Exploring the immune-tumor microenvironment using high resolution single-cell spatial transcriptomics

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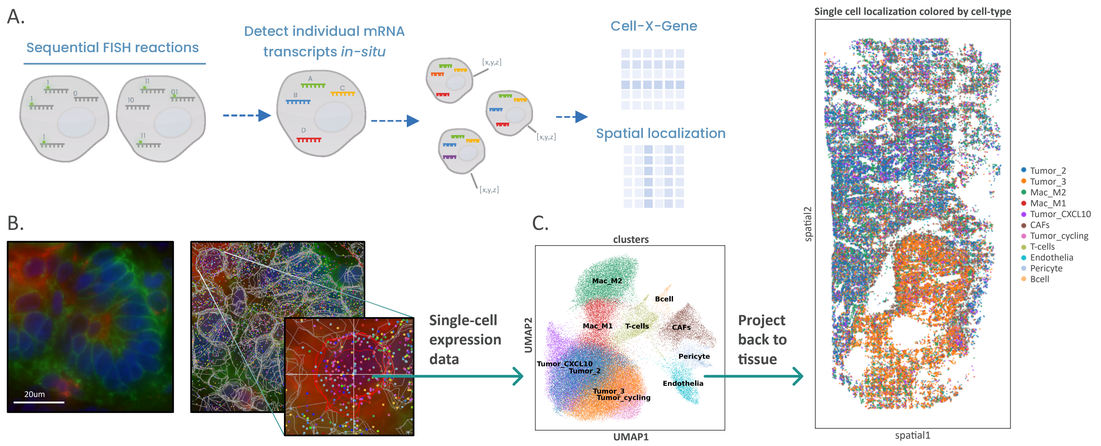
BACKGROUND

Cancer immunotherapies manipulate the immune system to repropagate anti-tumor response. Development of such treatments relies on the understanding of the interactions between tumor, stroma and immune cells found in the tumor microenvironment (TME); which are often based on direct cell-cell interactions and local secretion of factors. Therefore, high resolution spatial mapping of immune cells and adjacent cells is highly valuable resource for deciphering nature of interactions in the TME for the development of novel anti-cancer treatments. Here we harness advancements in MERFISH technology to detect the expression of 350 distinct mRNA transcripts at sub-cellular resolution directly on Ovarian & Colorectal tumor tissue sections.

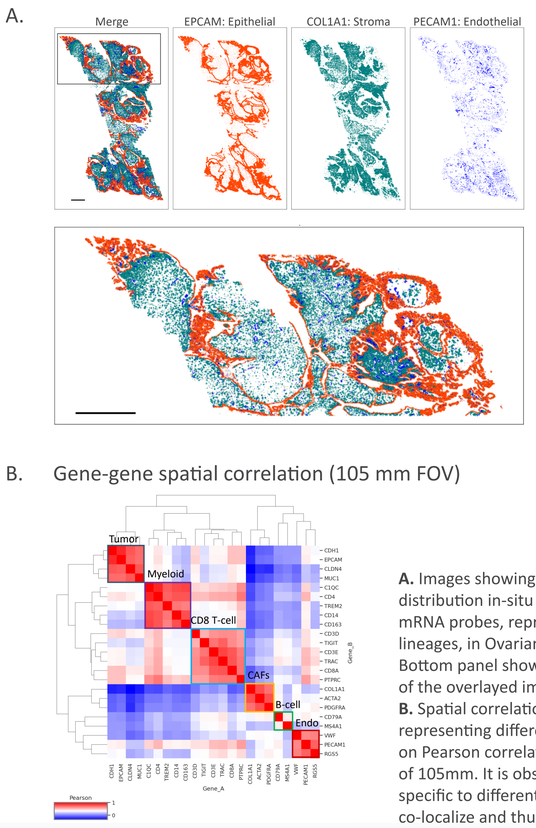
Methods:
MERFISH assay was conducted on FFPE or frozen sections using the Vizgen Inc protocol and images were captured using 60x microscope. Images were decoded to RNA spots with xyz and gene id using Vizgen's Merlin software. Single-cell analysis was conducted using Scanpy after filtering cells based on size and quality. Probe and spatial single-cell visualizations were done using custom code, MERSCOPE-viewer or Scanpy. Cellular neighborhood and gene-gene correlation was calculated using custom code. Ligand-receptor analysis was conducted using Squidpy.

TECHNOLOGY



A. Illustration of the MERFISH technology, using sequential FISH reactions each transcript is detected by series of binary barcodes which are later translated into distinct mRNA transcripts. Transcripts are then quantified per cell as detected by cell-border stain to obtain single-cell expression matrix and spatial localization map. **B.** Image showing cell boundary stain in CRC tumor (left) and the overlayed boundaries as detected algorithmically (gray line in the middle image) individual mRNA transcripts appear as colored dots. Example of individual cell and its associated transcripts (bottom left). **C.** (left) UMAP showing the clustering of cells based on the extracted single-cell data and later projected back onto the spatial localization (right). M1 – inflamatory macrophages. M2 – anti-inflamatory macrophages

