Single-cell sequencing of the developing lens

Abstract

The lens focuses light on the retina for optimal vision, and loss in its transparency is termed cataract. It has distinct cell populations, broadly classified as anterior epithelium (AE) and posteriorly located differentiated fiber cells (FCs). Further cell subpopulations are recognized in AE and FCs based on proliferative or differentiation status, respectively. Pathological changes in individual cells are hypothesized to cause human lamellar cataract. Thus, to gain insights into lens-cell-specific transcript heterogeneity, I developed a workflow to obtain viable isolated mouse embryonic and newborn lens cell suspensions for single-cell RNA-sequencing (scRNA-seq). 10x Genomics tools were used to assign unique molecular identifiers to expressed transcripts and identify lens marker genes. These data show that scRNA-seq identifies distinct new cell populations in the lens. Further, its application to cataract animal models can identify disease-specific changes in individual cell types.

Approaches to study eye-lens

Mouse lens epithelial and fiber cell organization

Big Data approaches in lens

Mouse lens transcriptome at various stages

RNA-seq data quality assessment

Self-organizing tree algorithm (SOTA)-based clustering reveals dynamics of lens-enriched genes

Conclusions and Future directions

1. The present study represents the first optimal method to dissociate lens tissue to make a viable single-cell suspension of embryonic and post-natal lens tissue for 10X chromium gene-expression single-cell sequencing.

2. Altogether, our new data show the feasibility of using single-cell sequencing to study transcriptomic differences between lens cell types and the heterogeneity in gene expression within one cell population.

3. Based on expression of marker genes, we were able to identify 5 (for E16.5) and 8 (P0) clusters of lens cells.

Key insights from single-cell sequencing of mouse lens

10X genomics Cell Ranger tool summary

- Cell Ranger output files were processed with Seurat R-package workflow
- Low-quality empty droplets were removed and data-normalization was performed
- Detection of variable features for downstream analysis, like PCA were identified

Future directions

- A graph based clustering K-nearest neighbor (KNN) method was implemented to identify similar expression of highly interconnected cells
- Single-cell sequencing of mouse embryonic and post-natal lens revealed multiple cell type clusters

Sample preparation for single-cell sequencing

1. Tissue collection
   - Microscopic dissection of E16.5 and P0 mouse lens
   - Lenses were collected in cold 1X PBS and kept on ice followed by step 2

2. Tissue dissociation
   - Dissection of lens using an enzyme mix solution at 37°C to get single cell suspension

3. Single cell viability and counting
   - Cells were counted using trypan blue dye exclusion test

4. Freezing and shipping samples
   - Cell suspensions were transfer to a freezing containers in -80°C
   - Samples were shipped to sequencing facility on dry ice

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