Multi-species single-cell transcriptomic analysis of ocular compartment regulons

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Abstract

The retina is a widely profiled tissue in multiple species by single-cell RNA sequencing studies. However, integrative research of the retina across species is lacking. Here, we construct the first single-cell atlas of the human and porcine ocular compartments and study inter-species differences in the retina. In addition to that, we identify putative adult stem cells present in the iris tissue. We also create a disease map of genes involved in eye disorders across compartments of the eye. Furthermore, we probe the regulons of different cell populations, which include transcription factors and receptor-ligand interactions and reveal unique directional signalling between ocular cell types. In addition, we check for conservation of regulons across vertebrates and zebrafish to identify common core factors. Here, we show perturbation of KLF7 gene expression during retinal ganglion cells differentiation and conclude that it plays a significant role in the maturation of retinal ganglion cells.

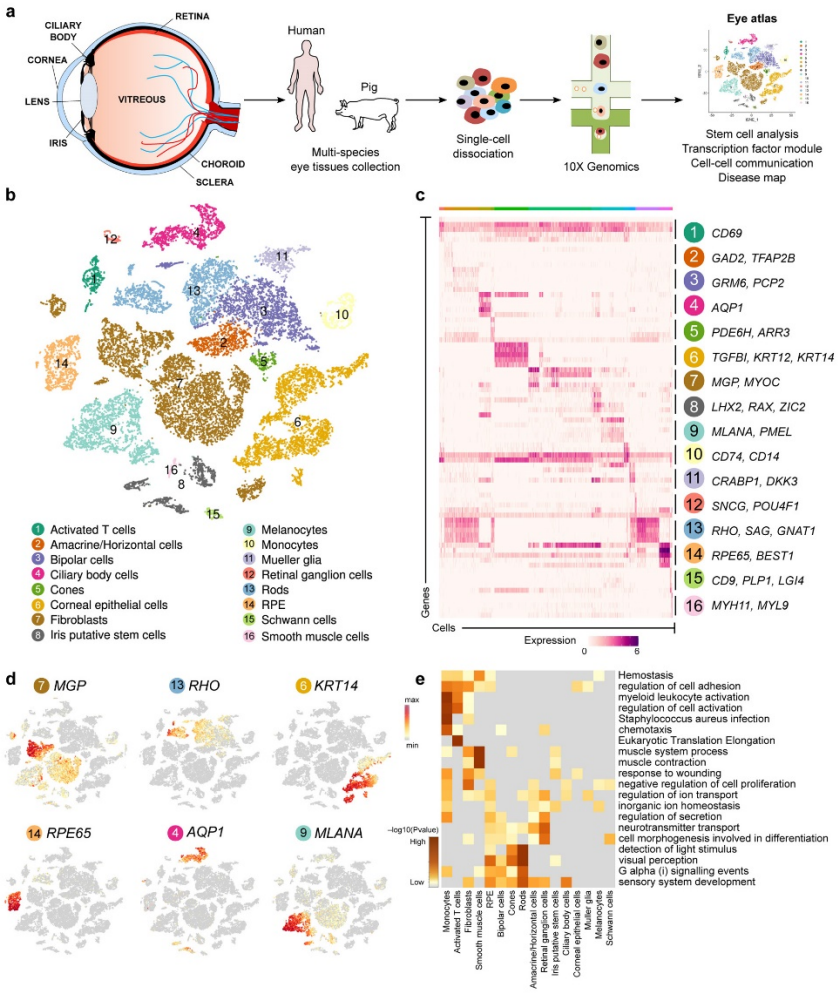

Figure legend: Preparation of single-cell transcriptome atlas of the human eye
a. Overview of single-cell RNA-seq libraries prepared from different sources. Postmortem human and pig eyes were enzymatically dissociated, and single cells were isolated. Approximately 50,000 single cells across the human eye of six individuals using droplet-based scRNA-seq platform were profiled.

b. tSNE plot visualisation of human eye cell-types coloured by 16 different transcriptionally distinct clusters.

c. Heatmap of differentially expressed genes (DEGs) used to classify cell types for each cluster. The top 5 genes were selected using the one-sided Wilcoxon rank-sum test (p-value < 0.01 & |avg_log2FC| > 0.25), and ranked based on their p-values within each identified cell type. Scaled expression levels for each cell are colour-coded.

d. tSNE plots showing expression of selected marker genes depicting major classes of cells in the human eye. Scaled expression levels for each cell are colour-coded and overlaid onto the t-SNE plot.

e. GO analysis of DEGs associated with distinct clusters. Metascape calculated the statistical significance of each GO term enrichment (p-value) based on the accumulative hypergeometric distribution. The Grey colour indicates a lack of significance.