University of British Columbia - School of Biomedical Engineering

It All Stems from Them: The First Step in the Framework of Understanding Hematopoietic Stem Cells in a Dynamic System

Kyle Palacios, Janet He, Hanson Sun, Qais Alsharif and Tiam Heydari

Introduction

- Stem cells can renew themselves and develop into different cell types. One type of stem cell is the hematopoietic stem cell (HSC), which can differentiate into any type of blood cell in the body such as erythrocytes (red blood cells), leukocytes (white blood cells), and platelets.
- The ability to regulate HSC behavior can be pivotal to sustaining proper homeostasis in an organism over its lifetime.
- This project is the beginning of a large analytical framework, which will ultimately be able to study dynamic systems of hematopoietic stem cells after extraction from laboratory data.

How do hematopoietic stem cells communicate, and what signaling factors cause them to differentiate into specific types of blood cells?

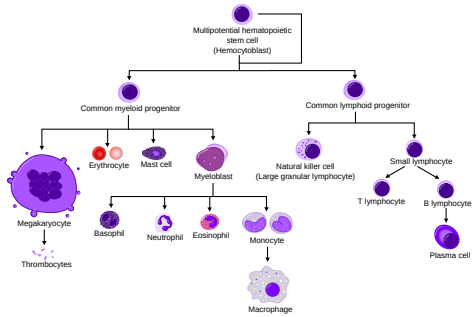

Figure 1

Methodology

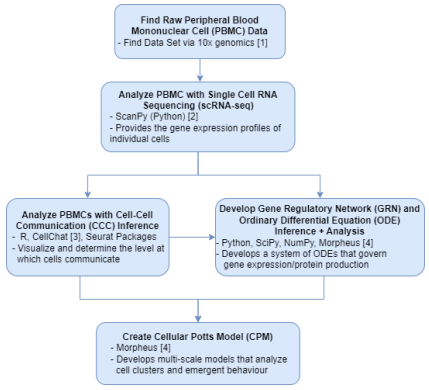

Figure 2

Results

SCRNA SEQUENCING

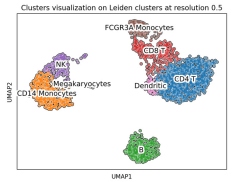

Figure 3

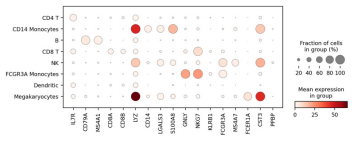

Figure 4

Figure 3 depicts the clustering of the PBMC cells into 8 separate clusters. Meanwhile, figure 4 is a dot plot of the gene expression profile across each cell cluster. Megakaryocytes seem to exhibit high levels of gene expression with LYZ being the strongest. CD14 Monocytes also have a strong expression.

CELL-CELL COMMUNICATION INFERENCE

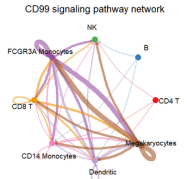

Figure 5

Figure 5 depicts the CD99 signaling pathway was one of the pathways that showed significant communication. Within this pathway, megakaryocytes and FCGR3A Monocytes showed high amounts of interaction.

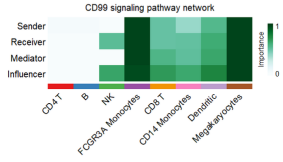

Figure 6

This heatmap in Figure 6 plots the importance of the cell clusters in their role(s) as senders, receivers, mediators, or influencers in the CD99 signaling pathway network. Megakaryocytes and FCGR3A Monocytes both showed high importance in all cell signaling roles.

GRN AND ODE DEVELOPMENT

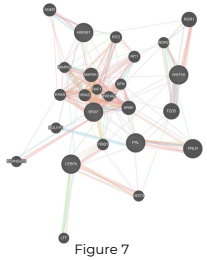

Figure 7

$$\frac{dDiff}{dt} = K\left[\frac{[h]^n}{1+[h]^n} - D_h[h] + \frac{[b]^m}{1+[b]^m} - D_b[b] + \frac{[c]^q}{1+[c]^q} - D_c[c]\right]$$

$$-M\left[\frac{1}{1+[p]^r} - D_p[p] + \frac{1}{1+[w]^s} - D_w[w]\right]$$

$$\frac{dp}{dt} = P_p - D_p \quad \frac{dh}{dt} = P_h - D_h$$

$$\frac{dw}{dt} = P_w - D_w \quad \frac{db}{dt} = P_b - D_b \quad \frac{dc}{dt} = P_c - D_c$$

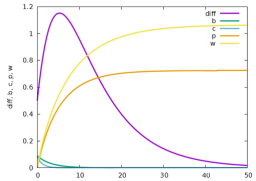

Figure 8

A system of ODEs was designed to model the Myeloid Progenitor Cell Proliferation using information from the scRNA-seq and CCC inference. Several key genes: ***Prl*** **(p)**, ***wnt5a*** **(w)**, ***cebpa*** **(c)**, ***hmgbl*** **(h)**, and ***braf*** **(b)** in the network were selected to create a set of ODEs. From further graphical analysis, we found the differentiation follows a skewed bell curve that peaks and flattens to zero as regulatory genes increase.

