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Establishment of spatial transcriptomics assay to find the mechanism of immune therapy against tumors

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ABSTRACT

BACKGROUND: Intra-tumor heterogeneity is one of the biggest challenges in cancer treatment today, not only on cell types and functions, but also on the spatial pattern of immune cells within the tumor tissue. While most of the single-cell RNA sequencing technologies could enable the exploration of gene expression heterogeneity at the single cell level, they cannot remain spatial information within the tumor for us to map the whole transcriptome with morphological context. Spatial transcriptomics is an in situ capturing technique, which profiles gene expression at RNA level, whilst preserving the spatial information of histological tissue sections. Thus we try to develop our spatial transcriptomics assay through a serial of validation experiments and to find the mechanism of immune therapy against tumors.

METHODS: Firstly, the tumor from mouse colorectal cancer syngeneic mode MC38 was frozen and sections were cryosectioned at 10 μm thickness. Sections processing including H&E staining, tissue optimization, permeabilization, reverse transcription and cDNA library preparation was carried out following the user guide of Visium Spatial Gene Expression Reagent Kits (10X Genomics). And the final cDNA libraries were sequenced using Illumina Novaseq instrument. Using gene expression matrix and aligned H&E image processed by Cell Ranger and Loupe Browser with default parameters, we performed data QC, normalization, dimensionality reduction and clustering by Seurat and cell components identification in tumor microenvironment based on a multistep approaches. Differential gene expression and spatial heterogeneity were analyzed in each clusters.

RESULTS: In this study, we established a reliable system for spatial transcriptomics assay to elucidate the mechanism of anti-PD1 treatment in mouse syngeneic model MC38. Using this system, we constructed the gene expression spatial patterns of mouse tumor cell and immune cell, including periphery and center of tumor cell, immune-cell enrichment area and tumor cell surrounding immune cell. Furthermore, we explored the cell-cell interactions in which the mouse tumor cells directly interacts with adjacent non-tumor cells, for example macrophages. Using this system, we tried to find the mechanism of anti-PD-1 against the tumors by comparing the spatial transcriptome profiles changed after the treatment with anti-PD-1 or isotype control in mouse colorectal cancer models. Interestingly, changes in both gene level and spatial level were observed, and it will help us to reveal how the immune therapy may shake up the complex ecosystem.

RESULTS FIGURE 1-3

Figure 1. Schematic workflow of single cell sequencing.

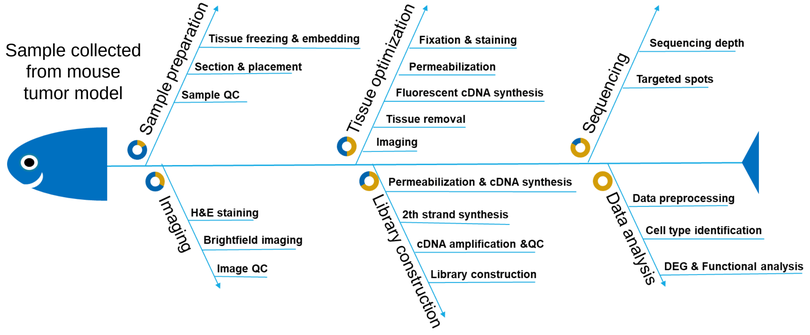
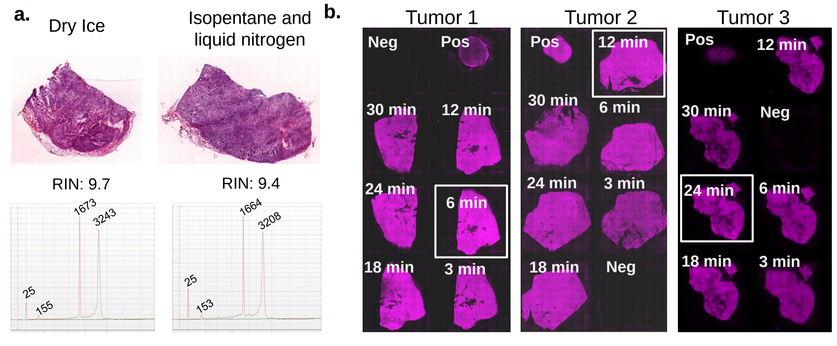
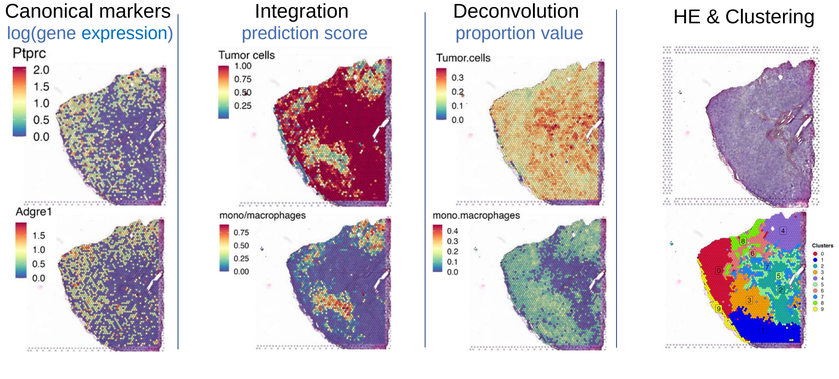


