Children's Medical Research Institute
Stem Cell Medicine Group
Dr Anai Gonzalez Cordero

Retina Australia Research Report 2020

Project Title: Single cell RNA sequencing to characterise cell diversity and molecular signatures of hiPSC-derived retinal organoids

Lay description: Eye diseases caused by genetic defects are numerous and devastating to patients and families. These conditions affect the neural retina at the back of the eye in particular the light sensing cells, the photoreceptors. The aim of this project was to enhance our understanding of inherited eye diseases by combining two important technologies, stem cells and single cell RNA sequencing. Here we used stem cells generated from patient's blood (iPSCs) to generate mini human retinas called organoids. Due to limited accessibility of human tissue samples, these organoids offer unlimited amounts of human retinal tissues that can be used in multiple applications in the laboratory, in a dish. In this study we characterised cells isolated from organoids from two distinct forms of diseases, named Usher2a and Stargardt's and compare these to normal organoids and the adult human retina transcriptome. We identified the cell populations within organoid cultures and established that they compare with published datasets from the human adult retina samples. This data demonstrated differences between disease and control organoids. Further bioinformatics analysis will elucidate the causes of cell damage in disease. Therefore, this study enhanced our understanding of eye Usher and Stargardt's retinal diseases.

Scientific Outcomes resulting from this funding:

Retinal organoids generated using varied methodologies have been comprehensively characterised using transcriptome analysis by RNA sequencing. Single cell RNA sequencing (scRNA-seq) has elucidated the cellular composition of organoids generated from a number of protocols. In this study, we aimed to utilised scRNA-seq to characterise retinal organoids generated using our 2D/3D method of differentiation (Gonzalez-Cordero et al., 2017) and compare these to the adult human retina as well as to organoids generated from iPSCs from the two most common form of inherited retinal degeneration, Usher2a and Stargartd's.

Using this technique, we confirmed our hypothesis that healthy control and diseased iPS cell lines generate retinal organoids contained similar cell types and gene expression similar to the human retina. We also established that iPSC-derived Usher2a and Stargardt's retinal organoids contain different proportion of cells and present different molecular characteristics when compared to control photoreceptor cells.

1) *Comparison of single cell RNA-sequencing of adult human retina datasets and iPSC-derived control organoids.*

We first investigated the cell-type composition of two control retinal organoids at day 210 of culture (~30 weeks of development). Previous studies have revealed that at this stage retinal organoids transcriptomes show maturation of retinal cell classes and reached a stable state (Cowan et al., 2020). TSNE plots have demonstrated the presence of discreet cells populations (**Figure 1A**). The expression profiles of key marker genes for each cell type population were

used to delineate specific cell clusters (**Figure 1B**). The presence of three distinct rod photoreceptor cells populations, might indicate differences in developmental stages of these cells as well as changing in health status. Further analysis of differential expressed genes will highlight the differences of these particular populations.

Cowan et al., 2020 has recently demonstrated that the transcriptome of the adult human retinal change rapidly post mortem in ischemia (Cowan et al., 2020). They have developed a procedure to obtain adult human retinas that were exposed to less than 5 min of ischemia and maintained light responses and functional retinal circuits for 16 h *ex vivo*. Therefore, this transcriptome dataset is considered gold standard and we aimed to compare this publicly available data and two other transcriptome datasets with our 'in house' dataset.

The three published human adult retina scRNA-seq transcriptome datasets (Cowan et al., *Cell*, 2020, Sridhar et al., *Cell Reports*, 2020 and Lukowski et al., *EMBO J*, 2019) were compared to our retinal organoids using the predefined cell type labels from the public datasets. Next, we measured the agreement between differential analysis statistics of all cell types across the multiple datasets and visualised the pairwise comparisons of the correlations on a clustered correlation heatmap (**Figure 1C**). We demonstrated that the same cell types show high correlation with one another (even though they are from different datasets) and distant cell types show relatively lack of correlation. Rod population 1 appears to correlate with the adult peripheral rods of Cowan et al., indicating that this population is indeed a more mature population than the one named Rods 2.

