNA-seq and T cell receptor capture, it is possible to profile the effects of many overexpressed genes across diverse T cell subsets and different contexts to identify shared or distinct pathways modulated by overexpressed constructs.

> "OverCITE-seq identified lymphotoxin beta receptor (LTBR) overexpression as a driver of profound transcriptional remodelling in T cells"

To demonstrate the utility of OverCITE-seq, we applied it to resting and stimulated primary human T cells that overexpressed ~30 candidate genes identified in the genome-wide screen focused on T cell expansion and persistence. Among others, OverCITE-seq identified lymphotoxin beta receptor (LTBR) overexpression as a driver of profound transcriptional remodelling in T cells, with thousands of genes differentially expressed. In lymphocytes, LTBR is not endogenously expressed. Its ectopic expression activates the canonical and non-canonical NF-κB pathways, which potentiates antigen-specific responses of activated T cells and preserves their capability for self-renewal and resistance to exhaustion. Thus, expression of LTBR could enhance the function of engineered T cell-based therapies.

In future experiments, we aim to expand the applicability of OverCITE-seq to enable combinatorial screening of overexpressed genes with the goal to identify synergistic combinations that might ultimately be capable of overcoming the various immunosuppressive mechanisms that are at play in the TME. We hope that OverCITE-seq proves to be a valuable tool for testing and developing new, more effective cellular immunotherapies, through scalable profiling of numerous genetic modifications in a single assay.

Mateusz Legut
OverT Bio, New York, NY, USA.
e-mail: mat@overt.bio

Competing interests
M.L. is an inventor on patent applications covering OverCITE-seq filed by the New York Genome Center and New York University. M.L. is an employee and shareholder of OverT Bio.

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