Leveraging Mitochondrial DNA Mutations for Macrophage Lineage Tracing in Primary Human Tissues

BACKGROUND

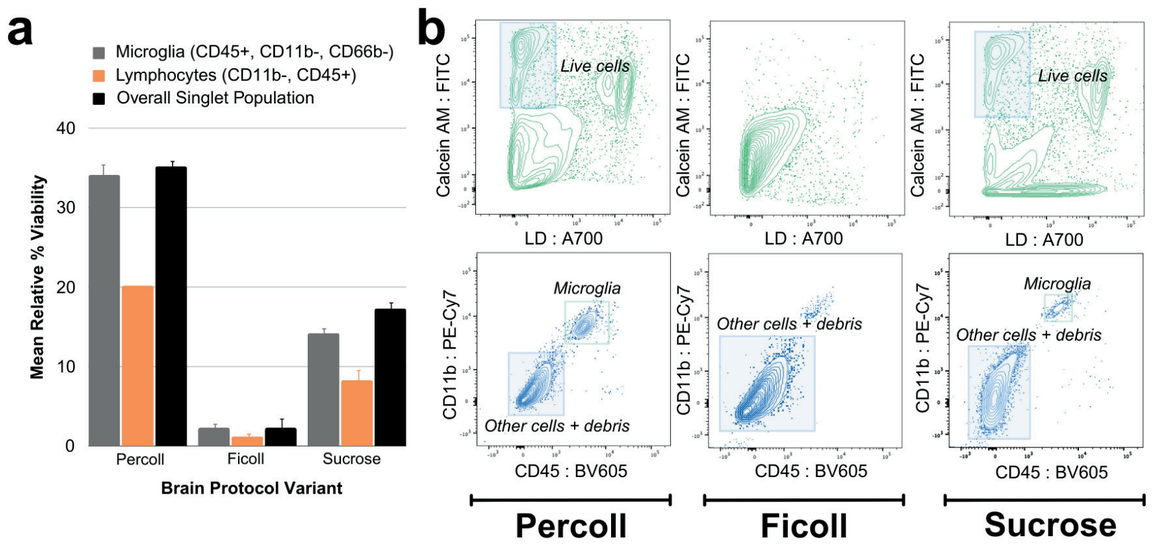
Characterization of macrophage development is essential to understand their role in disease settings. Macrophages, which play a role as the primary defense against pathogens and tumor cells, have specific functions based on the tissues they reside in. These are known as tissue-resident macrophages **(TRMs),** examples being **microglia** in the brain and **Kupffer cells** in the liver. Our current understanding suggests that multiple TRM populations originate during the **early stages of embryonic hematopoiesis** and **self-replicate independently** to maintain their populations A new study presents a possibility that certain TRM populations may undergo replenishment from hematopoietic stem cells (HSCs), especially in elderly populations. This study uses a genomics technique called the **mitochondrial single-cell Assay for Transposase Accessible Chromatin sequencing (mtscATAC-seq)**, a high-throughput method combining mitochondrial DNA analysis and chromatin accessibility profiling, to **trace and identify macrophages derived from bone marrow stem cells** in primary human tissues. **Project Hypothesis**: *As humans age, TRM populations are replenished from HSCs in their bone marrow.*

METHODS and WORKFLOW

Primary tissue samples were received from Donor Network West. Tissues were dissociated and filtered for optimal cell viability. Sample solutions were sorted through a process known as flow cytometry, which uses lasers to identify certain cell markers and sorts cells by droplet. For this study, I sorted for live cells, excluding neutrophils and red blood cells. The sorted samples underwent the mtscATAC-seq protocol which allowed for extraction and purification of mitochondrial DNA (mtDNA). DNA samples were sequenced and cell clusters were plotted to visually identify shared mutations across clusters.

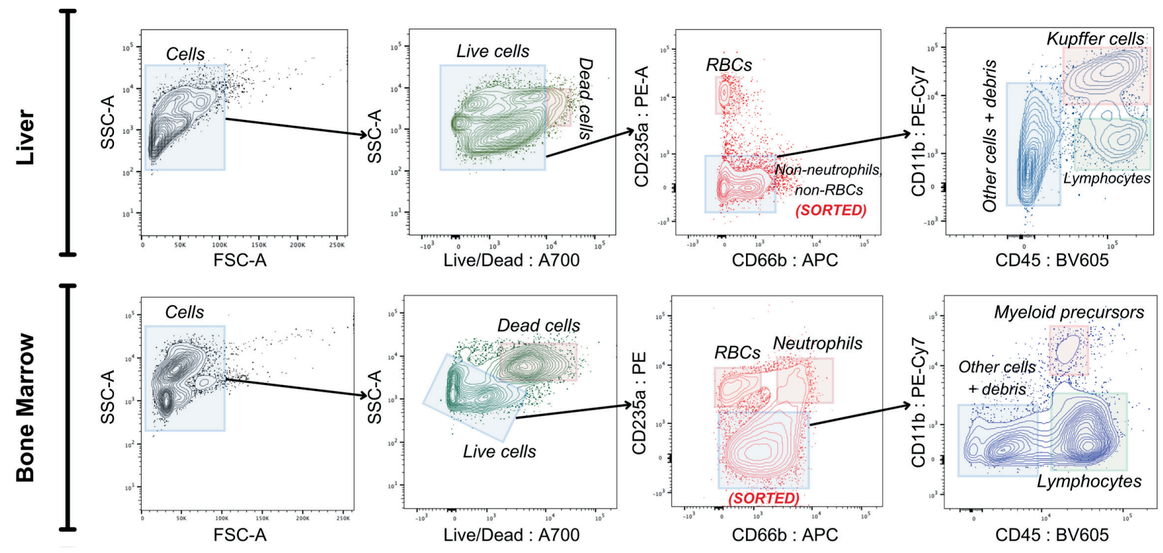
RESULTS

Figure 1: Mean Viabilities by Brain Cell Type From Different Density Centrifugation Gradients



