Development of a Novel Immuno-Metabolic Spatial Signature to Predict Response and Resistance to Immunotherapy in NSCLC Patients

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Introduction

Immune checkpoint blockade therapies (ICB) have led to durable benefits in a subset of patients in non-small cell lung cancer (NSCLC), however the majority of patients fail to respond. In this study, we spatially profiled the tumor-immune contexture and metabolic activity of first-line ICB treated patients to gain further understanding of the functional cell types, states, and interactions to predict treatment sensitivity and resistance.

Methods

- Utilizing a multiplexed immuno-fluorescence (mIF) panel of 44 proteins, covering both immune and metabolic cell states (Figure 1A), we profiled tumor microarrays of two independent retrospective cohorts of NSCLC patients treated with ICB (n=117, of which 72 are pre-treatment biopsies with clinical annotation).
- Deploying Nucleai's deep learning (DL)-based multiplex imaging analysis pipeline[1], marker positivity was determined by an automated DL-based classifier for each segmented cell, followed by assignment to 14 cell types by known marker expression profiles. Cell subtypes were established by clustering of positive prediction probabilities of metabolically and immunologically relevant markers (Figure 1B).
- Spatial features were calculated, Combining cell types, phenotypic states, and spatial context.
- To identify spatial features associated immunotherapy clinical outcome within each metabolic subgroup, we performed Welch's T-test between clinical benefiters (responders) and non-clinical benefiters (non-responders), defined as an objective response or prolonged progression free survival (PFS > 6 months) or overall survival (OS > 18 months).
- A multivariate model of clinical benefit prediction, was built based on differentially expressed spatial features within tumor subtypes in the training set (n=53) and was validated on an independent test set (n=19). Performance evaluation was done using ROC analysis, and the model predictions association with clinical outcome.

Results

- We identified five distinct tumor clusters which differed in their cellular composition, immune and metabolic states, and clinical outcomes following PD-1 blockade. Key proteins differentiating these clusters included CD44, G6PD, Hexokinase-1, HLA-A, and oxidative phosphorylation proteins. Within clusters, we observed varying levels of CD8 T cell infiltration and immune molecules, such as PD-L1 and IDO1, which were particularly higher in the HLA+ cluster (Figure 2A). All patients in the CD44+ tumor cluster had clinical benefit vs. ~40% in the G6PD+ and HEX1+ clusters, and exhibited prolonged PFS (median PFS of 23.2 months in the CD44+ tumor cluster, vs. 3.0 and 3.3 months in the G6PD+ and HEX1+ tumor clusters, p=0.02), and a trend towards prolonged OS (Figure 2B).
- Spatial feature distribution differed significantly between responders and non-responders across the whole cohort and within tumor clusters, as exhibited by poor correlation of the T-statistics of differentially expressed spatial features within tumor clusters (Figure 3A, B). Thus, we speculated that different tumor metabolic states were associated with different biomarkers of response and resistance to ICB. Indeed, within the G6PD+ and HEX1+ tumor clusters, positivity for oxidative phosphorylation proteins was associated with better clinical outcome, while in the HLA+ and OXPHOS- tumor clusters, high expression of immune activation related proteins, such as HLA-A and PD-L1, were more significantly associated with clinical outcome (Figure 3C).
- A multivariate ICB outcome predictor based on tumor subtypes demonstrated high AUC in an independent test set (0.93, p=0.001) with predicted responders demonstrating higher clinical benefit rate (84.6% vs. 16.7, p=0.01), as well as higher median PFS (9.4 vs 3.7 months, HR=0.2 (0.06-0.67), p=0.004) and median OS (Not reached vs. 6.0 months, HR=0.21 (0.06-0.69), p=0.005).

Conclusions

Our study highlights the significant impact of tumor metabolic subtype classification on the clinical outcome biomarker landscape of NSCLC patients undergoing PD-1 blockade therapy. Our analysis revealed that the clinical relevance of spatial features varies significantly between tumor clusters, suggesting that this metadata is essential for accurate ICB clinical outcome prediction. Despite being one of the largest clinically annotated immunotherapy datasets analyzed by mIF, we are still limited by the cohort size which might not fully capture the complexity of tumor states associated with immunotherapy response. Additionally, the use of tumor microarrays limited our analysis to the tumor area, restricting our ability to capture the full extent of tumor heterogeneity and immune cell distribution across the tumor stroma. Nonetheless, our research illustrates the potential of multiplex imaging to deepen our understanding of tumor states associated with immunotherapy response and its promise in biomarker identification and personalized treatment selection.

