





Single-cell analysis of human glioma and immune cells identifies S100A6 as A Novel immunotherapy target

A Novel Master Regulator of Myeloid-Driven Immunosuppression

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Abstract

This study presents a comprehensive single-cell RNA sequencing analysis of the human glioma microenvironment, identifying *S100A6* as a novel, specific marker of tumor and myeloid cells with significant implications for immunotherapy development. Through advanced bioinformatics methodologies trained under the Department of Genetics at the University of Cambridge, we analyzed data from 18 patient samples using Seurat and Harmony integration, revealing distinct cellular subpopulations and communication networks. Our analysis demonstrates that *S100A6* shows highly specific overexpression in glioma and myeloid cells, in contrast to the broader expression pattern of other S100 family members. Cell-cell communication inference using CellChat identified MIF signaling as the dominant immunosuppressive pathway, suggesting a mechanism by which *S100A6*-high cells may contribute to T cell exhaustion. These findings position *S100A6* as a promising therapeutic target that could simultaneously address both tumor cells and their immunosuppressive niche. The research framework established here provides a robust foundation for future functional validation and therapeutic development aimed at overcoming immunotherapy resistance in glioma.

1 Introduction

Gliomas, particularly glioblastoma (GBM), are devastating brain cancers with dismal prognoses due to an immunosuppressive TME. This study aims to deconvolve this cellular complexity to identify novel, master regulatory nodes that can be therapeutically targeted. Our primary objective was to leverage single-cell genomics to identify a novel, targetable master regulator of the immunosuppressive glioma TME.

2 Materials and Methodology

We used a robust analytical pipeline integrating various bioinformatics tools and custom scripts.

- **Data:** Processed scRNA-seq data from 18 human glioma samples (Neftel et al., 2022).
- **Preprocessing:** Quality control, normalization, and feature selection using Seurat.
- **Integration:** The Harmony algorithm was employed to integrate cells from different patients.
- **Clustering:** PCA, UMAP, and Leiden clustering (resolution = 0.6) identified distinct cell populations.
- **Annotation:** Cell clusters were annotated using canonical marker genes.
- **Differential Expression:** Wilcoxon rank-sum test to identify significantly upregulated genes.
- **Communication Inference:** CellChatDB to infer ligand-receptor interactions.

3 Results

Our analysis identified seven major cell types within the TME. Differential expression analysis identified *S100A6* as significantly upregulated in glioma cells and myeloid cells. CellChat analysis revealed the MIF signaling pathway as a dominant immunosuppressive axis, linking S100A6-expressing cells to T cell exhaustion.

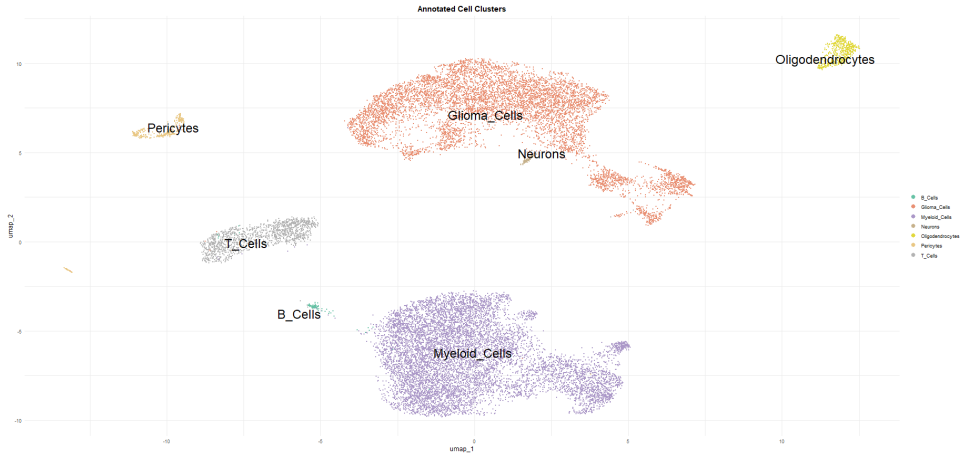


Figure 1: UMAP visualization showing seven distinct cell populations.

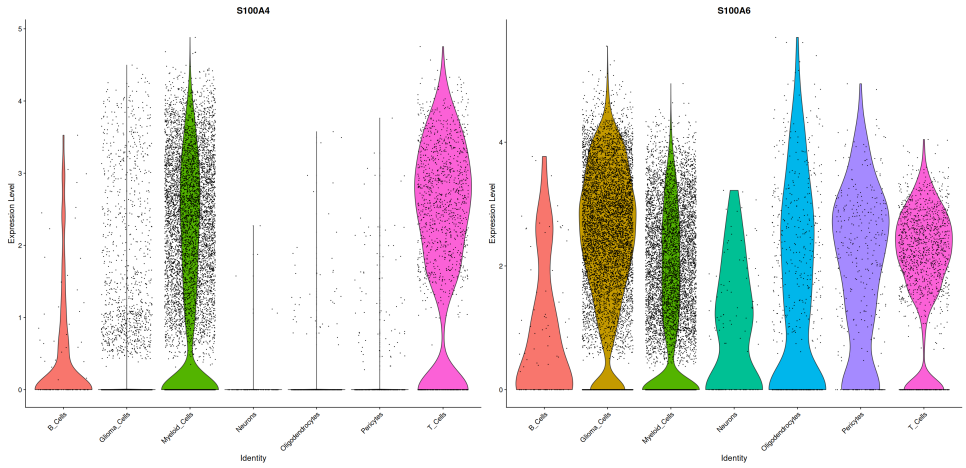


Figure 2: S100A6 demonstrates specific overexpression in glioma and myeloid cells.

