

Analysis of Cellular Heterogeneity, Gene Expression and Activation in the TME of Hodgkin's Lymphoma



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Abstract

Hodgkin's lymphoma is a cancer that most often originates from B cells, one of the two main types of lymphocytes, which produces antibodies that defend against pathogens. In order to understand the pathogenesis of these tumor cells, their genomic data has been widely studied. In this project, different cell types of a Hodgkin's Lymphoma dissociated tumor were identified and clustered using a whole transcriptome RNAseq dataset from 10x genomics. Following this, RNA velocity analysis, a novel approach was implemented in order to understand the transcriptional dynamics of the unique clusters identified. We found heterogeneity in the tumor microenvironment (TME), with several distinct T cell states based on expression analysis, which was then confirmed by our RNA velocity analysis. We also identified a high abundance of the Treg sub-cluster. Our clustering analysis will allow future comparative experiments between dormant, reactive, and malignant Lymph cells. For future analyses, we would recommend looking into drivers of this heterogeneity in Lymph cells.

Introduction

- Hodgkin's Lymphoma (HL) is a cancer with its etiology in lymphocytes
- Death rate: 4.2%-- and it disproportionately affects young adults of European ancestry
- HL has a variable tumor microenvironment (TME) and since it is heterogeneous: it is challenging to identify and subsequently treat it
- New technology and methods may help us examine HL's heterogeneity and the driver genes
- RNA velocity is a promising way to shed some light on this disease
- RNA velocity is the time derivative of the gene expression state

Research Objectives

- Aim 1: Identify and define cell subtypes in the TME of Hodgkin's Lymphoma according to cell transcriptomes
- Aim 2: Determine the transcriptional dynamics in the TME of Hodgkin's Lymphoma
- Aim 3: Identify gene activation and repression in the TME of Hodgkin's Lymphoma

Methods

1) Preparation

In the preparation stage, we processed, normalized, and filtered the scRNA-Seq data. We excluded cells with >10% mitochondrial genes and cells with gene and RNA counts outside of 3SD. We then sorted out the highly variable genes.

