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In-situ visualization and measurement of tumor-infiltrating lymphocytes (TILs) on intact FFPE renal cell carcinoma (RCC) tissue using the spatial molecular imager (SMI)

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Summary

Although cancer immunotherapies can effectively restore T cell-mediated immunity leading to sustained clinical responses, these responses are unpredictable partly due to highly heterogeneous phenotypes of tumor-infiltrating lymphocytes (TILs) between patients. Thus, understanding such TILs and their roles in the context of tumor microenvironments (TME) may lead to developing better immunotherapy solutions.

Many previous studies have highlighted the high degree of heterogeneity in the phenotypes of tumor infiltrating lymphocytes. We and others also reported evidence for an abundance of non-tumor specific T cells infiltrating tumors and we've shown that these "bystander" T cells do not express markers associated with terminal exhaustion, such as CD39.

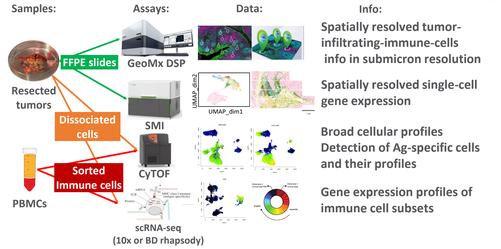
Here, using a combination of methodologies, we investigate relationships between lymphocyte phenotypic diversity, antigen-specificity and the spatial localization of tumor infiltrating immune cells.

Using CyTOF, scRNA-seq, and TCR sequencing analysis of dissociated, consistent with our previous reports, we found T cell populations could be segregated based on markers associated with chronic T cell receptor signaling and many T cells with an exhausted phenotype were clonally expanded in the tumor but not the blood.

To link multi-omics TIL profiling to spatial localization, we used the spatial molecular imager (SMI) , which is a novel spatial transcriptomics platform that allows spatially resolved high-dimensional cellular phenotyping for comprehensive TIL profiling. SMI uses fluorescent molecular barcodes to enable in-situ measurement of biological targets on an intact tissue sample.

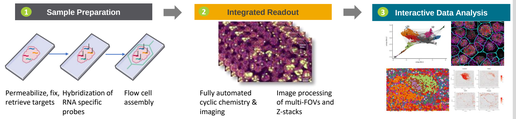
SMI analysis of matched tumor tissue was used to accurately quantify the densities and to compare the spatial organization of various T cell and other cellular subsets. Preliminary data show that T cells with the most terminally exhausted gene expression localize to areas of with a high degree of T cell infiltration into the tumor bed.

Integration of non-spatial single cell data to spatial single cell data



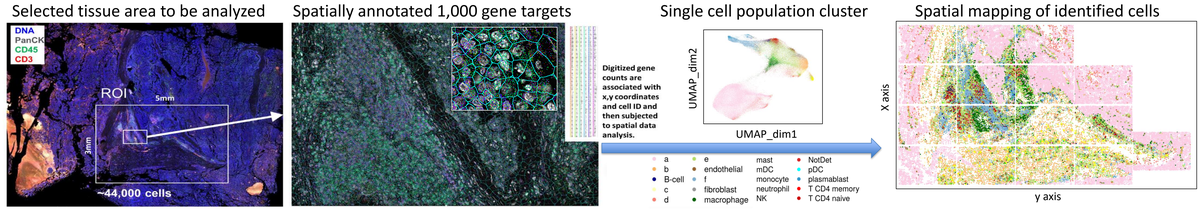
SMI assay overview

SMI is a single instrument solution for subcellular spatial analysis.

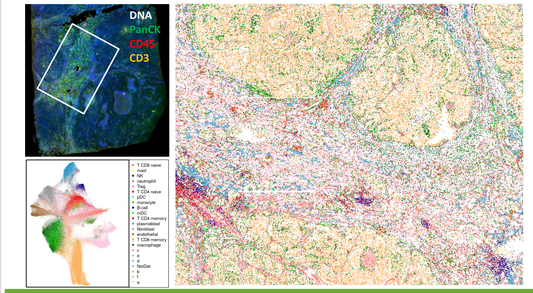


The Spatial Molecular Imaging (SMI) platform applied to an RCC tumor allows for subcellular 1000-plex spatial transcriptomics

Cell Typing and mapping of the renal cell carcinoma (kidney tumors) overview



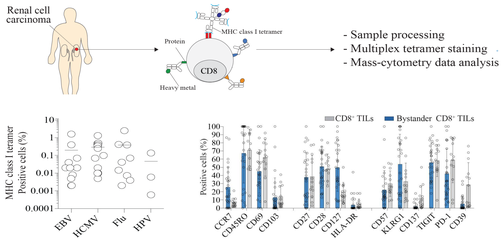
200K single cells of the renal cell carcinoma were spatially characterized and mapped back into the neative locations.



