651 MOLECULAR SIGNATURE OF NEOANTIGEN-REACTIVE CD4+ AND CD8+ T CELLS FROM METASTATIC HUMAN CANCERS ENABLES PROSPECTIVE ANTITUMOR TCR PREDICTION

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Background Autologous patient T cells engineered to express antitumor T cell receptors (TCRs) and chimeric antigen receptors (CARs) have been effective for the treatment of certain cancer types,[1–4] and tumor neoantigens encoded by cancer-specific mutations have emerged as major targets of CD4+ and CD8+ T cells in immune checkpoint blockade (ICB) and in adoptive cell therapy (ACT).[5–9] However, only a minority of intratumoral T cells are reactive to cancer antigens while the majority represent bystander cells.[10–12] Conventional approaches to isolate tumor-reactive T cells and identify their TCRs from tumors rely on T cell function and can be impaired due to T cell exhaustion and dysfunction.[13 14]

Methods We performed single-cell RNA and T cell receptor (TCR) sequencing (scRNA/TCR-seq) on over 46,000 T cells isolated from eleven archival metastatic tumor samples whose primary cancer types included colon, rectal, breast, anal, and melanoma. From these samples, 15 CD8+ and 17 CD4+ neoantigen-reactive TCR clonotypes (NeoTCRs) were known. We then performed transcriptomic clustering of these cells and mapped known NeoTCR clonotypes onto the transcriptomic map. Subsequently we predicted NeoTCRs from prospective metastatic colon cancer samples based on their presence within clusters sharing gene expression with NeoTCR+ clusters in the archival samples.

Results Projecting known NeoTCRs onto the TIL transcriptomic map, we observed 325 total T cells bearing these NeoTCRs, and the majority (>80%) of NeoTCRs were expressed by T cells within 2 clusters, one CD4+ and one CD8+, that included by expression of CXCL13, ENTPD1 (CD39), TOX, TIGIT, LAG3, and PDCD1 (PD-1), indicating a dysfunctional state. Reasoning that T cells sharing phenotypes with those within the NeoTCR clusters could be novel NeoTCRs, we developed gene signatures (NeoTCR4 and NeoTCR8) of CD4 and CD8 NeoTCR+ cells, respectively, and four prospective patients' TIL were analyzed by scRNA/TCR-seq and scored according to NeoTCR signatures. We expressed predicted NeoTCRs in healthy donor PBL and screened them with antigen presenting cells (APCs) expressing candidate neoantigens. 33/73 predicted NeoTCRs (including both CD4 and CD8) were reactive against patients' tumors or candidate neoantigens.

Conclusions This study enabled successful detection of tumor-specific NeoTCRs in the sequenced TIL of 14/14 patients for whom reactivity was studied. Deconvolution of NeoTCRs from bystander TCRs within the tumor-immune microenvironment represents an important step in the development of personalized immunotherapeutics, and prospective NeoTCR isolation based on TIL transcriptional phenotypes will allow for rapid development of personalized immunotherapy in the form of lymphocytes expressing these tumor-specific TCRs.

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