CELLULAR POTTS MODEL

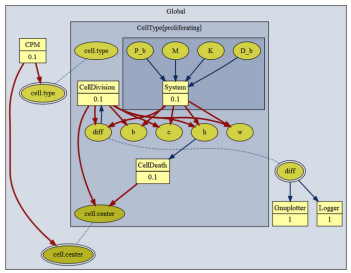

Figure 9

The CPM Model focused on the proliferation cycle of Myeloid Progenitor Cells before differentiation into mature monocytes and macrophages. It uses the ODE and asymmetrical differentiation to model the proliferation patterns in a batch of MPCs.

Myeloid Progenitor Cell Proliferation Cycle

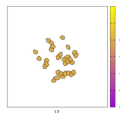

Figure 10a

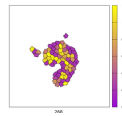

Figure 10b

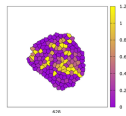

Figure 10c

Further Possibilities

CPM supports more complicated cell-cell interaction pathways:

- Paracrine signaling methods (Notch pathway)
- Diffusive Chemical Signaling (via PDE field)
- Differential adhesion and cell aggregation (CD99)

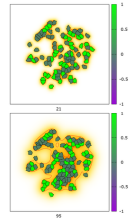

Figure 11

Discussion

ScRNA Sequencing:
Single-Cell RNA Sequencing (scRNA-seq) analyzed the gene expression of the Peripheral Blood Mononuclear Cells (PBMCs). The results from clustering and the generated dot plot from scRNA-seq determined that two of the major genes, among many, that were most expressed were LYZ and CST3. LYZ is a protein-coding gene expressed as an immune response to break bacterial walls. CST3 is a gene that encodes for cystatin C, a protein that regulates chemical reactions by inhibiting specific enzymes.

CCC Inference:
This analysis highlighted the activity of megakaryocytes (hematopoietic cells) in the CD99 signaling pathway. This pathway encodes for the *CD99* gene, which was shown to be a marker for acute myeloid leukemia (AML). [5] Such a result can be crucial to regulating not only megakaryocytes but potentially HSCs as well.

ODE Development:
From the graphs, we can see that the rate of proliferation increases sharply with low levels of p and w and then decreases as p and w increase. From the gene regulatory network, we can also see that this matches the interactions occurring.

Cellular Potts Modelling:
The CPM models analyzed the proliferative behaviour of Myeloid Progenitor Cells. Crucially, it gave a holistic view of the emergent and qualitative behaviour in a cell cluster. From the simulation, weak grouping was observed with a "self-sustaining" effect. Moreover, it also opened new opportunities for further research with other HSCs and interaction pathways.

Future Work

- Analyze a dataset containing HSCs and searching for specific trends of interest regarding these cells.
- The results of CCC inference will be able to lend direct information that will form the GRN and ODEs, which will then create a more comprehensive Cellular Potts model.
- Determine how we could manipulate the proliferation and differentiation behaviours of HSCs if they were to be implanted into different parts of the human body.
- The research pipeline that was developed in the project opens up many possibilities for the research of other cell types. A similar process can be extended to other stem cells to examine their differentiating and proliferating behaviours.

References

[1] "Datasets -Single Cell Gene Expression -Official 10x Genomics Support," support.10xgenomics.com. https://support.10xgenomics.com/single-cell-gene-expression/datasets/1.1.0/pbmc3k? (accessed Mar. 12, 2023).

[2] F. A. Wolf, P. Angerer, and F. J. Theis, "SCANPY: large-scale single-cell gene expression data analysis," Genome Biology, vol. 19, no. 1, Feb. 2018, doi: https://doi.org/10.1186/s13059-017-1382-0.

[3] S. Jin et al., "Inference and analysis of cell-cell communication using CellChat," Nature Communications, vol. 12, no. 1, Feb. 2021, doi: https://doi.org/10.1038/s41467-021-21246-9.

[4] J. Starruß, W. de Back, L. Brusch, and A. Deutsch, "Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology," Bioinformatics, vol. 30, no. 9, pp. 1331–1332, Jan. 2014, doi: https://doi.org/10.1093/bioinformatics/btt772.

[5] S. S. Chung et al., "CD99 is a therapeutic target on disease stem cells in myeloid malignancies," Science Translational Medicine, vol. 9, no. 374, p. eaaj2025, Jan. 2017, doi: https://doi.org/10.1126/scitranslmed.aaj2025.

Acknowledgements

We acknowledge that our research presentation at the MURC sits on the traditional, ancestral, and unceded territory of the xʷməθkʷəy̓əm (Musqueam) First Nation. We would like to thank our mentor, Tiam Heydari, for guiding us through this research project. We also thank the URO for developing the REX program to connect us with our mentor and with MURC to present our research.