Exploring the secretome of hematopoietic stem cells

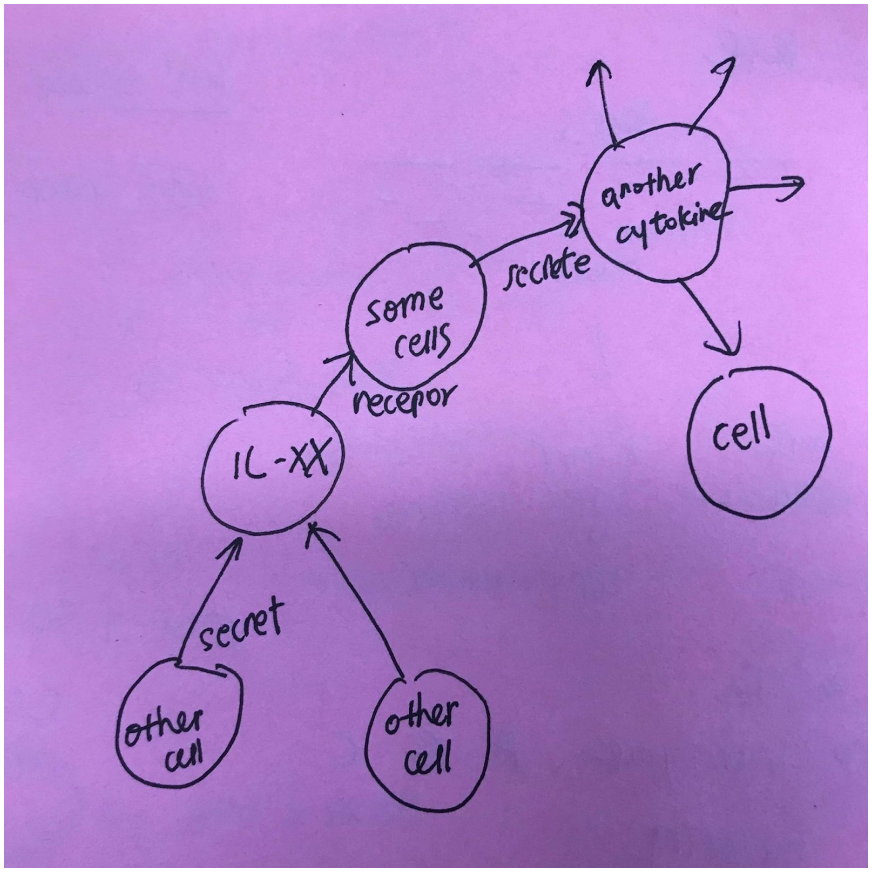
Background

Hematopoietic cell differentiation is tightly controlled through the stimulation of chemokines and cytokines secreted by the bone marrow microenvironment. Hematopoietic cancer cells also rely on signaling to progress. Proteomic quantification of the bone marrow secretome suffers from large number of contaminants from dead cells. To thoroughly understand crosstalk between all cellular components at we sought to study the secretome of the bone marrow microenvironment at a single cell level.

Objectives

The aim of this proposal is to build up a pipeline that can map single cell data onto secretory pathways and predict the secretome of each cell. Single cell datasets available are showed in the Table on the last page. To thoroughly understand the hematopoiesis microenvironment, we might need to join data that includes not only CD34+ or LIN- cells, but also those from the bone marrow stroma. We will start from raw count matrix, imputing dropouts, and joining different scRNA-seq datasets. To validate the imputation result, unique cellular fingerprints will be validated by comparing to (Chambers et al., 2007). Then, we will join the annotation from the human protein atlas to find out the secreted proteins including surface markers and cytokines. By constructing a network consisting of cells and secreted proteins, we can globally see the regulations that drive cell differentiation.

This is the network I am thinking of ↓ ↓



Challenges I will face will depend upon my understanding in processing single cell data. These models and data analytic strategies depend heavily on mathematical concepts my background lack. I expect to strengthen my weakness in quantitative science.

Expected results

This analysis is expected to secretome of cellular components in the bone marrow and identify novel cellular crosstalk between them. The regulation between them will serve as a foundation to engineer regenerative stem cell as well as understand the bone marrow microenvironment in hematopoietic diseases.