2) *Data analysis to establish cell composition and diversity of control, Usher2a and Stargardts hiPSC-derived retinal organoids*

Next, we compared the cell number and proportion of different retinal cell types present in the two WT healthy control organoids and four Usher2a retinal organoids. The overall cell type compositions were similar between control and Usher2a organoids. However, rod photoreceptors appear to be reduced in Usher samples. Furthermore, a clear increase in apoptotic cells was observed (**Figure 2A**). When controls were compared to Stargardt's organoids we observed similar proportion of cell populations (**Figure 2B**). However, no distinct differences between the six biological samples, in terms of cell composition were observed.

3) *Data analysis for each cell type to elucidate cellular mechanisms and signalling pathways of disease photoreceptor cells*

Gene ontology enrichment analysis demonstrated a downregulation of metabolic processes in in Usher2a organoids when compared to healthy control organoids. Differential gene set enrichment analysis between Rods 3 and Rods 1 in USHER organoids demonstrated up regulation of oxygen disregulation and mitochondrial pathways on the Rods 3 population.
Further studies are ongoing to reveal pathways in Stargartd's organoids and to further validate the pathways enriched in Usher organoids.

Acknowledgements: We would like to thank Retina Australia for funding and enabling us to study the molecular signatures of these important human retinal models of inherited retinal degeneration. The data generate here will be crucial for future grant applications and publications. We would like to acknowledge the Computational Systems Biology Group and Embryology Unit, especially Hani Kim, Pengyi Yang and Nader Aryamanesh for bioinformatics analysis.

