Integrative spatial and single-cell analysis elucidates tumor microenvironment heterogeneity in NSCLC

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Abstract

Background:

Tumor microenvironment (TME) heterogeneity plays a crucial role in cancer progression and therapeutic response. Advanced multi-omics spatial and single-cell technologies offer unprecedented resolution for dissecting TME complexity. However, the application of these technologies to formalin-fixed paraffin-embedded (FFPE) samples remains underexplored. This study aims to demonstrate the power and capability of these technologies in analyzing FFPE samples, benchmark and cross-validate different platforms/assays, and deepen our understanding of TME heterogeneity at the single-cell level and its spatial context.

Methods:

We performed following assays including 1) High-plex multiple IHC platform: PhenoCycler Fusion (PCF) protein-level spatial analysis (n=10), 2) FFPE Single-Cell RNA Sequencing (FFPE scRNA-seq) (n=4) and 3) Bulk RNAseq RNA-level analysis with FFPE NSCLC samples

Results:

Firstly, to evaluate the PCF marker expression pattern, cell density of PanCK+PD-L1+ cell ratio (%) was compared to PD-L1 IHC TC score, which showed a high correlation. Further, By image co-registration between H&E with ML-based tumor/stromal classification and PCF PanCK-PD-L1 staining, PD-L1+ cell density in tumor cell specifically can be precisely calculated.

To further evaluate the marker gene expression consistency, high gene expression concordance was observed across PhenoCycler Fusion (PCF), formalin-fixed paraffin-embedded (FFPE) single-cell RNA sequencing (scRNA-seq), and bulk RNA sequencing (bulk RNA-seq) data. This high concordance (average pearson r=0.84) validates the consistency and reliability of our multi-omics approach, ensuring that the data obtained from different sample types are comparable and robust.

A diverse TME was identified, highlighting the complexity and heterogeneity of TME components. This diversity was evident in the range of immune cell subsets and cancer-associated stromal cells detected, underscoring the importance of comprehensive TME analysis even in small cohorts.

In addition, FFPE scRNAseq enabled a deeper profiling at transcriptome level with a median gene recovery rate at 1130 genes per cell, which contribute to a more detailed cell lineage identification including small populations like Mast cells and neutrophils.

Conclusions:

Overall, the study demonstrates the effectiveness and reliability of PCF in evaluating PD-L1 expression and the TME. The high concordance across multi-omics approaches and the detailed transcriptome profiling provided by FFPE scRNAseq highlight the robustness of these latest methodology on FFPE samples. Despite the limited sample size, the diverse TME identified emphasizes the need for comprehensive and personalized TME analysis. These findings provide a foundation for further investigations into the TME.