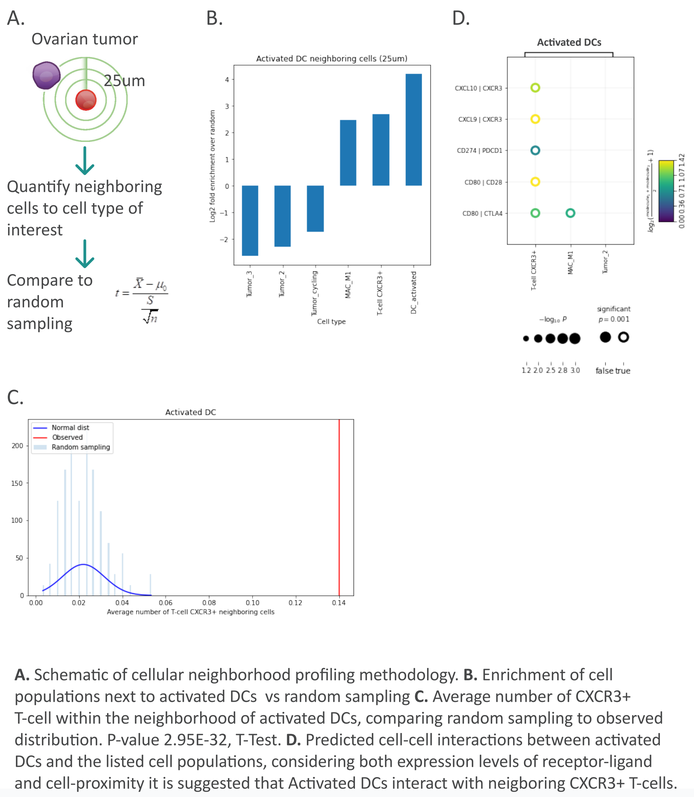
SPATIAL mRNA CORRELATION PROVIDES MEANS TO STUDY GENE ASSOCIATION



A. Images showing the spatial distribution in-situ of selected mRNA probes, representing different lineages, in Ovarian tumor sample. Bottom panel shows enlarged region of the overlayed image. Scale bar 1mm **B.** Spatial correlation of selected genes representing different lineages based on Pearson correlation in window size of 105mm. It is observed that genes specific to different cell type spatially co-localize and thus cluster together.

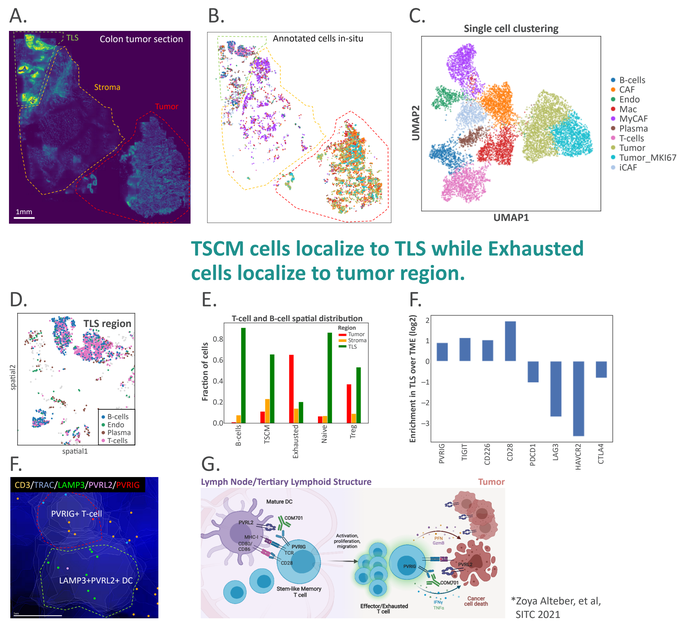
PROFILING CELLULAR NEIGHBORHOOD AT THE SINGLE CELL LEVEL

Activated DCs secreting CXCL10 are found adjacent to CXCR3+ CD8 T-cells



A. Schematic of cellular neighborhood profiling methodology. **B.** Enrichment of cell populations next to activated DCs vs random sampling **C.** Average number of CXCR3+ T-cell within the neighborhood of activated DCs, comparing random sampling to observed distribution. P-value 2.95E-32, T-Test. **D.** Predicted cell-cell interactions between activated DCs and the listed cell populations, considering both expression levels of receptor-ligand and cell-proximity it is suggested that Activated DCs interact with neigboring CXCR3+ T-cells.

DNAM-1 AXIS SHOW DOMINANT PRESENCE IN TERTIARY LYMPHOID STRUCTURES (TLS)



*Zoya Alteber, et al, SITC 2021

A. DAPI pseudo-coloring of colorectal tumor section highlighting tumor, stroma and TLS regions. **B.** Spatial localization of single cells corresponding to (A) **C.** UMAP showing single-cell clustering of single cells. **D.** Localization of selected cell types in the TLS region showing the unique co-localization. **E.** Relative abundance of types of T-cells and B-cells in the three regions noted in (A) **F.** Fold enrichment of selected checkpoints shows preferential expression of PVRIG, TIGIT and DNAM-1 at the TLS region vs TME **F.** Image showing individual PVRIG+ T-cell adjacent to LMAP3+PVRL2+ DC in the TLS region. **G.** Summarizing illustration showcasing the activity of PVRIG in the TLS regions and potential contribution of COM701 (anti-PVRIG) treatment to activating immune response.

CONCLUSION

High-resolution Spatial Transcriptomics allows in-depth profiling of the TME at the single-cell level. We have used this method to reaffirm aspects of known biology of the TME, such as association of CXCL10+ Activated DCs and CXCR3+ T-cells. We also demonstrated the presence of DNAM-1 pathway members in TLS regions. The presented methods could be used to uncover new biological findings that would enhance the understanding of the TME and possibly support the development of new cancer immunotherapy treatments.