A Spatial Analysis of the Hematopoietic Stem Cell Niche in Mice Fetal Liver

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Introduction

Hematopoietic stem cells (HSCs) in the fetal liver are known for their rapid proliferation and high engraftment potential. Despite a general understanding of the organ’s cell type makeup, the fetal liver's spatial architecture is poorly understood. A recent study documented the HSC niche in mice using Multiplexed Error- Robust Fluorescence in Situ Hybridization (MERFISH) for the first time. However, important biological properties of the niche available in this dataset, such as cell type co-localization, cell to cell interaction, and genetic expression remain largely unexplored.

Methods and Data

MERFISH data was processed using the following steps in the Giotto spatial analysis software (developed by the Dries lab):

1. Dimension Reduction (Principle Component Analysis and Uniform Manifold Approximation and Projection) to identify cells with similar gene expression.
2. Clustering via Leiden’s algorithm, an unsupervised machine learning algorithm, to identify cell groups.
3. Differential gene expression analysis using the Gini method to identify the top 5 genes expressed within each cluster.

Through a computational analysis of the existing MERFISH dataset, we aimed to understand several components of the fetal liver niche:

1. What cell types exist in the dataset, and do our results using Giotto agree with previously published results?
2. What cell types localize together within the tissue, i.e., which cell types are communicating? And what biologically relevant patterns exist?
3. What genes show high expression among the fetal liver niche? How are these genes differentially expressed among cell types?
4. Which genes are the most informative and could be used in future data collection?

We identified 9 distinct clusters and their cell types by comparing differentially expressed genes to existing literature. We replotted the original 2D spatial image with our cell type annotations and produced the following results:

For each cell type we found the following genes to be most highly expressed:

- Prox —> Hepatocyte Marker
- Lmo2 —> EC Marker
- Ezfl —> Erythroid Marker
- Myh10 —> Erythroid
- Fgfr1 —> Pre-B cell Marker
- Adger1 —> Macrophage Marker
- Kdr —> SEC Marker
- Col4a1 —> AEC Marker
- Gnas —> Megakaryocyte Marker
- Cd34 —> Myeloid Marker

In order to determine patterns of co-localization, we identified neighbor cells for each cell in our 2D plot (defined as any cell within a 30 micrometers radius). We produced the following heat map, identifying cell types which were frequently identified as each other’s neighbors.

Our results indicated:

1. Self pairs were very common among all identified cell types and frequently took the same type trend to aggregate in space.
2. Erythroid cells are frequently within a 30 micrometer radius of macrophage cells.
3. Other cell types such as Myeloid, AEC, and SECs did not seem to have obvious patterns of frequent neighbors.

We also observed several “Erythroblastic islands” (a single macrophage cell surrounded by erythroid cells). Such as in this image:

Cluster results from the original data release, Lu et al 2021

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Possible reasons for lack of spatial pattern:

1. Errors in data collection of original MERFISH sequencing
2. Technical errors such as small sample size or number of genes
3. Size of slide is unable to detect a pattern
4. There is really no spatial organization of the fetal liver

Future Directions and Acknowledgements

• Analyze associated single-cell RNA sequencing data and compare highly expressed genes
• Analyze the other publicly available dataset (TET2 knockout)
• Compare clusters and expressed genes to the wild type samples
• Designing a gene probe list from genes found in this analysis

This work was funded, in part, by NSF grant DMR-1949968, awarded to the Boston University Bioinformatics BRITÉ REU program.

Thank you to everyone at the Dries Laboratory and my peers in the BRITÉ Program!

Special thanks to Christina Ennis, Dr. Ruben Dries, and Dr. Gary Benson


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