Single-cell sequencing of the developing lens

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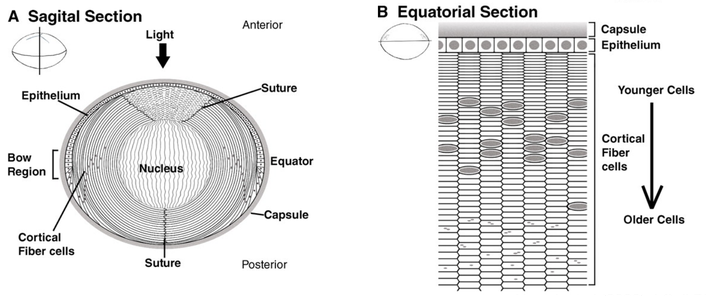
Abstract

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 sRNA-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.

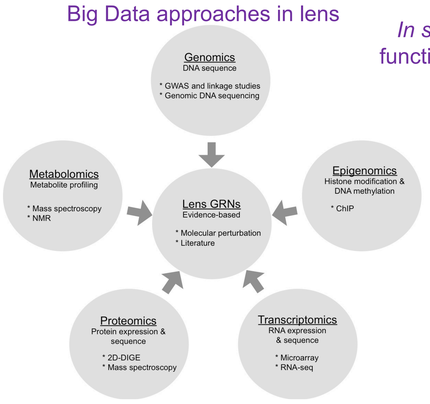
Approaches to study eye-lens

Mouse lens epithelial and fiber cell organization



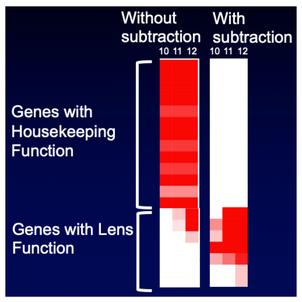
Nowak et al (2009) J Cell Biol

Big Data approaches in lens



Anand, Lachke (2017) Exp Eye Res

In silico subtraction identifies genes that function in lens development and pathology

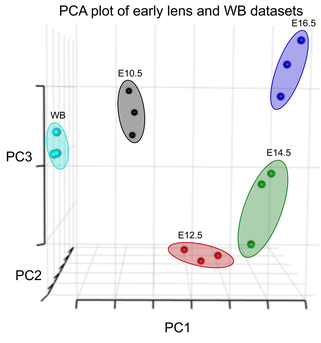


Lachke et al (2012) Invest Ophthalmol Vis Sci

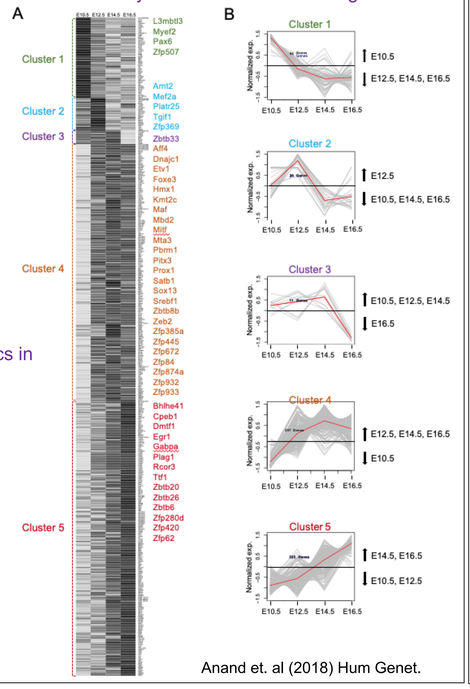
Mouse lens transcriptome at various stages