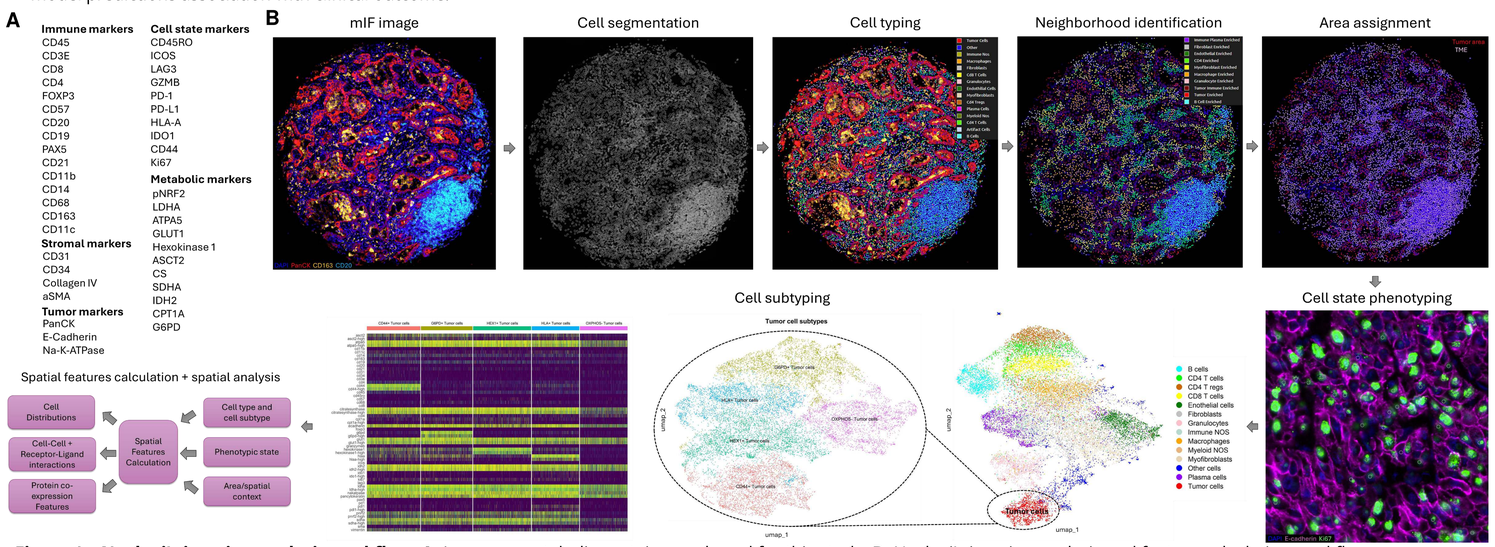

Figure 1 – Nucleai's imaging analysis workflow. A, Immuno-metabolic protein panel used for this study. **B,** Nucleai's imaging analysis and feature calculation workflow.

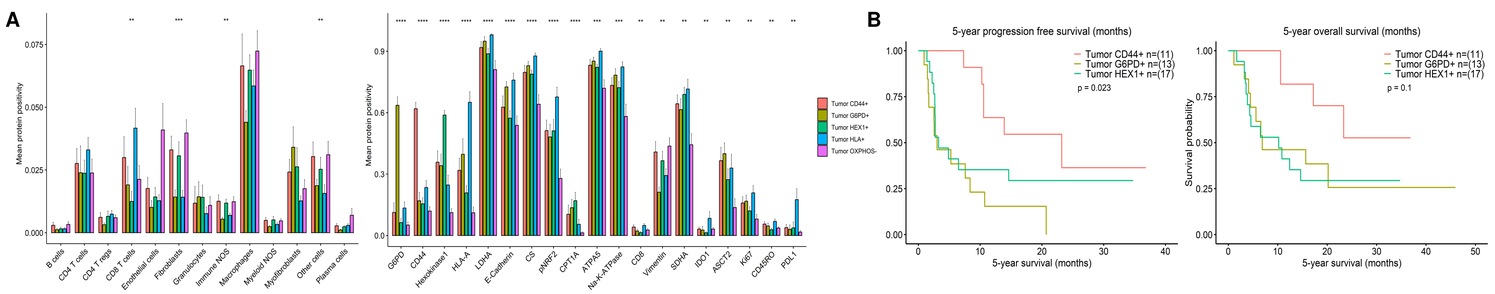

Figure 2 – Tumor metabolic clusters differ in their cellular composition, protein positivity, and in ICB-related clinical outcome. A, differential cell type distribution and marker positivity within the tumor area of the tumor subtypes. **B,** Progression free and overall survival comparison between the CD44+, G6PD+ and HEX1+ tumor clusters.

