Visualizing the p53 Regulatory Networks: A Pathway Enrichment Analysis

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Introduction

- Malaria remains a critical global health threat, with high morbidity and mortality, especially in regions affected by *Plasmodium falciparum* (Pf).
- Pf malaria accounts for the highest severity and mortality rates, particularly in children under five years of age.
- Previous studies have shown that p53 is able to mitigate parasite-induced inflammation and fever during malaria infection[1].
- p53, known primarily as a tumor suppressor, is now recognized for its role in controlling inflammation and promoting immune tolerance.
- This project investigates p53's immunoregulatory role by analyzing single-cell gene expression patterns in the peripheral blood immune cells of malaria-exposed children.
- Using a pseudobulk scRNA-seq approach and enrichment tools (CAMERA and mroast), we assess p53-regulated pathways across immune cell types.
- Our goal is to understand how p53 influences immune response dynamics and to uncover potential host-directed therapeutic strategies to reduce malaria clinical illness.

Practicum Scope

- Curated comprehensive p53-regulated gene signatures by collating p53 target genes from literature (PubMed) and curated databases, including Fischer et al.[2] and additional referenced datasets[3]
- Applied pseudobulk differential expression analysis using edgeR on malaria-exposed peripheral blood mononuclear cells (PBMC) single-cell RNA sequencing (scRNA-seq) data.
- Compared *Plasmodium falciparum* (Pf)-positive and Pf-negative samples to identify infection-responsive transcriptional changes.
- Conducted gene set enrichment analysis using both CAMERA and mroast methods:
- CAMERA accounts for inter-gene correlation and competitive gene set testing.
- mroast provides a self-contained test evaluating whether genes within a set are consistently up- or down-regulated.
- Used two methods to cross-validate findings, as competitive (CAMERA) and self-contained (mroast) tests offer complementary insights into pathway regulation.
- Identified key immune pathways modulated by p53 during malaria infection.
- Revealed novel p53-dependent mechanisms potentially contributing to antimalarial immunity.
- Established transcriptional signatures as potential biomarkers for future host-directed intervention strategies.

Preceptor Details

- Our laboratory employs systems-based approaches to study host responses to Pf infection and malaria vaccines.
- Our research focuses on understanding malaria immunity mechanisms, particularly in pediatric populations from endemic regions.
- The lab is funded by NIAID for multiple malaria research projects.
- Administrative structure includes Principal Investigator (Dr. Tran), Assistant Research Professors (Dr. Prasida Holla) (Dr. Jyoti Bhardwaj) and technical support staff (Erik Gaskin).
- Research capabilities span systems biology, single-cell sequencing, transcriptomics, and antibody response characterization.

Learning Objectives

- Investigate the role of p53 in immune regulation during malaria infection.
- Perform pseudobulk sampling of scRNA-seq data from and conduct differential gene expression analysis between children who are Pf-positive and Pf-negative.
- Apply enrichment analyses (CAMERA and mroast) to interpret immune cell behavior in the context of p53 regulation of inflammation.
- Strengthen bioinformatics skills in R for next-generation sequencing and single-cell genomics data analysis and visualization.
- Explore host-targeted strategies to improve malaria outcomes.

Timeline

- **Week 1–2:** Conducted literature review on p53 in immunity and curated a high-confidence p53 target gene list.
- **Week 3–4:** Pre-processed scRNA-seq data and generated pseudobulk counts by cell type and sample.
- **Week 5–6:** Performed differential gene expression (DGE) and pathway enrichment analysis using edgeR, CAMERA, and mroast.
- **Week 7–8:** Created visualizations, finalized analyses, and prepared poster content.