RNA-seq data quality assessment

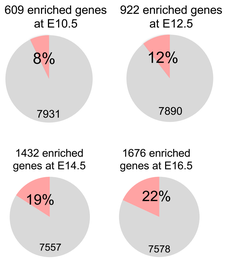
PCA plot of early lens and WB datasets



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



Lens-enriched gene expression dynamics in mouse embryonic development



counts >5 at significant Fold change >1.5 and p-value <0.05

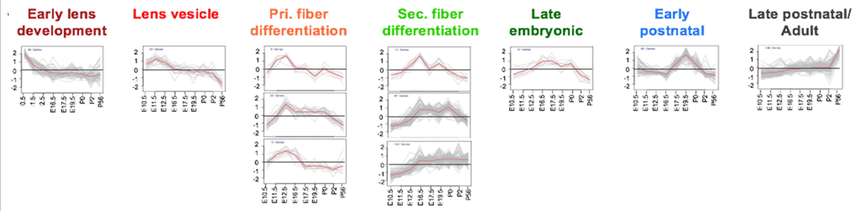
Anand et. al (2018) Hum Genet.

iSyTE 2.0 provides insights into lens expression dynamics

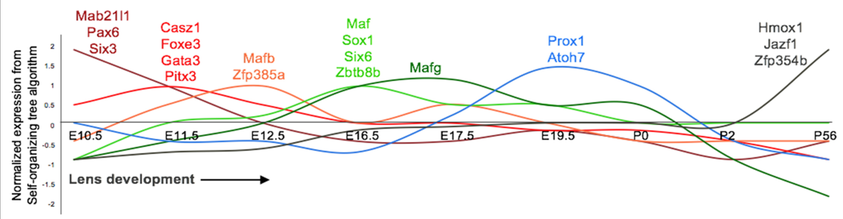
iSyTE (integrated Systems Tool for Eye gene discovery) based enrichment analysis of lens development and differentiation genes

Kakrana, Anand, Lachke et al (2018) Nucleic Acids Res.

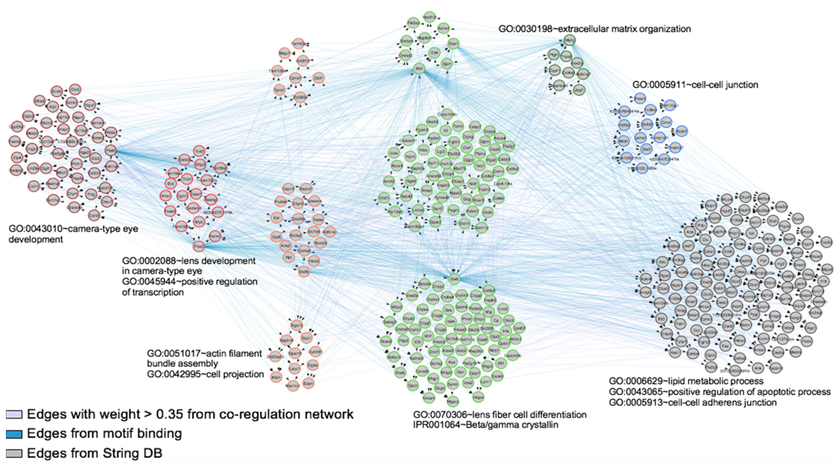
Expression based clustering (SOTA clustering) of the top 200 lens-enriched genes from lens development stages



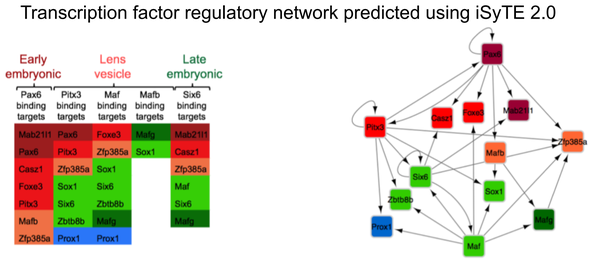
Combined expression dynamics of TF-genes across all eleven lens development stage clusters



A combined network of eleven cluster genes derived from WGCNA correlation network (purple edges), transcription factor binding motif analysis (blue edges) and String DB (gray edges) across the different developmental stages



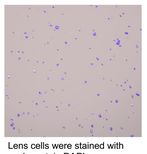
Transcription factor regulatory network predicted using iSyTE 2.0



