Molecular dissection of the retina to elucidate early changes in diabetes

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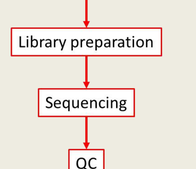
INTRODUCTION

- Diabetic retinopathy (DR) is a leading cause of blindness globally
- More effective treatments aimed at the earlier stages of the disease are required.
- Although often considered primarily a vascular disease, there is evidence of early glial and neuronal dysfunction.
- Recently developed single cell RNA-Sequencing (scRNA-Seq) technology can measure gene expression in individual cells
- Measuring how the diabetic environment impacts these different cell types would direct development of new therapies



Single cell RNA-Seq is like a fruit salad, letting us examine all the individual cells rather than a blended tissue extract

METHODS

- Streptozotocin-induced diabetes model (see above)
- Retinas dissected at 3 time points & single cells isolated
- Single cell RNA sequencing performed using the 10X Genomics and Illumina platforms (see schematic below)
- Data analysed using the Seurat package in R to define cell types and perform differential gene expression

Workflow for Single cell RNA-Seq

RESULTS

- Over 30,000 thousand cells, representing all the main cell types, were profiled from control and diabetic retinas (Figure 1)

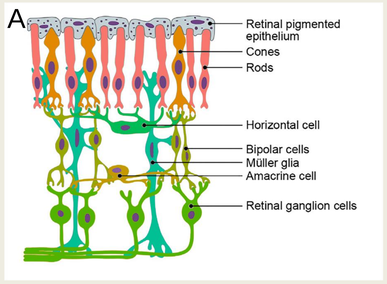
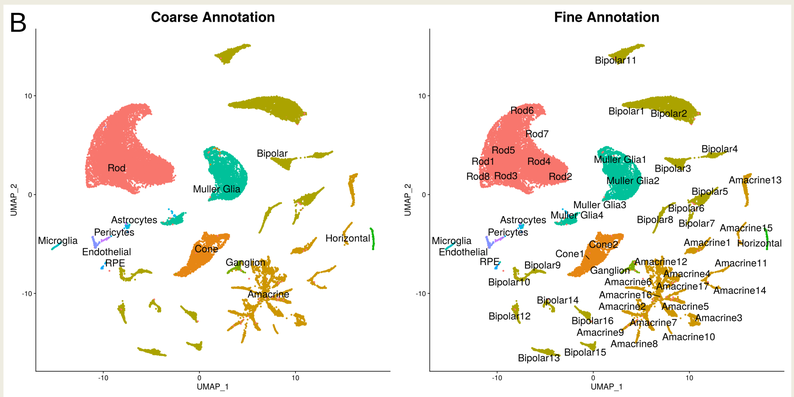


Figure 1. Retinal cell types identified. **A**. Schematic illustrating the different cell types of the retina. **B**. Two dimensional representations (UMAPs) of the relationship between cell types. Each individual cell is represented by a dot and located close to others with similar gene expression. The clusters represent cell types and are coloured according to the schematic in A. Main cellular populations are indicated (left) – most celltypes could be further divided into subpopulations based on gene expression profile.



- No significant changes in cell proportions were observed (Figure 2), but diabetes caused significant differential gene expression in most cell types (Figure 3).

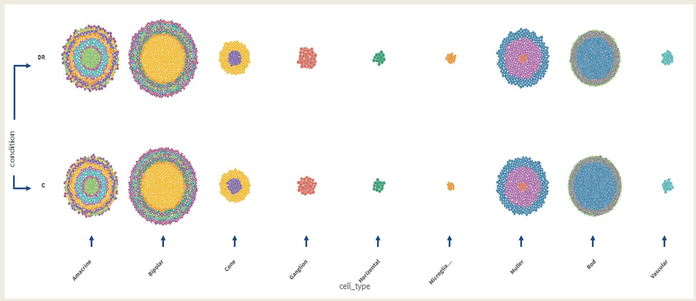


Figure 2. Proportions of each cell type in normal and diabetic retina. Each dot represents a cell, with the colour indicating the sub-type (cell cluster) and the size the number of genes expressed. All samples from the late timepoint are combined for illustration, but there were no significant differences.

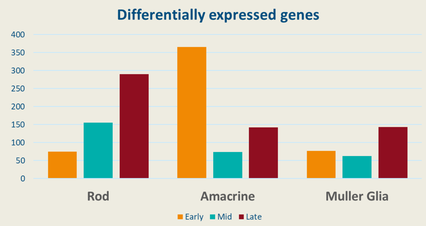
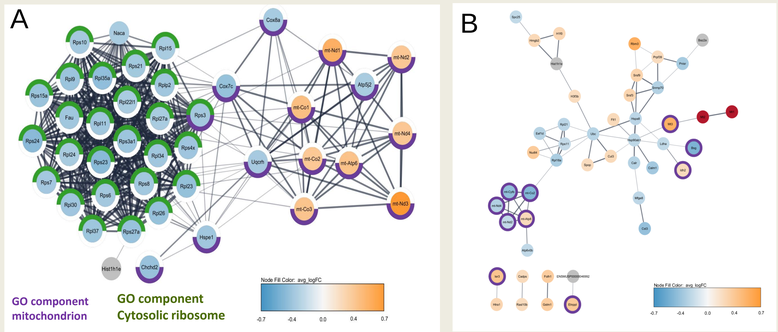


Figure 3. Differentially expressed genes in the most abundant cell types after differing periods of diabetes. Amacrine cells displayed many early changes with rods more affected at later timepoints.

- Prominent changes in protein synthesis and oxidative phosphorylation occurred in many cells (Figure 4).
- Muller cells also showed an increase in metallothionein genes, potentially offering protection against oxidative stress (Figure 4).



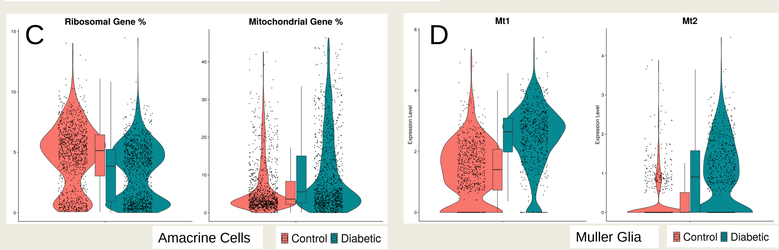


Figure 4. Functional changes in the diabetic retina. A. Network of the genes most significantly altered in diabetes in Amacrine cells at the early timepoint. The genes circled in purple are involved in mitochondrial oxidative phosphorylation and decrease in expression, while those circled in green are involved in protein synthesis and increase in expression. **B**. Network of Muller cells at the late timepoint indicating pronounced enrichment of metallothionein genes in diabetes. **C**. Expression plots of the aggregated ribosomal and mitochondrial gene sets (4A) altered in Amacrine cells in diabetes, shown as a percentage of total cellular expression. **D**. Strong enrichment of Mt1 and Mt2 genes in the Muller glia of diabetic retinas.

CONCLUSIONS

- **This study has confirmed early molecular changes in neural and glial cells during diabetes and as expected, suggested changes within vascular cells.**
- **Enrichment of the relatively rare endothelial cells and pericytes will be required to provide more conclusive data regarding vascular effects.**
- **We have identified many novel potential targets for early interventions to prevent or slow diabetic retinopathy.**
- **The upregulation of metallothionein genes is consistent with an increase in oxidative stress is occurring within diabetic Muller glia, suggesting potential therapeutic avenues.**

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