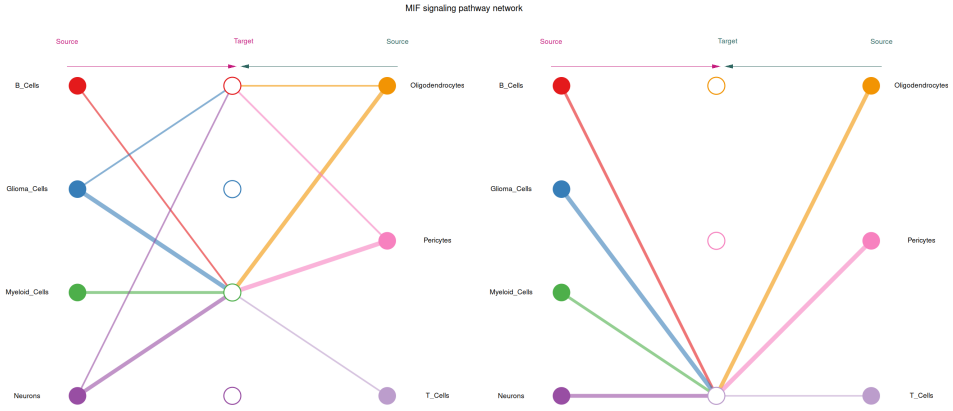


Figure 3: MIF signaling as the dominant immunosuppressive pathway.

4 Skills and Knowledge Gained

- Advanced proficiency in single-cell RNA sequencing analysis using Seurat.
- Expertise in data integration techniques using Harmony.
- Comprehensive understanding of cell-cell communication inference.
- Mastery of differential expression analysis.
- Enhanced R programming and scientific communication skills.

5 Challenges and Solutions

- **Large Dataset Management:** Addressed by implementing data filtering strategies.
- **Batch Effect Resolution:** Successfully implemented the Harmony algorithm.
- **Cell Type Annotation Complexity:** Addressed by using multiple marker genes and literature comparison.
- **Communication Network Interpretation:** Addressed through literature review and expert consultation.

6 Next Steps and Future Work

Building on the findings of this study, several strategic directions are proposed for future research:

6.1 Functional Validation of S100A6

- Conduct *in vitro* knockdown/knockout experiments using siRNA or CRISPR-Cas9 in glioma cell lines to validate S100A6's role in maintaining immunosuppressive phenotypes
- Perform co-culture assays to investigate how S100A6 expression in glioma cells influences T-cell function and myeloid cell polarization
- Develop patient-derived organoid models to test S100A6 inhibition in a more physiologically relevant context

6.2 Advanced Microenvironment Deconvolution

- Apply high-resolution clustering to identify novel cellular subpopulations within major cell types, particularly focusing on:
  - Myeloid cell diversity (e.g., M1 vs M2 macrophages, dendritic cell subsets)
  - T-cell exhaustion states and functional heterogeneity
  - Cancer stem cell populations within glioma cells
- Integrate spatial transcriptomics data to validate cell-cell communication predictions and understand spatial organization of S100A6-high cells
- Incorporate multi-omic data (ATAC-seq, proteomics) to understand regulatory mechanisms controlling S100A6 expression

6.3 Therapeutic Development

- Develop and characterize monoclonal antibodies or small molecule inhibitors targeting S100A6
- Investigate combination therapies pairing S100A6 inhibition with existing immunotherapies (anti-PD-1/PD-L1)
- Establish preclinical models to evaluate efficacy and safety of S100A6-targeted approaches
- Explore biomarker potential of S100A6 expression for patient stratification and treatment response monitoring

6.4 Mechanistic Studies

- Investigate downstream signaling pathways activated by S100A6 using phosphoproteomics and pathway analysis
- Identify binding partners and receptors that mediate S100A6's immunomodulatory effects
- Explore epigenetic regulation of S100A6 expression in glioma and immune cells

7 Conclusion

This research identified *S100A6* as a novel and specific marker of tumor and myeloid cells with potential as an immunotherapy target. We revealed MIF-mediated communication as a key immunosuppressive mechanism. Future work should focus on the functional validation of S100A6 and the development of S100A6-targeting therapeutic strategies.

8 Acknowledgments

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9 References

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Code: https://github.com/AiBJ/Single-cell-analysis-of-human-glioma-and-immune-cells-i
git