Figures

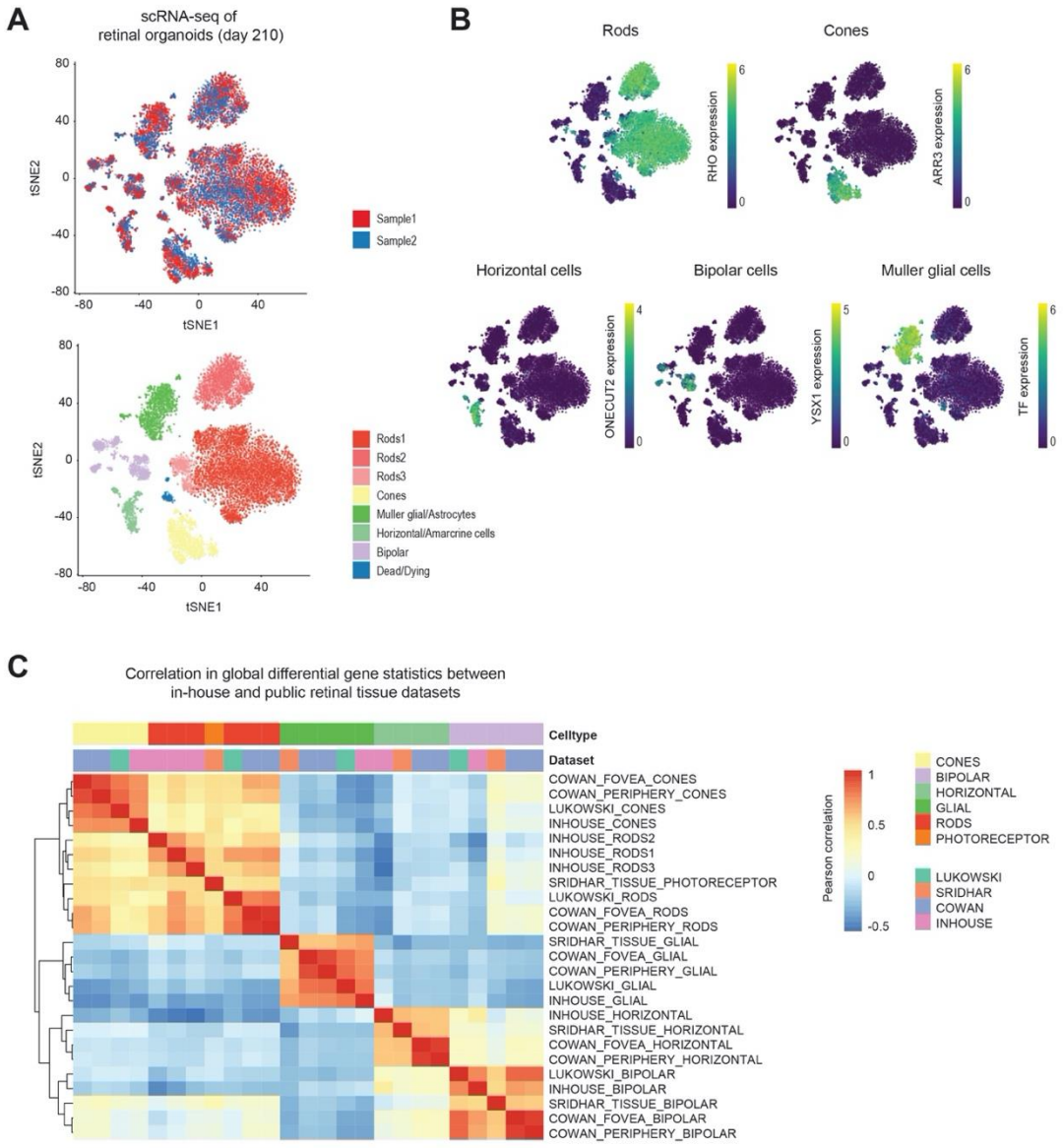

Figure 1. A) TSNE plot of single cells from 210 day old healthy retinal organoids. Upper TSNE plot is colored by biological samples and lower TSNE pot is colored by cell types that were manually annoted through marker gene analysis. B) Gene expresssion (log2 normalised expression) of key cell type marker genes overlaid on TSNE. C) Correlation heatmap of global differential analysis statistics derived from public retinal tissue datasets and in-house retinal organoid datasets. Pearson correlation was uesd to calculate the correlation across approximately 5600 features. Color bar denotes cell types (upper panel) and the original dataset (lower panel).

