173 DECIPHERING THE TUMOR MICROENVIRONMENT OF RENAL CELL CARCINOMA: INTEGRATING SINGLE-CELL SEQUENCING AND *IN SITU* SPATIAL TRANSCRIPTOMICS FOR COMPREHENSIVE CHARACTERIZATION OF TUMOR-INFILTRATING T CELLS

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Background Renal cell carcinoma (RCC) is a prevalent and aggressive form of kidney cancer, with variable response rates to checkpoint blockade immunotherapies. While high T cell infiltration often indicates a favorable response to immunotherapies, this correlation is inconsistent in RCC. To accurately identify RCC patients who will benefit from immunotherapies, additional markers are needed to differentiate between subsets of tumor-infiltrating T cells. Recent advances in single-cell sequencing and subcellular spatial transcriptomics have revolutionized cancer immunology, enabling the analysis of individual cells and their interactions within the tumor microenvironment.

Methods In this study, we used comprehensively analyzed the molecular profiles of individual cells in RCC to understand the interplay between tumor cells and neighboring immune cells. Utilizing patient-matched peripheral blood mononuclear cells, cryopreserved dissociated tumor, and adjacent normal cells, we performed single-cell RNA, antibody-derived tag, and TCR sequencing on the blood and dissociated cells (n=10 patients). 10x Genomics Xenium *in situ* spatial transcriptomics with single-cell resolution was conducted using a custom designed gene panel targeting kidney, tumor specific genes and various immune related genes with emphasis on those relevant to the analysis of T cells, which was guided by single-cell RNA sequencing data. Formalin-fixed paraffin-embedded samples from tumor and adjacent normal tissue were subjected to *in situ* spatial transcriptomics (n=4 patients).

Results By aligning single-cell RNA sequencing and *in situ* spatial transcriptomics with single-cell level resolution, our findings revealed highly heterogeneous yet distinct T cell subpopulations within renal cell tumors compared to adjacent normal tissues. Notably, tumor-infiltrating CD8 and CD4 T cells expressing *CXCL13*, which is often associated with T cell exhaustion program, demonstrated a highly organized spatial distribution. These cells were positioned in close proximity to tumor cells, suggesting antigen-specific engagement. Furthermore, we tracked corresponding T cell subsets and clones in patient-matched blood samples, assessing their phenotypes as potential biomarkers for tumor-reactive T cells.

Conclusions Through the integration of single-cell multi-omics analyses and single-cell *in situ* spatial transcriptomics, we addressed critical questions concerning tumor heterogeneity, spatial organization, and the dynamic interplay between cancer cells and their microenvironment in RCC. These advancements hold promise for the development of improved therapeutic strategies, enhancing the effectiveness of cancer treatments and facilitating patient stratification for checkpoint blockade immunotherapy.

Acknowledgements This project was supported by funding from an IIRC post-doctoral fellowship (to HR), a post-doctoral training fellowship from the National Research Foundation of Korea (to HR), and Aldarra foundation (to EN).

Ethics Approval The samples were provided by Northwest BioSpecimen and the analysis was performed according to the IRB file/approval number IR File #10422.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0173