Figure 2. Fit-for-purpose sample processing optimization



a. HE staining and RNA quality assessment of tumors frozen by dry ice or isopentane and liquid nitrogen. **b.** To optimize permeabilization conditions for tumors.

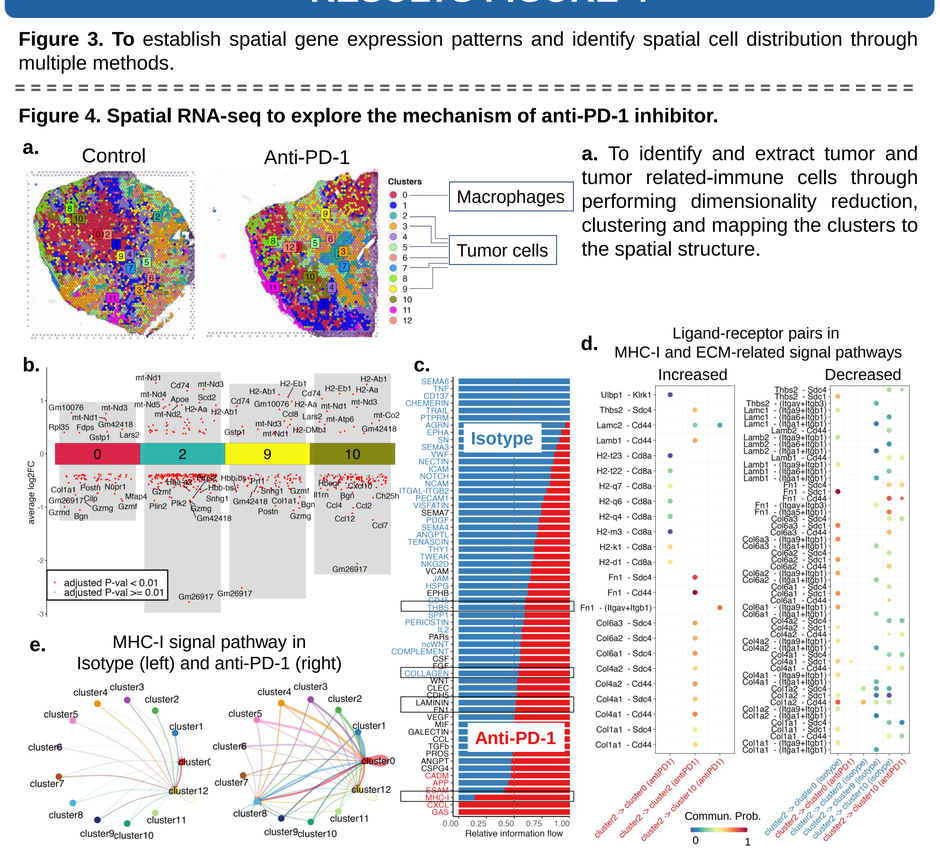
Figure 3. Identification of major cell populations by multiple methods.



RESULTS FIGURE 4

Figure 3. To establish spatial gene expression patterns and identify spatial cell distribution through multiple methods.

Figure 4. Spatial RNA-seq to explore the mechanism of anti-PD-1 inhibitor.



a. To identify and extract tumor and tumor related-immune cells through performing dimensionality reduction, clustering and mapping the clusters to the spatial structure.

b-e. Changes in both gene level and spatial level after anti-PD-1 treatment. Through differential gene expression analysis and cell-cell communication analysis between adjacent tumor cells and immune cells, we found that: **1)** many antigen presentation related genes were unregulated in tumor cells after anti-PD-1 treatment **(b); 2)** anti-PD-1 inhibitor may remodel the tumor microenvironment by enhancing MHC-I related antigen presentation signal pathways **(c,e)** and decreased ECM-related signal pathways **(c,d)** between tumor cells and surrounding immune cells.

SUMMARY

We have optimized and established a reliable system for spatial transcriptomics analysis, to dissect the complicated changes of TME after immune checkpoint blocker treatment. Spatial RNA-seq technology could be a powerful method to help us to find the potential mechanism after immunotherapy and seek the possible therapeutic targets.