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The Cancer Control Challenge:
Better Understanding It to Better Face It

A SINGLE-CELL AND SPATIAL TRANSCRIPTOMICS-INFORMED APPROACH FOR TUMOR ANTIGEN PRIORITIZATION IN IMMUNE CHECKPOINT BLOCKADE COHORTS

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Introduction: The identification of tumor antigens is crucial for advancing immunotherapies, including cancer vaccines, T cell therapies, and antibody-based approaches. Furthermore, understanding tumor antigenicity can assist in prognosis predictions and the development of personalized treatments. To enable these applications, single-cell and spatial transcriptomics technologies provide unprecedented resolution to characterize tumor heterogeneity and prioritize targets within the tumor–immune context. **Objectives**: Here, we aimed to characterize the antigenic landscape in patient cohorts undergoing immune checkpoint blockade (ICB) therapy, including both responders and non-responders. Furthermore, we also sought to validate tumor antigen expression and assess its immunogenic potential using spatial transcriptomic. **Methods**: We leveraged several publicly available datasets encompassing distinct tumor types, including skin-related cancers, as well as breast, kidney, and liver tumors. Raw count matrices were filtered based on transcript abundance (mean reads per cell ≥25,000), molecular complexity (median UMIs ≥1,000), gene coverage (median genes per cell ≥900), and cell throughput (300–7,500 cells per sample). Next, we retained only cells with less than 25% mitochondrial gene content. Potential doublets were identified and removed using scDblFinder. After quality control, high-quality cells were normalized using Seurat, followed by dimensionality reduction and clustering. Cell type annotation was performed using Celltypist. To distinguish malignant from non-malignant populations, we applied inferCNV for copy number variation inference. Finally, spatial transcriptomics data from 10X Genomics, comprising breast and melanoma tumor samples, were collected to validate the expression and spatial distribution of antigens within the tumor microenvironment. Results: In total, we identified 24 cell populations across the cancer types. The most abundant were T cells (n=161,299), fibroblasts (n=30,194), macrophages (n=47,112), endothelial cells (n=14,439), and B cells (n=29,011). Additional populations included dendritic cells (n=5,799), plasmacytoid dendritic cells (n=1,642), plasma cells (n=6,408), innate lymphoid cells (n=11,661), monocytes (n=3,111), mast cells (n=868), and smaller subsets such as erythroid precursors and granulocytes. Rare populations, such as early HSC/MPP, were also detected in specific samples. Malignant clusters displayed transcriptional signatures consistent with tumor identity. For instance,

in skin cancer samples, expression of known markers such as MART-1, BCAM, and TP63 confirmed their classification. Preliminary antigen profiling revealed the expression of PRAME and SPAG9 in malignant subsets. However, SPAG9 exhibited broad expression across non-malignant cells, which may limit its therapeutic value. We are currently developing a protocol to perform neighborhood analysis on spatial transcriptomics data, aiming to identify immunogenic hotspots — regions enriched in $CD8^+$ T cells — that are spatially associated with antigen expression. **Conclusion**: Our study demonstrates the combined potential of single-cell and spatial transcriptomics to refine tumor antigen prioritization. By integrating spatial data, we validated candidate expression and explored their spatial relationship with immune infiltration, particularly $CD8^+$ T cells. These findings support the discovery of tumor antigens as a crucial element in next-generation cancer immunotherapies, particularly in light of recent advances in RNA vaccine platforms.

Keywords: Tumor antigens; single-cell RNA-seq; tumor microenvironment.