115 SPATIAL DISSECTION OF T CELL CLONOTYPE IDENTITY, TRANSCRIPTIONAL PROFILES, AND CELL-CELL INTERACTIONS IN THE TUMOR MICROENVIRONMENT AND TERTIARY LYMPHOID STRUCTURES

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Background Determining the spatial interactions of T cell clonotypes in tumor microenvironments and tertiary lymphoid structures (TLSs) is essential to understanding adaptive antitumoral immune responses, but existing spatial sequencing methods remain unable to profile the TCR repertoire at high resolution.

Methods We previously described the development of Slide-TCR-seq, which integrates Slide-seqV2 (spatially-resolved RNA capture by a DNA-barcoded bead array with 10µm resolution) with rhTCRseq (highly sensitive targeted capture of TCR sequences)[2,3,4] to facilitate amplification of the TCR transcript from CDR3 to the 3' end. This approach enables the simultaneous measurement of cellular transcriptomes, T cell clonotypes, and spatial location at 10µm resolution. We examined melanoma and renal cell carcinoma (RCC) tumors with Slide-TCR-seq to understand the spatial relationships between T cell clonotypes, tumor cells, and immune cells in the tumor microenvironment and TLSs.

Results We applied Slide-TCR-seq to melanoma and RCC metastases because of the well-characterized roles played by T cell phenotype and TCR repertoire in their immune microenvironments.[5,6] By histology and Slide-TCR-seq, we identified TLSs in both melanoma and RCC metastases. We observed that T cells located within the tumor regions were more clonally expanded than those in the TLSs. Furthermore, T cells in TLSs tended to be CD4+ T cells, while those infiltrating into tumor tended to be CD8+ T cells with an exhausted phenotype ($p<0.05$ by FDR-corrected t-test)—together suggesting unique immunological roles of TLSs in tumors.

In the melanoma metastasis, one T cell clone (CDR3 sequence CASRASNEQFF) was preferentially enriched in one of the two tumor lobes that were examined ($p=1\times10^{-102}$). Compared to other clonotypes, *GZMB* ($p=1\times10^{-8}$; associated with cytotoxic T cell function) and *STAT3* ($p=7\times10^{-7}$; associated with activated T cells' survival) were prominently upregulated in CASRASNEQFF.

CASRASNEQFF T cells additionally exhibited unique cell non-autonomous mechanisms: monocytes neighboring CASRASNEQFF T cells displayed elevated *CXCL10* chemokine expression ($p=5\times10^{-21}$), which can recruit tumor-reactive effector T cells.[7] Notably, monocytic expression of *CXCL10* was higher in the same lobe that was enriched with CASRASNEQFF T cells, implicating a preferential interaction between the two. Melanoma cells neighboring the CASRASNEQFF T cells also displayed differential gene expression, including downregulated *MGST1* expression ($p=3\times10^{-15}$).

Conclusions Slide-TCR-seq enables spatially-resolved transcriptomics and TCR clonotyping. Our findings suggest that TLS T cells' phenotype and TCR repertoire are distinct from tumor-infiltrating T cells, and that the transcriptional profiles of T cells, monocytes, and tumor cells may depend on their spatial relationships to one another in the tumor microenvironment.

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