A REPRODUCIBLE PIPELINE FOR ANALYSIS OF MULTIPLEX IMAGING (MIBI) DATA WITH APPLICATION TO UNCOVERING NOVEL FEATURES OF THE TUMOR-IMMUNE MICROENVIRONMENT

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Background Although immune checkpoint inhibition (ICI) has been transformational, tumor-associated factors regulating response have not been elucidated. High-dimensional spatial profiling technologies have enabled simultaneous investigation of many protein targets on individual cells within the spatial context of the tumor microenvironment (TME). Analysis of these data to uncover immune and tumor profiles relies on identification of individual cells and characterization of their specific marker expression to classify lineage and functional state. However, robust automated cell type assignment remains a key challenge in multiplex image data analysis. Here, we describe a reproducible pipeline for single cell identification and typing from multiplex image data utilizing lineage protein expression, which has applications in the context of precision immunotherapy and beyond.

Methods Biopsies from melanoma (n = 80) or non-small cell lung cancer (NSCLC, n = 38) patients treated with anti-PD-1 were profiled by MIBI for spatial analysis of 32 protein markers. Following cell segmentation, an automated pipeline was developed to assign cell types based on bootstrap sampling of lineage-specific marker expression within each region of interest (n = 395), on a per-pixel basis. This pipeline was further optimized by integrating marker lineage-specificity to improve the identification and segmentation of cells with irregular shapes or sizes (e.g., fibroblasts, macrophages) by expanding cell outlines based on pixel type identity. The overall approach was benchmarked against existing automated and manual methods. Feature extraction and downstream harmonization with clinical and other molecular data was facilitated by our multi-omic data integration platform (CANDEL).

Results Our cell typing and segment expansion approach outperformed several alternatives in proportion of cell types confidently assigned, increasing the proportion of identified segments by 15-55% across all samples tested. The additional improvement in segmentation demonstrated by the pixel expansion method enabled clearer visualization of TME context and cellular structure, increasing the overall inclusion of macrophage and fibroblast markers by 14.9% and 30.47%, respectively. Exploration of the association between image features and clinical response to anti-PD-1 is currently underway.

Conclusions Multiplex imaging data can provide new insights into mechanisms, targets, and biomarkers for cancer immunotherapy. These insights depend on reliable segmentation and cell typing, and manual methods can be unreliable and do not scale. Our toolchain provides a foundation for reliable and scalable analysis of high-dimensional spatial profiling data. These analytical improvements will facilitate generation of new insights into mechanisms of PD-1 resistance and features of the TME that correlate with clinical outcomes.

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