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Establishment and Optimization of scRNA-seq assay to find the mechanism of immune therapy against tumors

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ABSTRACT

BACKGROUND: Tumors are complex ecosystems composed of different cell types with different phenotypes, status and gene profiles. Commonly used GEP tools like bulk RNA Sequencing could only display the gene expression profiles as a whole, and cannot reflect the heterogeneous tumor cell change or immune composition in a tumor. Single cell RNA Sequencing can be a good tool to implement it as single cell level. However, the method and experiment system should be optimized to make sure the result is reliable and interpretable. Thus we try to develop and optimize our scRNA-seq assay through a series of validation step to make sure the reliability of the results, to find the mechanism of immune therapy against tumors.

METHODS: Sample processing were validated for each step separately, including tumor dissociation, dead cell removal and red blood cell lysis. Single-cell 3′ library generation was performed on the 10x Genomics Chromium Controller following the manufacturer's protocol for the Single Cell 3' Reagent Kits v3.1 (10x Genomics), and libraries were sequenced using Illumina Novaseq instrument. Using gene expression matrix processed by Cell Ranger with default parameters, we performed data QC, normalization, clustering by Seurat and cell type identification based on a multistep approaches. Differential gene expression were analyzed in each clusters.

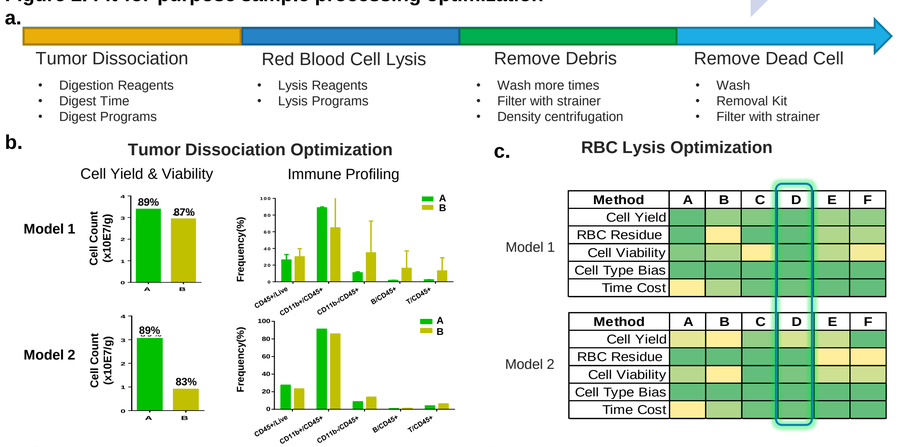
RESULTS: In this study, we established a reliable system for scRNA-seq analysis to elucidate the mechanism of anti-PD-1 treatment, and the interaction between the tumor and immune cells. We optimized the system from the following 3 aspects: 1) sample processing method to make sure the cell yield, viability and no bias introduced. 2) Library size that sensitive enough for our sub-population analysis. And 3) whether to sort immune cells before the library construction. Using this system, we tried to find the mechanism of anti-PD-1 against the tumors by comparing the scRNA-seq profiles changed after the treatment in two breast cancer models, EMT-6 and 4T1, which are responsive and non-responsive to anti-PD-1 treatment, respectively. Both models were treated with anti-PD-1 or isotype control and tumors were collected for scRNA-seq analysis. Interestingly, changes in both tumor cells and tumor micro-environment were observed, and it will help us to reveal how the immune therapy may shake up the complex ecosystem.

SUMMARY: We optimized and established a reliable system for scRNA-seq analysis to explore the mechanism of anti-PD-1 treatment against the breast cancer..