Single-cell gene expression of mouse lens

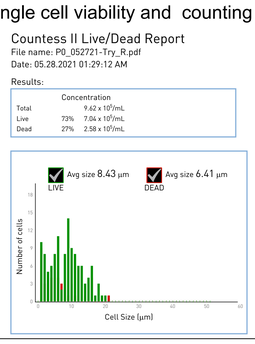
Sample preparation for single-cell sequencing

1. Tissue collection
   - Microscopic dissection were performed to isolate E16.5 and P0 mouse lens
   - Lenses were collected in cold 1XPBS and kept on ice followed by step 2
2. Tissue dissociation
   - Dissociation of lens using a enzyme-mix solution at 37C to get single cell suspension



Lens cells were stained with nuclear stain DAPI

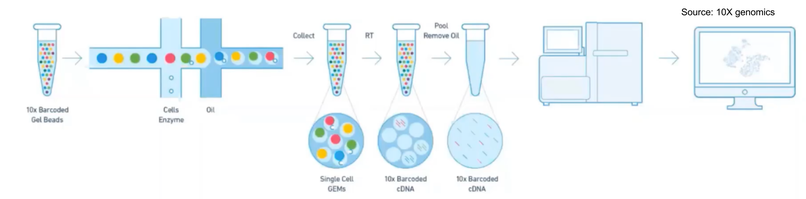
3. Single cell viability and counting



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

Key insights from single-cell sequencing of mouse lens

10X chromium single-cell gene-expression workflow

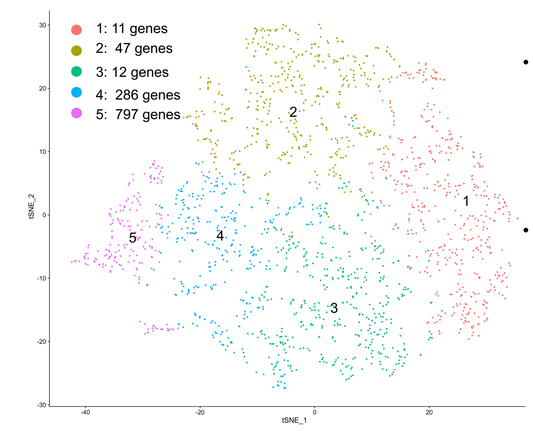


10X genomics Cell Ranger tool summary

Data summary	Embryonic 16.5	Post-natal P0
Estimated no. of cells	21,031	14,108
Mean reads per cell	26,548	24,587
Median genes per cell	295	276
Total genes detected	17,689	17,986
Median UMI counts per cell	1,610	1,648
Reads mapped to genome	92.6%	87.4%

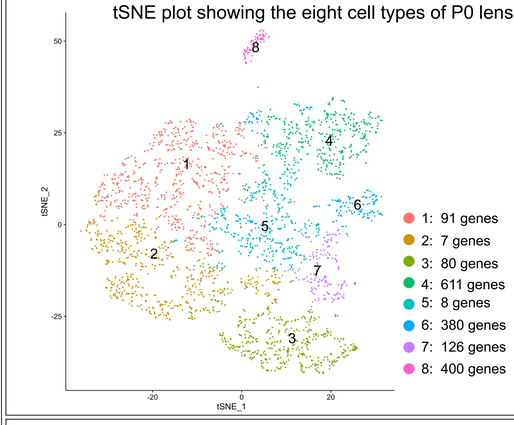
- Cell Ranger output files were processed with Seurat R-package workflow
- Low-quality empty droplets were removed and data-normalization was performed
- Detection of variable feature for downstream analysis, like PCA were identified

tSNE plot showing the eight cell types of E16.5 lens



- 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

tSNE plot showing the eight cell types of P0 lens



- The cell types identified in E16.5 and P0 samples were based on expression of known lens epithelium and fiber-cell marker genes
- Several new genes in individual cell-types were also identified

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

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