Spatially resolved cell profiling unveils tumor metabolic states associated with immunotherapy response in NSCLC

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Introduction

- Multiplex imaging offers a high-resolution approach to comprehensively profile the tumor microenvironment (TME), capturing both tissue architecture and functional states.
- As only a subset of non-small cell lung cancer (NSCLC) patients benefit from immune checkpoint inhibitors (ICIs), there is a clinical need to develop predictive biomarkers for ICIs and to deepen our understanding of response and resistance mechanisms.
- In this study, we leveraged multiplex imaging techniques to investigate functional profiles within cell subtypes in both responders and non-responders to immune checkpoint inhibitors (ICIs).

Methods

- We profiled a retrospective cohort of 39 NSCLC tissue cores from 27 patients treated with ICI, using highly multiplexed immunofluorescence (mIF) stained by the Phenocycler Fusion platform (Akoya Biosciences) capturing 45 proteins.
- Utilizing our previously published deep learning-based multiplex imaging analysis pipeline[1] (Figure 1), cells were classified to 15 cell types by known protein expression.
- To identify functional cell subtypes, each cell type was further subclassified by unsupervised clustering of the expression vector of metabolic and cell state proteins.
- Following cell typing, cellular neighborhoods were designated as previously described[2]. Tumor cell-enriched neighborhoods were defined as the tumor area, whereas all other neighborhoods were defined as the TME.
- We compared the distribution of cell types and subtypes within tumor areas between responders and non-responders to ICI therapy using Fisher's exact and logrank tests.

References

1. Markovits, E. et al. A novel deep learning pipeline for cell typing and phenotypic marker quantification in multiplex imaging. bioRxiv 2022.11.09.515776 (2022).
2. Schürch, C. M. et al. Coordinated Cellular Neighborhoods Orchestrate Antitumoral Immunity at the Colorectal Cancer Invasive Front. Cell 182, 1341-1359.e19 (2020).

Results

- Unsupervised cell subtyping of the 15 cell types identified 43 cell subsets, which were mostly segregated by their metabolism and activation status (Figure 2).
- In lymphocytes, we found a connection between metabolic state, effector functions and tissue localization as the metabolically active lymphocytes exhibited higher levels of PD-1, MHC class I and II and CD44 positivity, and were more abundant within tumor infiltrating lymphocytes (TILs) and tertiary lymphoid structures (Figure 3).

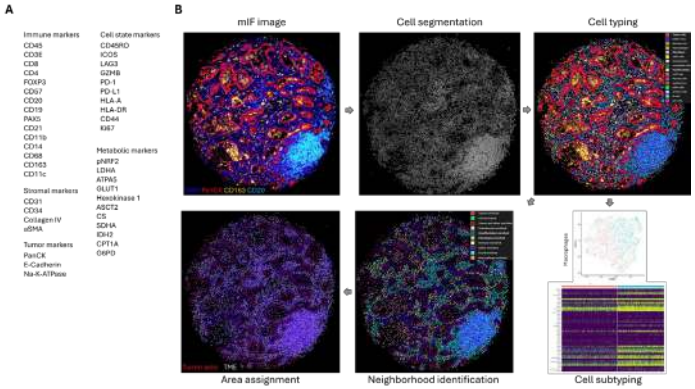

Figure 1: (A) Protein panel used in this study. (B) Image structuring workflow diagram.

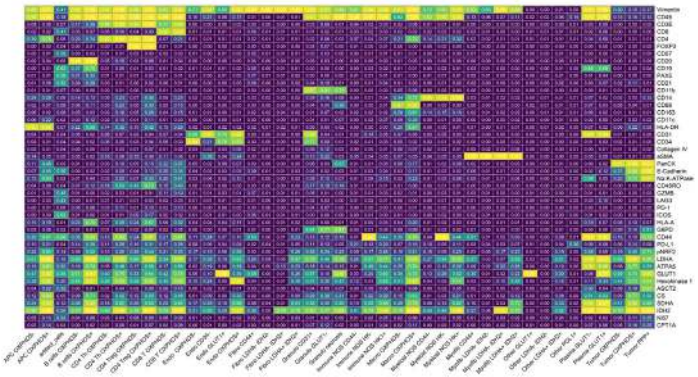

Figure 2: Mean marker positivity within the 43 cell subtypes.

- Tumor cells clustered to three main metabolic states - OXPHOS+, OXPHOS- and a third cluster (PPP+), which was characterized by upregulation of ASCT2, a glutamine transporter, as well as pNRF2 and G6PD, regulators of the pentose phosphate pathway. These cells exhibited higher proliferation rate and upregulation of CD44, a tumor stemness marker (Figure 4A).
- Tumors with high content of PPP+ tumor cells (>40%) were resistant to PD-1 blockade and showed reduced overall survival (OS) rates (Figure 4B).

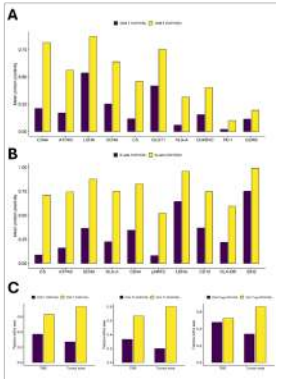

Figure 3: A-B) Differentially expressed proteins between CD8 T-cells (A) and B-cells (B) subtypes. (C) Fraction of cell subtypes by area.

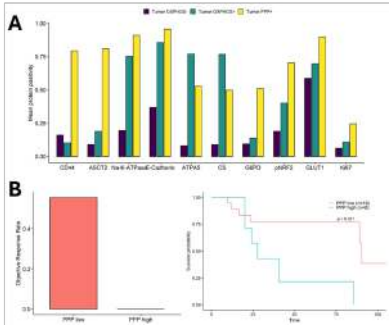

Figure 4: (A) Differentially expressed proteins between tumor cells subtypes. (B) Objective response rate and overall survival between the PPP high and low groups.

Conclusion

- Our novel approach for cell typing and subtyping combining supervised and unsupervised methods has unveiled a nuanced landscape of distinct cell subsets in the tumor microenvironment, primarily categorized by metabolism and activation states.
- This comprehensive profiling of lymphocytes and tumor cells provides critical insights into the interplay between metabolic state, effector functions, and tissue localization, potentially reshaping our understanding of immune response mechanisms.
- The identification of a unique PPP+ metabolic state in tumors, associated with resistance to PD-1 blockade and reduced overall survival, underscores the clinical importance of metabolic profiling in predicting treatment outcomes and patient prognosis.
- These findings not only advance our knowledge of tumor biology but also pave the way for personalized therapeutic strategies targeting specific metabolic pathways to enhance treatment efficacy and patient outcomes.