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

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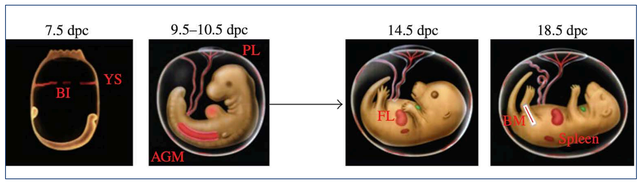
Introduction

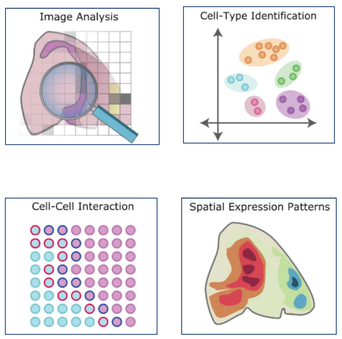
Hematopoietic stem cells (HSCs) in the fetal liver are known for their rapid proliferation and thus high engraftment potential. Despite a general understanding of the organ's cell type makeup, the fetal liver's spatial architecture is poorly understood. A recent study documented the HSC niche in mice 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.



In mouse embryos, HSC development begins in the yolk sac and travels to the fetal liver before reaching the bone marrow. Adapted from Lewis, K., Yoshimoto, M. & Takebe, T. Fetal liver hematopoiesis: from development to delivery. Stem Cell Res Ther 12, 139

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?

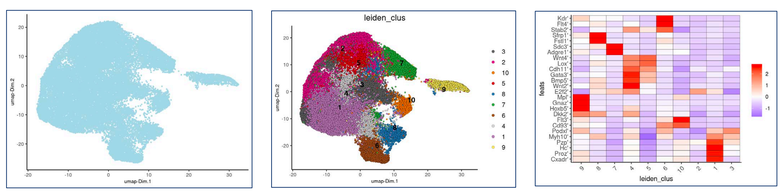


Dries, et al 2021 Giotto Suite

Methods and Data

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

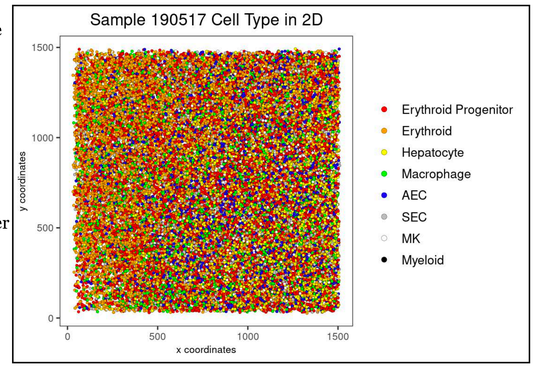
1. Dimension Reduction (Principle Component Analysis and Uniform Manifold Approximation and Projection) to identify cells with similar gene expression.
2. Clustering via Leiden's algorithm, an unsupervised machine learning algorithm, to identify cell groups.
3. Differential gene expression analysis using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



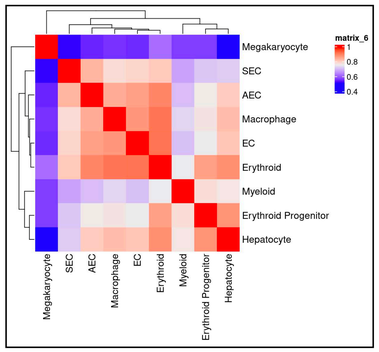
We identified 9 distinct clusters and their cell types by comparing differentially expressed genes to existing literature. We replotted the original 2D spatial image with our cell type annotations and produced the following results:

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

Proz —> Hepatocyte Marker
Lox —> EC Marker
E2f2 —> Erythroid Marker
Myh10 —> Erythroid Progenitor Marker
Adgre1 —> Macrophage Marker
Kdr —> SEC Marker
Col4a1 —> AEC Marker
Gnaz —> Megakaryocyte Marker
Cd34 —> Myeloid Marker



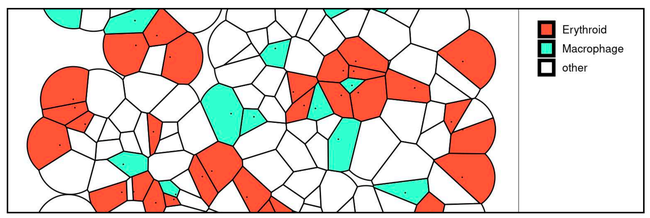
In order to determine patterns of co-localization, we identified neighbor cells for each cell in our 2D plot (defined as any cell within a 30 micrometers radius). We produced the following heat map, identifying cell types which were frequently identified as each other's neighbors.



Our results indicated:

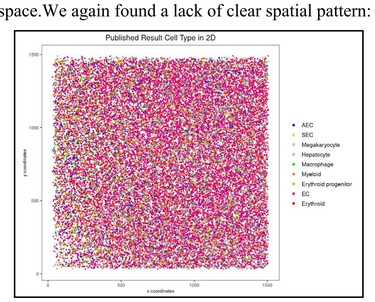
1. Self pairs were very common among all identified cell types (cells of the same type tend to aggregate together in space)
2. Erythroid cells are frequently within a 30 micrometer radius of macrophage cells
3. Other cell types such as Myeloid, AEC, and SEC's did not seem to have obvious patterns of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

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



However, largely speaking our results did not indicate any clear spatial pattern in the fetal liver samples. Given the complex organization required for development, we theorized that this was likely an artifact of our data/analysis and not in fact an indication that the liver has no spatial pattern.

To validate our results, we found the original published clustering results and plotting them in 2D space.We again found a lack of clear spatial pattern:



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

Possible reasons for lack of spatial pattern:

1. Errors in data collection of original MERFISH sequencing
   - When conducting the cell segmentation, fluorescence images of the transcripts could have been assigned to inappropriate cells
2. Technical errors such as small sample size or number of genes
   - Only 140 genes (45 cell markers) were used to differentiate cell types, this may not be enough for this deep of an analysis
3. Size of slide is unable to detect a pattern
   - The 2 mm square image we examined is too small to properly showcase a structural pattern in the tissue of the fetal liver
   - The slicing axis does not allow for proper pattern recognition. Tissue preparation along another axis may have produced spatially coherent patterns
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)
  - Cells in the sample lack TET2 gene (cancer associated)
  - Compare clusters and expressed genes to the wild type samples
  - Does this sample have any coherent spatial pattern?
- Our own analysis of the Human fetal liver using MERSCOPE technology
  - Designing a gene probe list from genes found in this analysis
  - Similar downstream analysis using Giotto

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- Lu, Y., Liu, M., Yang, J. *et al* (2021). Spatial transcriptome profiling by MERFISH reveals fetal liver hematopoietic stem cell niche architecture. *Cell Discov* 7, 47.
- Xia, C., Babcock, H.P., Moffitt, J.R. *et al* (2019). Multiplexed detection of RNA using MERFISH and branched DNA amplification. *Sci Rep* 9, 7721.
- Dries, R., Zhu, Q., Dong, R. *et al* (2021). Giotto: a toolbox for integrative analysis and visualization of spatial expression data. *Genome Biol* 22, 78.
- Vanuytsel, K., Villacorta-Martin, C., Lindstrom-Vautrin, J. *et al* (2022). Multi-modal profiling of human fetal liver hematopoietic stem cells reveals the molecular signature of engraftment. *Nat Commun* 13, 1103.