RESULTS FIGURE 1-3

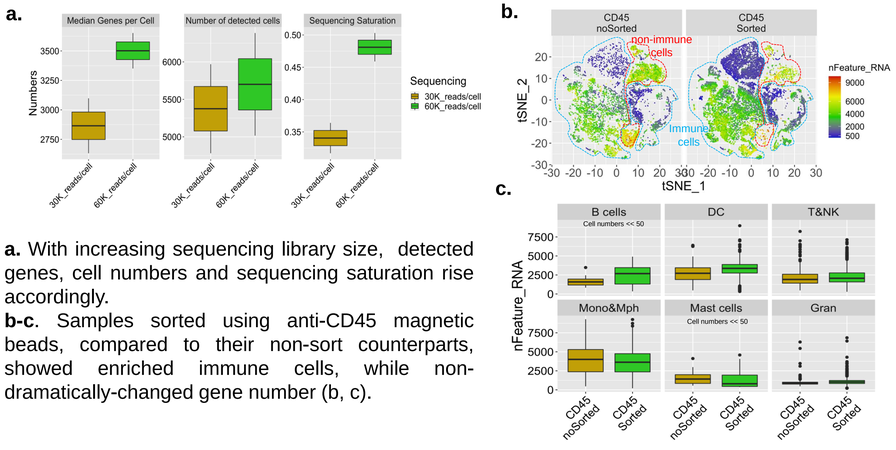
Figure 1. Schematic workflow of single cell RNA sequencing

Figure 2. Fit-for-purpose sample processing optimization



a. Schematic diagram of tumor tissue processing. **b**. Develop and optimize the tumor dissociation method that could be apply to all models in one study, to minimize the difference introduced by sample processing. Improper tumor dissociation could cause unstable or artificial results in the following scRNA-seq analysis. **c.** Optimize red blood cell lysis method according to cell yield, RBC residue, cell viability, cell type bias and time cost.

Figure 3. cDNA library construction and sequencing library size exploration



a. With increasing sequencing library size, detected genes, cell numbers and sequencing saturation rise accordingly.
b-c. Samples sorted using anti-CD45 magnetic beads, compared to their non-sort counterparts, showed enriched immune cells, while non-dramatically-changed gene number (b, c).

RESULTS FIGURE 4

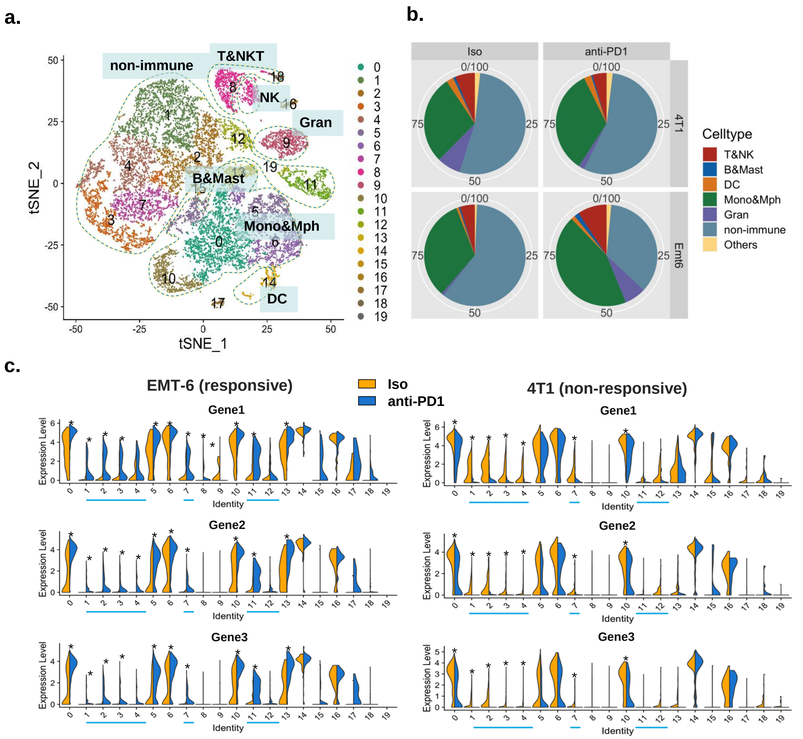


Figure 4. Single cell RNA-seq data analysis to explore the mechanism of Anti-PD-1 antibody resistance in 2 different models responsive differently to the treatment
a. Identification of dominated cell populations by canonical markers.
b. The composition change of major cell types detected was consistent with the drug responsiveness in two models respectively.
c. Three genes from the same family were found regulated the opposite way in these two models, which indicated their potential roles in anti-PD-1 antibody resistance.

SUMMARY

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