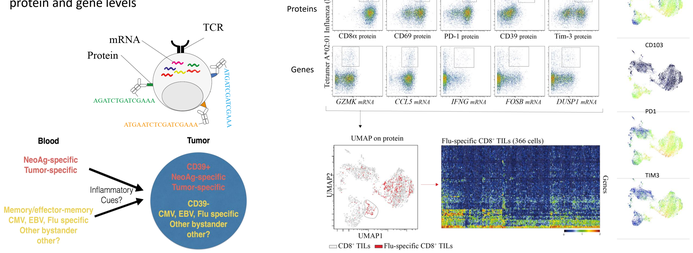
Marker-gene expression of each cell types are visualized in a spatial map.

Non-spatial single cell RNA-seq data were used to characterize subsets and gene-expression signatures of tumor-infiltrating lymphocytes

Mass cytometry analysis of RCC tumor infiltrating CD8 T cells highlights variable abundance of bystander tumor infiltrating T cells:

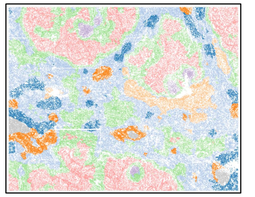


Single-cell sequencing based analysis of RCC tumor infiltrating CD8 T cells allows for determination of T cell subset transcriptional profiles:



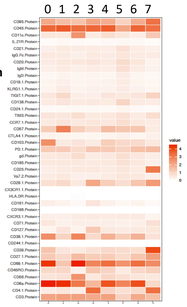
Integration of non-spatial scRNA-seq data to spatial SMI single cell data helps to visualize rare population of cell types of interests.

Individual cells were scored for the abundance of each cell type among their 200 closest neighbors. Cells were then clustered according to this neighborhood data. In this figure, cells are shown in physical space and colored by their neighborhood cluster

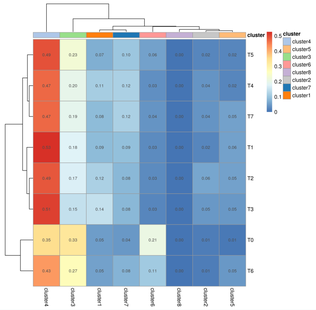


We used CITE-seq to profile and cluster T cell subtypes. Based on protein expression, we identified 8 clusters of T cell subsets. From the data, we determined gene expression signatures of T cell subsets.

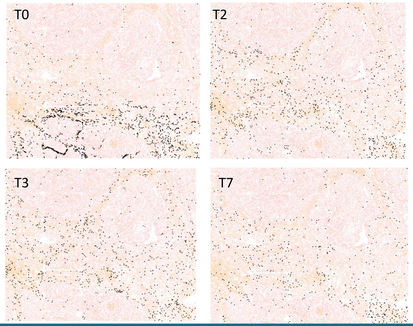
Notable clusters:
0 – CD8⁺CD69⁺CD103⁺CD39⁺
2 – CD8⁺CD69⁻CD103⁻CD39⁻
3 – CD4⁺ non-Treg
7 – CD4⁺ Treg



The following figure shows where those 8 T cell subsets are inriched in spatial clusters. As expected, most of T cell subsets are found in spatial cluster 4 except T0 and T6.



Each T cell subset is spatially mapped out.



Conclusion

As we previously reported for lung and colon cancer, virus-specific bystander T cells can be readily detected in kidney (RCC) tumors and display diverse phenotypic profiles.

The Spatial Molecular Imager (SMI) is a single instrument solution for subcellular spatial analysis. Provides sub-cellular resolution of 1000+-plex transcriptomic information.

Distinct niches of the tumor microenvironment can be delineated within RCC tumor sections. By this analysis, tumor-rich, stroma, and various regions with high densities of immune cell infiltrates (e.g., regions co-enriched for T and B cells) can be clearly identified.

Cellular subsets with gene expression profiles defined using single-cell sequencing-based multi-omics can spatially defined using SMI. T cells with an exhausted phenotype are preferentially enriched in tumor-dense regions in contrast to non-exhausted cells with bystander-like phenotypes.