● PERSPECTIVE

Using single cell transcriptomics to study the complexity of human retina

The human retina is a specialized multilayered structure composed of numerous cell types. The process of vision relies on a robust network integrated by rod photoreceptors, cone photoreceptors, bipolar cells, horizontal cells, amacrine cells and retinal ganglion cells, which detect, process and relay the visual information to the brain. Additionally, structural and metabolic support is provided by Müller glia, retinal astrocytes and microglia. Over 200 genes have been implicated in inherited retinal diseases (RetNet: https://sph.uth.edu/retnet/). However, in many cases, the retinal cell types that express these disease-associated genes remain to be identified. The complexity of the human retina represents a major challenge for the molecular profiling of all retinal cell types. Many previous studies utilised bulk RNA-seq to profile the whole human adult retina, which only analysed the averaged gene expression levels across all retinal cell types. As such, knowledge of the transcriptome profile in specific cell types within the retina would help us to unravel the heterogeneity of retinal cells, advance understanding of the pathogenesis of inherited retinal diseases, and to develop gene therapies that could improve treatment options.

Single cell RNA sequencing (scRNA-seq) is a powerful technique that enables a thorough analysis of the gene expression profile at a single cell level, thus fostering the understanding of the complex biological diversity of tissues at an unprecedented resolution. Using scRNA-seq, we recently reported the generation of a single cell transcriptome atlas of the human adult neural retina, by profiling 20,009 neural retinal cells from three healthy donors (Lukowski et al., 2019). This work was conducted as part of the Human Cell Atlas Project (Regev et al., 2017) and the Australia and New Zealand Human Eye Cell Atlas Consortium. Notably, our human retina transcriptome atlas identified the transcriptome of all major neural retinal cells, including rod photoreceptors, cone photoreceptors, Müller glia, bipolar cells, amacrine cells, retinal ganglion cells, horizontal cells, astrocytes and microglia. This dataset can be accessed through the EMBL Single Cell Expression Atlas or the Human Cell Atlas Data Portal (https://data.humancellatlas.org/explore/projects/8185730f-4113-40d3-9cc3-929271784c2b). Collectively, this gene atlas provide unprecedented insights into the gene expression that enable the specialised function of individual cells in the retina and contribute to healthy vision.

A powerful feature of scRNA-seq is the ability to resolve heterogeneous subpopulations within known retinal cell types based on similarities in transcriptome profiles. In our study, we were able to distinguish between some subtypes of neural retinal cells in this dataset. For example, photoreceptors are the largest cell population in the retina and we could determine the transcriptome of photoreceptor subtypes, including rods, L/M cones and S cones. We identified a role of *MALAT1* in putative rod degeneration, which can be used as a marker to assess the donor retina quality in future retina scRNA-seq studies. Also, we were able to distinguish some bipolar cell subtypes, including ON-bipolar, OFF-bipolar, rod bipolar and DB4 bipolar cells. However, the current dataset is limited in power to accurately identify differences in the transcriptomes of subtypes that are less frequently represented in the retina, such as amacrine cells and the retinal ganglion cells which are known to be highly complex. Future scRNA-seq studies with larger number of profiled retinal cells would improve the resolution of this retina transcriptome atlas and allow us to accurately distinguish and resolve subtypes of human retinal cells. In particular, the use of surface markers for cell enrichment prior to scRNA-seq would provide a useful strategy to increase the sensitivity to profile cell types that are less frequently represented.

Our transcriptome atlas of the healthy human retina can be used as a reference map to understand the genes that cause cellular defects associated to visual impairment and blindness. For instance, many of the genes causing retinitis pigmentosa are expressed in rod and cone photoreceptors. Further studies that utilize scRNA-seq to profile donor retina from patients with retina diseases, would provide a comprehensive characterization of the molecular basis of pathology in specific retinal cell types. For instance, a recent scRNA-seq study has profiled the retinal pigment epithelium and choroid tissue from patients with age-related macular degeneration, which identified specific expression of *RGCC* in choriocapillaris endothelial cells (Voigt et al., 2019). It would be very interesting for future studies to extend this work to a larger patient cohort, as well as profiling the neural retina to determine the pathological changes in age-related macular degeneration in specific retinal cell types.