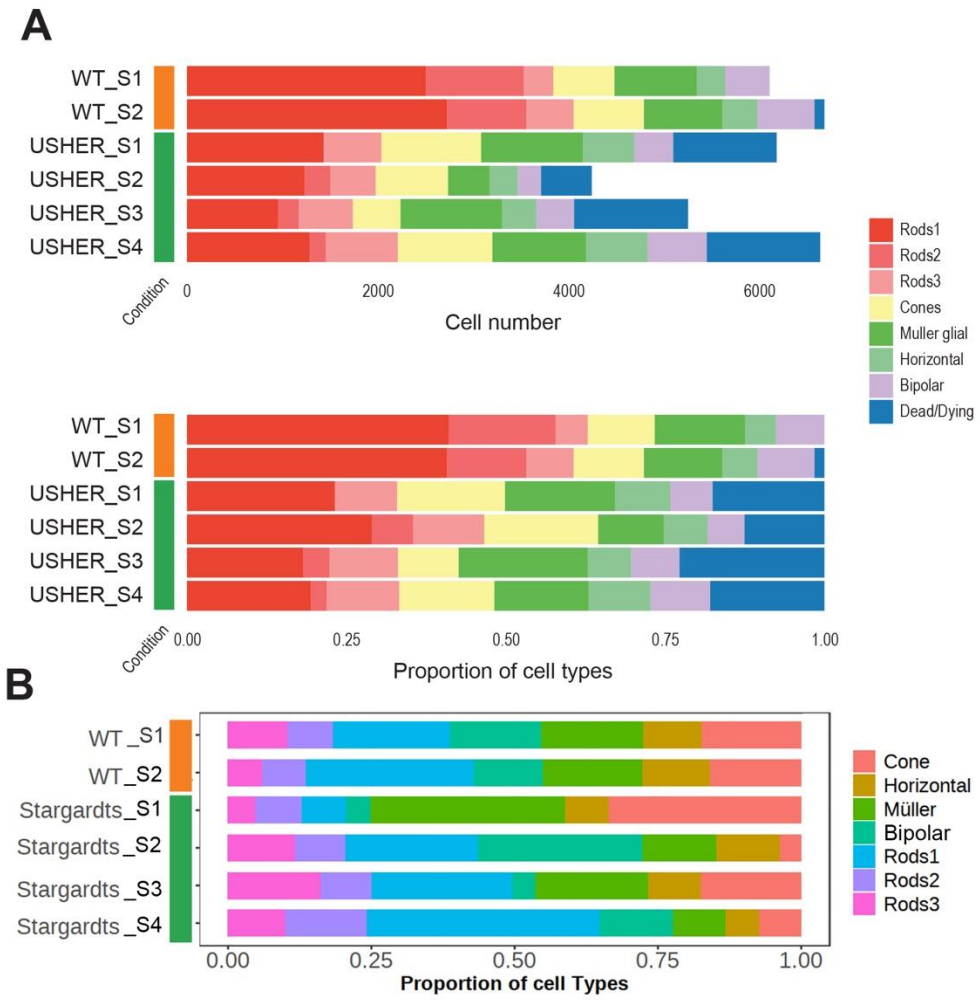

Figure 2. A) Bar plots of cell number (uppper) and proportion (lower) of cell types across six biological samples of healthy (orange) and diseased organoids (green). Each bar represents the number or proportion of cell types present in each organoid samples. B) Bar plot showing proportion of cell types in Stargardt' organoids across six biological samples of healthy (orange) and diseased organoids (green).

References

Gonzalez-Cordero A, Kruczek K, Naeem A, Fernando M, Kloc M, Ribeiro J, Goh D, Duran Y, Blackford SJI, Abelleira-Hervas L, Sampson RD, Shum IO, Branch MJ, Gardner PJ, Sowden JC, Bainbridge JWB, Smith AJ, West EL, Pearson RA, Ali RR. Recapitulation of Human Retinal Development from Human Pluripotent Stem Cells Generates Transplantable Populations of Cone Photoreceptors. Stem Cell Reports. 2017 Sep 12;9(3):820-837. doi: 10.1016/j.stemcr.2017.07.022. Epub 2017 Aug 24. PMID: 28844659; PMCID: PMC5599247.

Cowan CS, Renner M, De Gennaro M, Gross-Scherf B, Goldblum D, Hou Y, Munz M, Rodrigues TM, Krol J, Szikra T, Cuttat R, Waldt A, Papasaikas P, Diggelmann R, Patino-Alvarez CP, Galliker P, Spirig SE, Pavlinic D, Gerber-Hollbach N, Schuierer S, Srdanovic A, Balogh M, Panero R, Kusnyerik A, Szabo A, Stadler MB, Orgül S, Picelli S, Hasler PW,

Hierlemann A, Scholl HPN, Roma G, Nigsch F, Roska B. Cell Types of the Human Retina and Its Organoids at Single-Cell Resolution. Cell. 2020 Sep 17;182(6):1623-1640.e34. doi: 10.1016/j.cell.2020.08.013. PMID: 32946783; PMCID: PMC7505495.

Lukowski SW, Lo CY, Sharov AA, Nguyen Q, Fang L, Hung SS, Zhu L, Zhang T, Grünert U, Nguyen T, Senabouth A, Jabbari JS, Welby E, Sowden JC, Waugh HS, Mackey A, Pollock G, Lamb TD, Wang PY, Hewitt AW, Gillies MC, Powell JE, Wong RC. A single-cell transcriptome atlas of the adult human retina. EMBO J. 2019 Sep 16;38(18):e100811. doi: 10.15252/embj.2018100811. Epub 2019 Aug 22. PMID: 31436334; PMCID: PMC6745503.

Sridhar A, Hoshino A, Finkbeiner CR, Chitsazan A, Dai L, Haugan AK, Eschenbacher KM, Jackson DL, Trapnell C, Bermingham-McDonogh O, Glass I, Reh TA. Single-Cell Transcriptomic Comparison of Human Fetal Retina, hPSC-Derived Retinal Organoids, and Long-Term Retinal Cultures. Cell Rep. 2020 Feb 4;30(5):1644-1659.e4. doi: 10.1016/j.celrep.2020.01.007. PMID: 32023475; PMCID: PMC7901645.