Steps

1. Align to the mouse genome using mm10
2. UMI collapse using the cellRanger by 10X genomics
3. UMI < 200 / cell might need to be removed; < 500
4. Gene expression calculation
    a. CPTT(counts per million)
    b. Fraction of UMI/all UMI per cell * 10000
5. Batch effect
6. Cell population clustering
    a. Capable of identify transitional cell https://www.pnas.org/content/116/2/466
    b. kNN Clustering of the pCA reduced data tSNE
7. Trajectory analysis
    a. SPRING
8. Inferring differentiation relationshp
    a. correlations of average expression profiles between clusters(Figure 1F),
    b. graph abstraction (Wolf et al., 2017) (Figure 1G),
    c. and diffusion map analysis
9. Cell population annotation
    a. If dataset if FACs sorted then we have the surface marker
    b. http://software.broadinstitute.org/gsea/msigdb/index.jsp Broad Institute molecular signaure DB, are based on cell cultures and according to (Xue et al., 2019) it is contaminated by stromal cell
    c. (Xue et al., 2019) uses bulk RNA-seq and microarray data on GEO Marker Finder GO Elite
    d. ToppFun
    e. Literature review
    f. Seuraut findVariableGenes

Review of single cell data available

Paper	Species	disease	Tissue	Cell selection	Finding	preprocessing
(Pellin et al., 2019)	1 human	healthy	Bone marrow	CD34+ Then label by FAC(fig 1b markers)	Baso, eos, mono are missing	
	1 human	healthy	Bone marrow	LIN-	Find baso, eos, mono	
(Zhao et al., 2017)	9 human	4 healthy, 5 bone marrow failure(AA, FMS..) with chr abnormality	Bone marrow	CD34+		GSE99095
(Xue et al., 2019)	Zebra fish		Caudual haematopoietic tissue		Neibouring supporting cells share signaling pathways (co-regulated)	GSE120581
(Nestorowa et al., 2016)	mouse				Spike in control for absolute RNA level	GSE81682
(Tikhonova et al., 2019)	mouse		Bone marrow	of VE-Cad+, LEPR+ and COL2.3+ (bone marrow niche)		GSE108892
(Wolock et al., 2019)	mouse		Long bone	by sorting viable CD45_Ter119_ (non-hematopoietic) and CD31_ (non-endothelial) cells.(stroma)		GSE132151
(Zheng, Papalexi, Butler, Stephenson, & Satija, 2018)	human		Cord blood			GSE75478, GSE74912(ATAC-seq), GSE97104
(Freeman, Jung, & Ogle, 2015)	Mouse bone marrow stem cell culture					GSE70930
(Kowalczyk et al., 2015)	Mouse with age difference				Cell cycle has shorter G1 in aged cells	GSE59114.
	Mouse embryonal cells				Key factors contributing to endothelium or hematopoietic differentiation	GSE96986.
(Buenrostro et al., 2018)	human		Bone marrow	Seemed all CD34+	**Integrate chromatin accessibility with scRNA-seq**	GSE96772
(Baryawno et al., 2019)	mouse		Bone marrow	Not CD34+ (bone marrow stroma)		GSE128423
	mouse					https://www.ncbi.nlm.nih.gov/pubmed/31149730

(Oetjen et al., 2018)	20 healthy human with wide age range		Bone marrow	Not mentioned		GSE120221, GSE120446
(Hay, Ferchen, Chetal, Grimes, & Salomonis, 2018)	8 healthy human from Human cell atlas					Human cell atlas

Baryawno, N., Przybylski, D., Kowalczyk, M. S., Kfoury, Y., Severe, N., Gustafsson, K., … Scadden, D. T. (2019). A Cellular Taxonomy of the Bone Marrow Stroma in Homeostasis and Leukemia. *Cell*, *177*(7), 1915-1932.e16. https://doi.org/10.1016/j.cell.2019.04.040

Buenrostro, J. D., Corces, M. R., Lareau, C. A., Wu, B., Schep, A. N., Aryee, M. J., … Greenleaf, W. J. (2018). Integrated Single-Cell Analysis Maps the Continuous Regulatory Landscape of Human Hematopoietic Differentiation. *Cell*, *173*(6), 1535-1548.e16. https://doi.org/10.1016/j.cell.2018.03.074