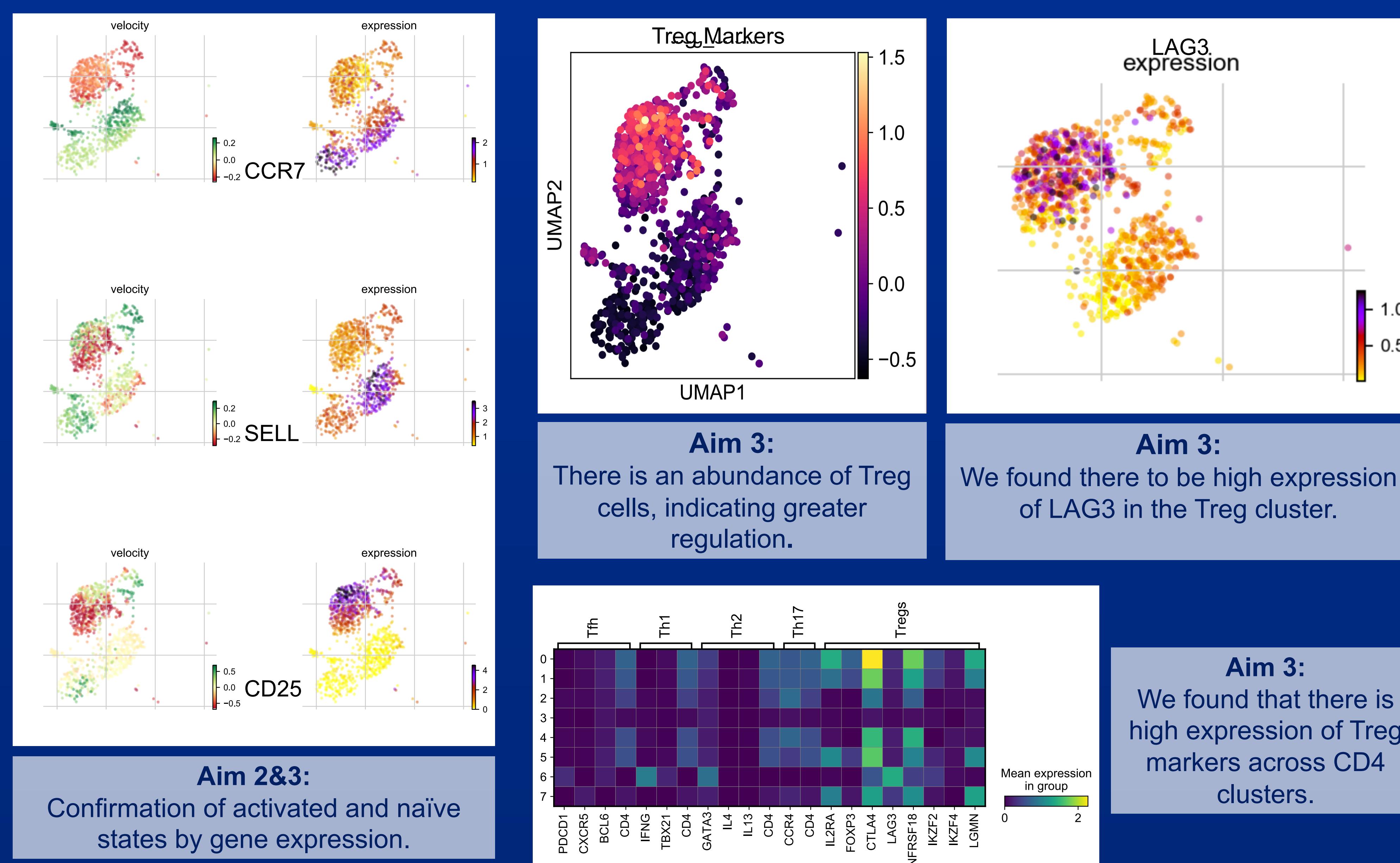
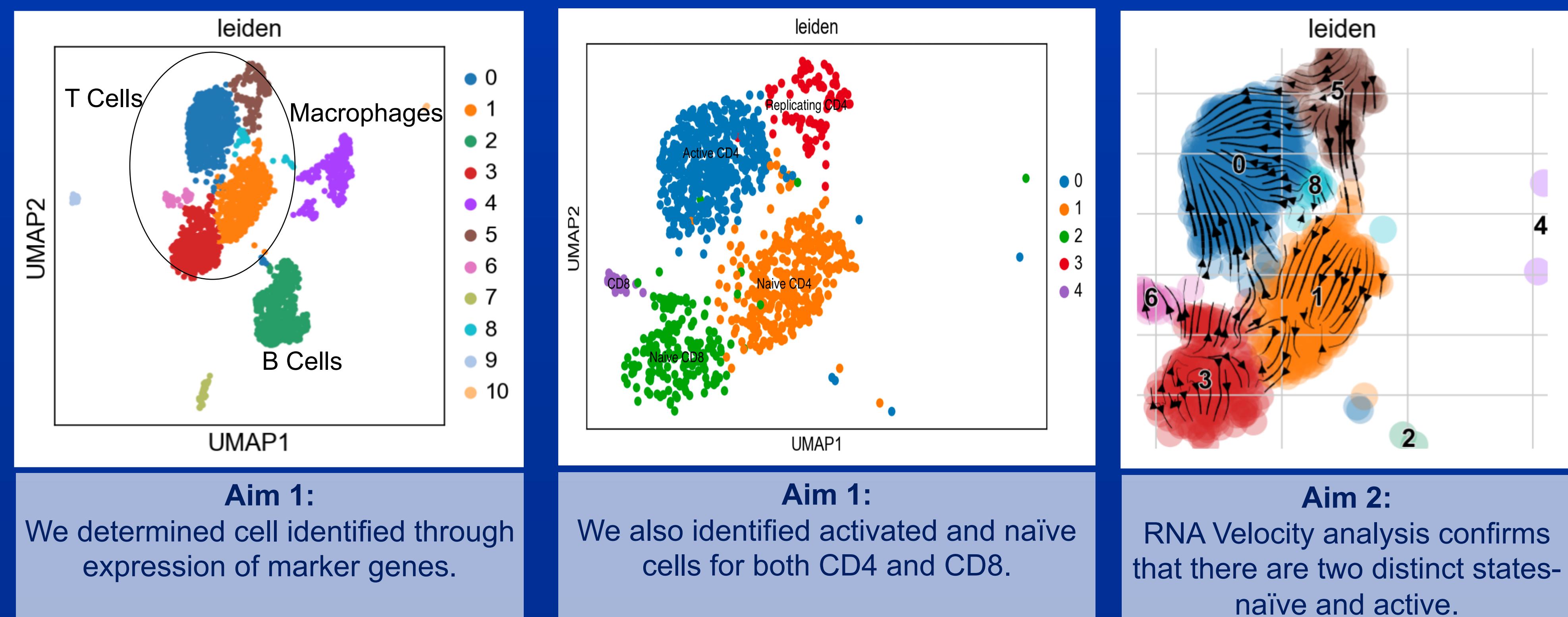
2) Clustering

In the clustering stage, we used Principal Component Analysis (PCA) to reduce the dimensionality of the data. We then generated neighborhood graphs in 2 dimensions using the Leiden algorithm and identified highly differential genes in each cluster using logistic regression.

3) Comparative analysis

In the comparative analysis stage, we compared genes within clusters and across clusters. We generated heat maps to visually demonstrate the expression patterns in each cluster. Here, we also rationalized the marker genes for each cluster with knowledge in immunology and literature research. Then, we generated spliced/unsPLICED matrix to infer RNA velocities.

Results



Conclusions

- 1.) We corroborated past findings related to clustering and heterogeneity of T cells, along with where we saw preferential enrichment of Tregs.
- 2.) Velocity analysis confirmed cell states.
- 3.) We also found unique CD4 and CD8 double positive cells, which are not usually found in lymph nodes, corroborating flow cytometry based experiments on Lymph nodes with Hodgkin's Lymphoma.

Future Directions

- 1.) Comparative experiments between dormant, reactive, and malignant Lymph cells
- 2.) Further identify drivers of this heterogeneity in Lymph cells.

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