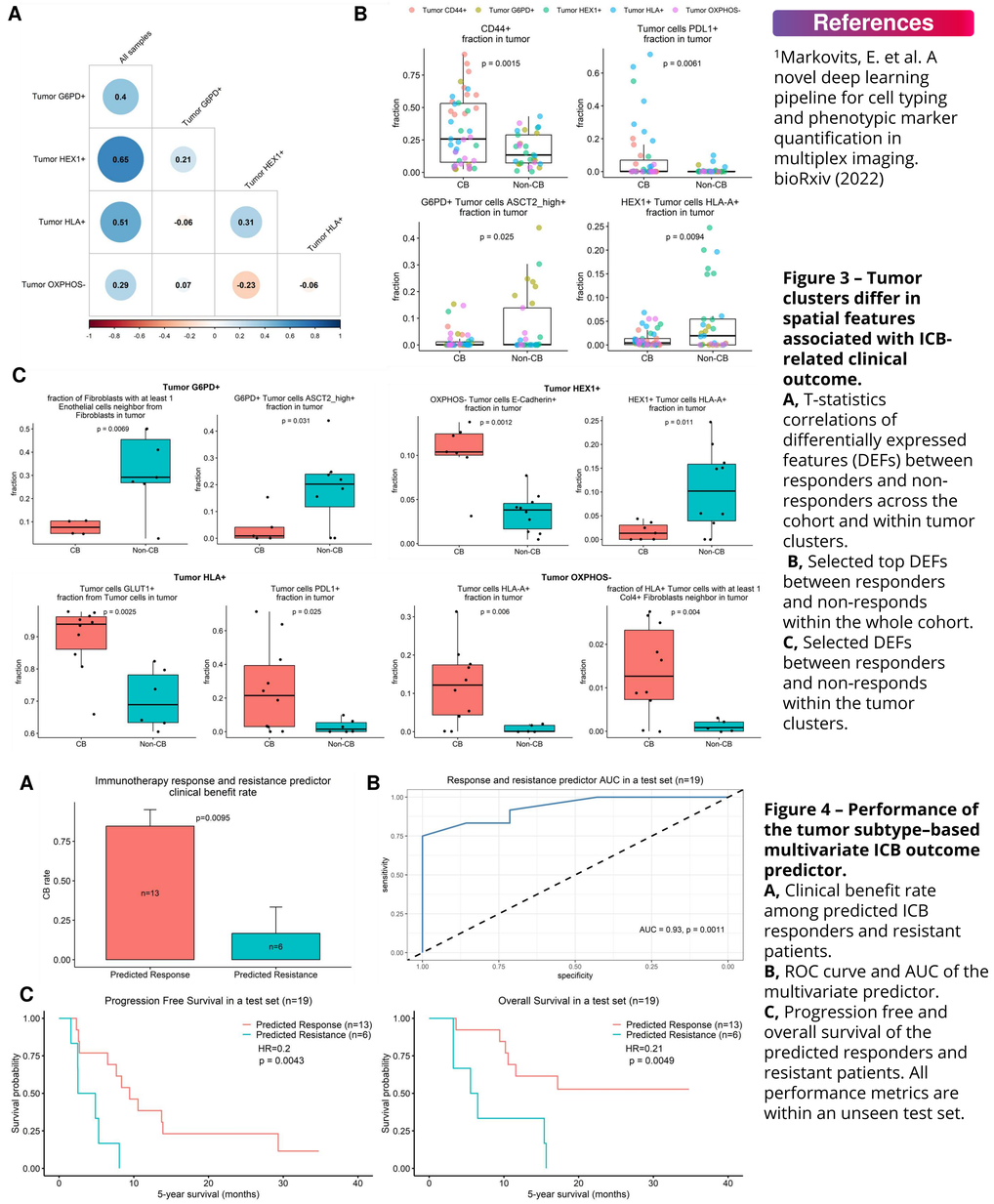

Figure 3 – Tumor clusters differ in spatial features associated with ICB-related clinical outcome.
A, T-statistics correlations of differentially expressed features (DEFs) between responders and non-responders across the cohort and within tumor clusters.
B, Selected top DEFs between responders and non-responds within the whole cohort.
C, Selected DEFs between responders and non-responds within the tumor clusters.

Figure 4 – Performance of the tumor subtype–based multivariate ICB outcome predictor.
A, Clinical benefit rate among predicted ICB responders and resistant patients.
B, ROC curve and AUC of the multivariate predictor.
C, Progression free and overall survival of the predicted responders and resistant patients. All performance metrics are within an unseen test set.

References

[1]Markovits, E. et al. A novel deep learning pipeline for cell typing and phenotypic marker quantification in multiplex imaging. bioRxiv (2022)