- After brain tissue was dissociated, the myelin fraction of the sample needed to be removed in order to prepare the sample for flow cytometry
- Conducted **three identical** brain digest preparations comparing Percoll, Ficoll, and Sucrose
- Percoll density gradient was most effective in removing myelin debris based on mean quantitative cell viabilities (**Figure 1a**) and visual population separation (**Figure 1b**)

Figure 2: Representative Flow Cytometry Data From Human Liver And Bone Marrow



The donor presented in this study is from Donor Network West, **referred to as 'DN3.'** Donor DN3's liver and liquid bone marrow were dissociated and sorted using flow cytometry, confirming the presence of the following (**Figure 2**):

- **DN3 Liver**: 364,970 total viable cells
  - 159,203 Kupffer cells and 25,573 lymphocytes
- **DN3 Bone Marrow**: 327,788 total viable cells
  - 134,932 lymphocyte progenitors and 70,293 myeloid precursor cells

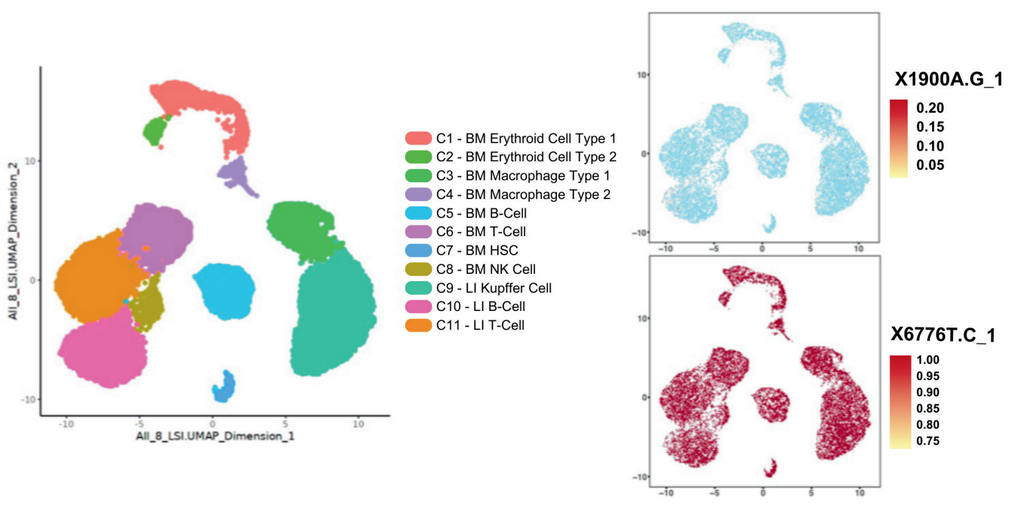


Figure 3: UMAP Clustering of DN3's Liver Samples Over Matching Bone Marrow Samples From mtscATAC Libraries

- DN3's liver and bone marrow (BM) samples showed **uniform expression of mitochondrial DNA mutations across cell clusters**
  - The top right panel of **Figure 3** displays all cell clusters lacking in expression of a particular mtDNA mutation, while the bottom right panel shows all cell clusters expressing another mtDNA mutation
  - This pan-expression indicates a germline mutation, suggesting ALL cell populations, including Kupffer cells, arose from embryonic hematopoiesis

CONCLUSIONS

This project presents a **new narrative regarding the origins and replenishment dynamics of TRMs** in adults.

- Liver and corresponding bone marrow findings illustrate uniform expression
  - Evidence suggests that the Kupffer cells in this subject were predominantly derived from embryonic hematopoiesis, aligning with traditional perspectives on Kupffer cell replenishment
  - However, **more liver samples** from patients of different **ages, sexes, and other variables** will be needed to confirm this analysis
- Using the effective methods from this study, macrophage lineage data from other tissues such as brain, lung, and spleen tissues have potential to shed light on how TRM replenishment plays a role in tissue-degenerative and age-related disease

FUTURE DIRECTIONS

C — **Compare** the macrophage lineage in **healthy subjects versus those with specific health conditions.**

U — **Understand** the relationship between **CHIP and the development of Alzheimer's Disease.**

R — **Research** the potential for adult bone marrow-derived replenishment of TRMs in more primary tissues, **such as the lung and spleen.**

E — **Expand** this study to include a broader, more diverse cohort of subjects, looking at **ages, sexes, and health conditions**.

S — **Streamline** the overall tissue dissociation pipeline to allow for **higher quality genomics data and comparable results**.