Chambers, S. M., Boles, N. C., Lin, K.-Y. K., Tierney, M. P., Bowman, T. V., Bradfute, S. B., … Goodell, M. A. (2007). Hematopoietic Fingerprints: An Expression Database of Stem Cells and Their Progeny. *Cell Stem Cell*, *1*(5), 578–591. https://doi.org/10.1016/j.stem.2007.10.003

Freeman, B. T., Jung, J. P., & Ogle, B. M. (2015). Single-Cell RNA-Seq of Bone Marrow-Derived Mesenchymal Stem Cells Reveals Unique Profiles of Lineage Priming. *PLOS ONE*, *10*(9), e0136199. https://doi.org/10.1371/journal.pone.0136199

Hay, S. B., Ferchen, K., Chetal, K., Grimes, H. L., & Salomonis, N. (2018). The Human Cell Atlas bone marrow single-cell interactive web portal. *Experimental Hematology*, *68*, 51–61. https://doi.org/10.1016/j.exphem.2018.09.004

Kowalczyk, M. S., Tirosh, I., Heckl, D., Rao, T. N., Dixit, A., Haas, B. J., … Regev, A. (2015). Single-cell RNA-seq reveals changes in cell cycle and differentiation programs upon aging of hematopoietic stem cells. *Genome Research*, *25*(12), 1860–1872. https://doi.org/10.1101/gr.192237.115

Nestorowa, S., Hamey, F. K., Pijuan Sala, B., Diamanti, E., Shepherd, M., Laurenti, E., … Gottgens, B. (2016). A single-cell resolution map of mouse hematopoietic stem and progenitor cell differentiation. *Blood*, *128*(8), e20–e31. https://doi.org/10.1182/blood-2016-05-716480

Oetjen, K. A., Lindblad, K. E., Goswami, M., Gui, G., Dagur, P. K., Lai, C., … Hourigan, C. S. (2018). *Human Bone Marrow Assessment by Single Cell RNA Sequencing, Mass Cytometry and Flow Cytometry* [Preprint]. https://doi.org/10.1101/416750

Pellin, D., Loperfido, M., Baricordi, C., Wolock, S. L., Montepeloso, A., Weinberg, O. K., … Biasco, L. (2019). A comprehensive single cell transcriptional landscape of human hematopoietic progenitors. *Nature*

Communications, *10*(1), 2395. https://doi.org/10.1038/s41467-019-10291-0

Tikhonova, A. N., Dolgalev, I., Hu, H., Sivaraj, K. K., Hoxha, E., Cuesta-Domínguez, Á., ... Aifantis, I. (2019). The bone marrow microenvironment at single-cell resolution. *Nature*, *569*(7755), 222–228. https://doi.org/10.1038/s41586-019-1104-8

Wolock, S. L., Krishnan, I., Tenen, D. E., Matkins, V., Camacho, V., Patel, S., ... Welner, R. S. (2019). Mapping Distinct Bone Marrow Niche Populations and Their Differentiation Paths. *Cell Reports*, *28*(2), 302-311.e5. https://doi.org/10.1016/j.celrep.2019.06.031

Xue, Y., Liu, D., Cui, G., Ding, Y., Ai, D., Gao, S., ... Liu, F. (2019). A 3D Atlas of Hematopoietic Stem and Progenitor Cell Expansion by Multi-dimensional RNA-Seq Analysis. *Cell Reports*, *27*(5), 1567-1578.e5. https://doi.org/10.1016/j.celrep.2019.04.030

Zhao, X., Gao, S., Wu, Z., Kajigaya, S., Feng, X., Liu, Q., ... Young, N. S. (2017). Single-cell RNA-seq reveals a distinct transcriptome signature of aneuploid hematopoietic cells. *Blood*, *130*(25), 2762–2773. https://doi.org/10.1182/blood-2017-08-803353

Zheng, S., Papalexi, E., Butler, A., Stephenson, W., & Satija, R. (2018). Molecular transitions in early progenitors during human cord blood hematopoiesis. *Molecular Systems Biology*, *14*(3). https://doi.org/10.15252/msb.20178041