"Dissecting the Impact of Immune Checkpoint Inhibitors on Differential Expression and Abundance in Single-Cell Data of Advanced Renal Cell Carcinoma: A Comprehensive Analysis"

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Section 1: Introduction and Description of Dataset

The paper, “Tumor and immune reprogramming during immunotherapy in advanced renal cell carcinoma” by Bi, et al. investigates the tumor microenvironment of advanced renal cell carcinoma (RCC) during therapy, specifically focusing on immune checkpoint blockade (ICB) treatment. By examining single-cell transcriptomes from RCC patients before and after ICB, the research identifies various immune and cancer cell populations, their responses to therapy, and potential mechanisms of treatment resistance. Their findings underscore the importance of exploring immunomodulatory pathways beyond the PD-1 axis and provide insights into potential therapeutic targets and combinations to combat treatment resistance in RCC.

In this study, tumor tissue samples from renal cell carcinoma patients were collected and transported in specific media on ice to maintain their freshness. The experimental approach involved mechanically and enzymatically dissociating the tissue samples to create single-cell suspensions. This process included incubating the tissue and processing it for dissociation, followed by centrifugation and resuspension of the cells. To remove red blood cells, a lysis step was performed. The single-cell isolation technology used for this study was the 10x Genomics Chromium platform, which employed Single Cell 3' (v2 Chemistry) reagents to ensure accurate and reliable results.

Section 2: Methodology

In this study, we followed several steps for data exploration, quality control, pre-processing, dimensionality reduction, and cell type annotation. The quality control and batch correction methods included calculating the percentage of mitochondrial UMI, removing cells with less than 200 genes, and removing cells with more than 25% mitochondrial RNA. For data pre-processing, we normalized, scaled by 10,000, and log-transformed the data, selecting 10,000 variable features.

Dimensionality reduction was performed using PCA, selecting the first 15 PCs, and UMAP for visualization. Potential batch effects were assessed and harmonized using the Harmony algorithm. Data was clustered using the Louvain algorithm with a resolution of 0.5 as was done by Bi, et al . The Wilcoxon test was employed to identify marker genes that were highly expressed in each cluster.

We annotated the cell type clusters based on the marker genes, validated by the paper and Panglao DB, including plasma cells, dendritic cells, TP1 cells (tumor cells), fibroblasts, NK cells, monocytes, hepatocytes, cycling cells, B cells, and endothelial cells. These cells encapsulate the microenvironment of the renal cell carcinoma and the tumor itself.

Section 3: Further Analyses

In this study, we performed further analyses focusing on differential expression and differential abundance of cell types. For differential expression, we aimed to identify genes differentially expressed in patients who received immune checkpoint blockade (ICB) treatment compared to those who did not. We conducted a differential expression analysis between ICB-exposed and non-exposed groups and identified the top 10 differentially expressed genes. A heatmap was then plotted to visualize the expression patterns across the groups.

For differential abundance, we first assigned cell type names to the clusters in the metadata, based on our previous cell type annotations. We then plotted the cell type abundance to examine the distribution of cell types within the dataset. To investigate the relationship between cell type abundance and ICB response, we calculated the proportion of each cell type for different ICB response groups and plotted the results. Lastly, we performed Pearson's Chi-squared test to determine whether there was a significant difference in cell type abundance between the ICB response groups.

Despite the limited scope of our differential expression analysis, we observed a significant increase in the expression of the IGKC gene in patients who received immune checkpoint inhibitors and experienced stabilized disease progression. The IGKC gene was used to annotate the plasma cell cluster, representing antibody-producing cells that signify the activation of the immune system in response to cancer cells, thereby stabilizing disease progression. These findings underscore the effectiveness of immune checkpoint inhibitors in patients with advanced renal cell carcinoma. The results from our differential abundance analysis further support this finding, as we observed a significantly higher proportion of B and plasma cells in the favorable response groups to immune checkpoint blockers compared to the no ICB group. Additionally, it was noted that patients who experienced stabilized disease progression had the lowest proportion of tumor cells among all response groups.

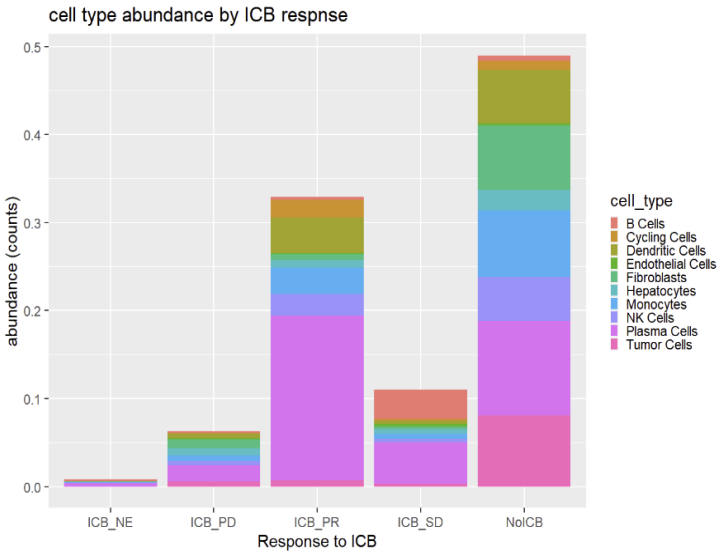

Fig 1. Stacked bar chart showing cell type proportions for each ICB response group

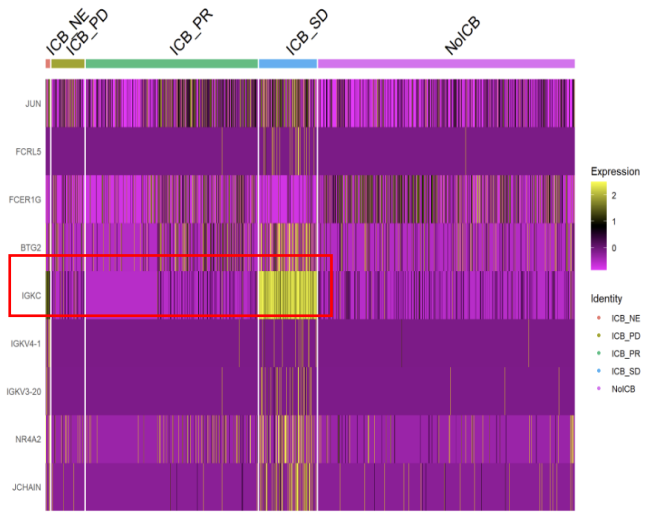

Fig 2. Heat map showing differential expression of Top gene markers for each ICB response group

Cell Type Cluster	Marker Genes
Plasma cells	IGLC2, IGLC3, IGKC
B cells	CD79A, MS4A1
Tumor cells	PDK4, VEGFA, ACSM2A
Fibroblasts	IGFBP5, NDUFA2L2, PPDPFL
NK cells	KLRC1, KLRB1, GNLY
Monocytes	S100A8
Hepatocytes	SAA1, RARRES2
Cycling T cells	STMN1, HIST1H4C, TUBB
Endothelial cells	VWF, MGP, CLDN5

Table 1. Cell type clusters and the top gene markers used for annotation, validated by utilizing Panglao DB and the paper by Bi, et al.

References

Bi, K., He, M. X., Bakouny, Z., et al. (2021). Tumor and immune reprogramming during immunotherapy in advanced renal cell carcinoma. Cancer Cell, 39(5), 649-661.e5. https://doi.org/10.1016/j.ccell.2021.02.015

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