Practicum Duties

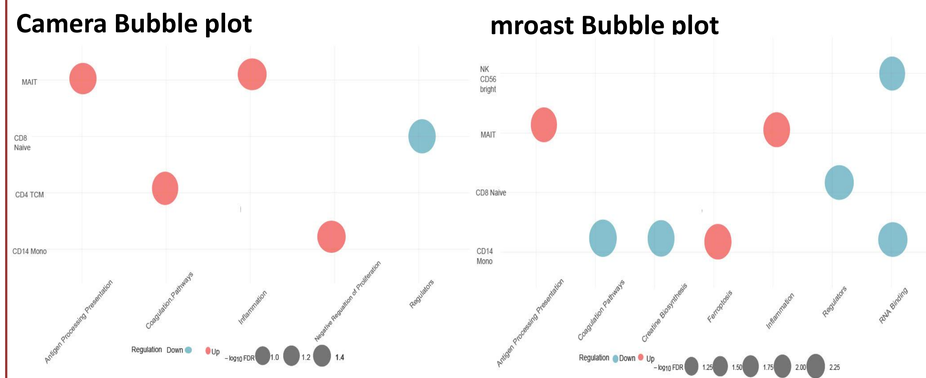
Objectives:
- Identify DEGs between Pf-positive and Pf-negative samples using pseudobulk scRNA-seq.
- Apply a curated p53 gene set for pathway enrichment analysis.
- Investigate how p53 influences immune responses during malaria infection.

Methodology:
- Aggregated single-cell data by cell type and sample for pseudobulk DGE using edgeR.
- Conducted gene set enrichment with CAMERA and mroast based on the curated p53 gene set.
- Visualized enriched pathways across cell types with bubble plots.

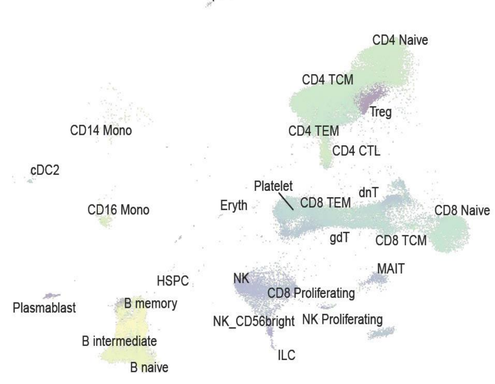
Tools Used:
- R programming language (Seurat, edgeR, limma, SingleCellExperiment, Matrix.utils)
- Visualization: ggplot2, ggpubr, viridis, patchwork
- Google Drive API (data access)
- Custom p53 gene set (.rds) curated from MSEQDB, Fisher's p53 List, and PubMed resources.

Specific Tasks:
- Compiled and validated a functional list of p53-regulated genes from databases and literature.
- Preprocessed, normalized, and pseudobulked scRNA-seq data.
- Ran differential gene expression and pathway enrichment analysis.
- Interpreted p53-driven immune activity through structured visual reports.

Practicum Outcomes – Professional



Red = Higher in Pf-positive (Pf+)
Blue = Higher in Pf-negative (Pf-)
False Discovery Rate (FDR) = 0.2



Results:
Pathway enrichment analysis revealed CD14 Monocytes exhibit elevated levels of ferroptosis and negative regulation of proliferation, supporting prior findings on their immune-regulatory role. In contrast, MAIT cells (Mucosal-Associated Invariant T cells) show a strong signal for p53-mediated inflammation, suggesting a unique contribution to the inflammatory response in malaria-exposed individuals.

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REFERENCES

[1] Tran, T. M., Guha, R., Portugal, S., Skinner, J., Ongoiba, A., Bhardwaj, J., Jones, M., Moebius, J., Venepally, P., Doumbo, S., DeRiso, E. A., Li, S., Vijayan, K., Anzick, S. L., Hart, G. T., O'Connell, E. M., Doumbo, O. K., Kaushansky, A., Fay, M. P., Kappe, S. H. I., … Crompton, P. D. (2019). A molecular signature in blood reveals a role for p53 in regulating malaria-induced inflammation. Immunity, 51(4), 750–765.e10.
[2] Fischer, M. (2017). Census and evaluation of p53 target genes. Oncogene, 36(28), 3943–3956. https://doi.org/10.1038/onc.2016.502(Referenced via ESBL Database: https://esbl.nhlbi.nih.gov/Signaling-Pathways/p53-Targets/)
[3] Beck, J., Turnquist, C., Horikawa, I., & Harris, C. (2020). Targeting cellular senescence in cancer and aging: roles of p53 and its isoforms. Carcinogenesis, 41(8), 1017–1029. https://doi.org/10.1093/carcin/bgaa071