In addition, our retina transcriptome atlas provide an important tool to benchmark the quality of retinal cells derived from stem cells *in vitro*. In our study, we have demonstrated the use of our retina transcriptome atlas to assess the quality of cone photoreceptors derived from human pluripotent stem cells (Welby et al., 2017). When compared to all the major adult retinal cell types, our results showed that stem cell-derived cones exhibit the highest transcriptome similarity to cones, though they are more similar to fetal cones than adult cones. Collectively, our retinal dataset in adult state can be used together with other datasets in fetal state (Welby et al., 2017; Hu et al., 2019) as a reference to assess the maturity of derived retinal cells, which would have implications in stem cell research. Identification of molecular differences between stem cell-derived cells and their adult counterpart could lead to new differentiation methods to improve the maturity of the derived cells.

As single cell genomic technology continues to improve, it is now possible to address more complex questions using spatial transcriptomics to perform transcriptome profiling while retaining spatial information. This is particularly important for the study of human retinal biology. For instance, it is well known that the distribution of both the cell number and subtypes of photoreceptor varies greatly at different location of the retina. The fovea at the macula region, which is responsible for maximum visual acuity in central vision, is exclusively populated by cones in high density. Histologically, the cones in the central fovea are thinner and more compact in morphology, which is different to cones found in other parts of the retina. In contrast, the peripheral retina contains predominantly rod photoreceptors, which are responsible for low light detection. Unlike human and other primates, rodents lack the macula region, which is a key limitation for retinal biology studies due to species differences. Previous studies have dissected the primate retina in the macular and peripheral region for scRNA-seq analysis and characterized the region-specific expression profiles in retinal cells (Peng et al., 2019). However, important information such as the accurate location and cell-cell interactions is lost with tissue dissection. Spatial transcriptomics, such as Slide-seq and sequential fluorescence *in situ* hybridization (seqFISH+, Eng et al., 2019; Rodriques et al., 2019) are innovative techniques that address these issues. In Slide-seq, a tissue section is placed on top of a glass coverslip that was previously coated with 10 μm barcoded beads. The RNA is released from the cells and captured by the beads, which allows the retention of the spatial information through the generation 3' barcoded RNA-seq libraries. One advantage of Slide-seq is that it can be easily integrated with large-scale scRNA-seq studies, thus it could foster the novel identification of spatially defined gene expression profiles and markers. On the other hand, seqFISH+, an improved version of multiplexed sequential FISH, enables the visualisation and sequencing of up to 10,000 pre-selected genes. This technique can identify the spatial organisation of distinct cell clusters, as well as information about the subcellular localisation of the transcripts within each cell profiled. Future use of these technologies could complement the single cell retina transcriptome atlas by identifying region-specific expression profiles and interactions between neighbouring cells, such as ligand-receptor pairs, which would advance our understanding of retinal anatomy in humans.

Another interesting direction is the use of scRNA-seq to understand the species differences in retinal biology which could potentially uncover the genetic signals that dictate retinal regeneration. For instance, while zebrafishes exhibit a remarkable ability for retinal regeneration following injury, while mammals do not possess the same regenerative capability (Lenkowski and Raymond, 2014). In this regards, the proneural transcription factor Ascl1 is known to play a key role in Müller glia for retinal regeneration in zebrafishes following injury, and an elegant study by Thomas Reh's lab showed that Ascl1 can be used to promote reprogramming of mouse Müller glia into new retinal neurons *in vivo* (Jorstad et al., 2017). This finding provided an exciting approach to stimulate retinal regeneration in the eye. Future studies that uncover retinal subpopulations, and their trajectories from precursor cells, would potentially enable the identification of new master regulators that can reprogram retinal cell linages.

In summary, advances in transcriptomic technologies have provided a promising approach to generate a human retinal transcriptome atlas at a single cell level (**Figure 1**). This is clearly an attractive research

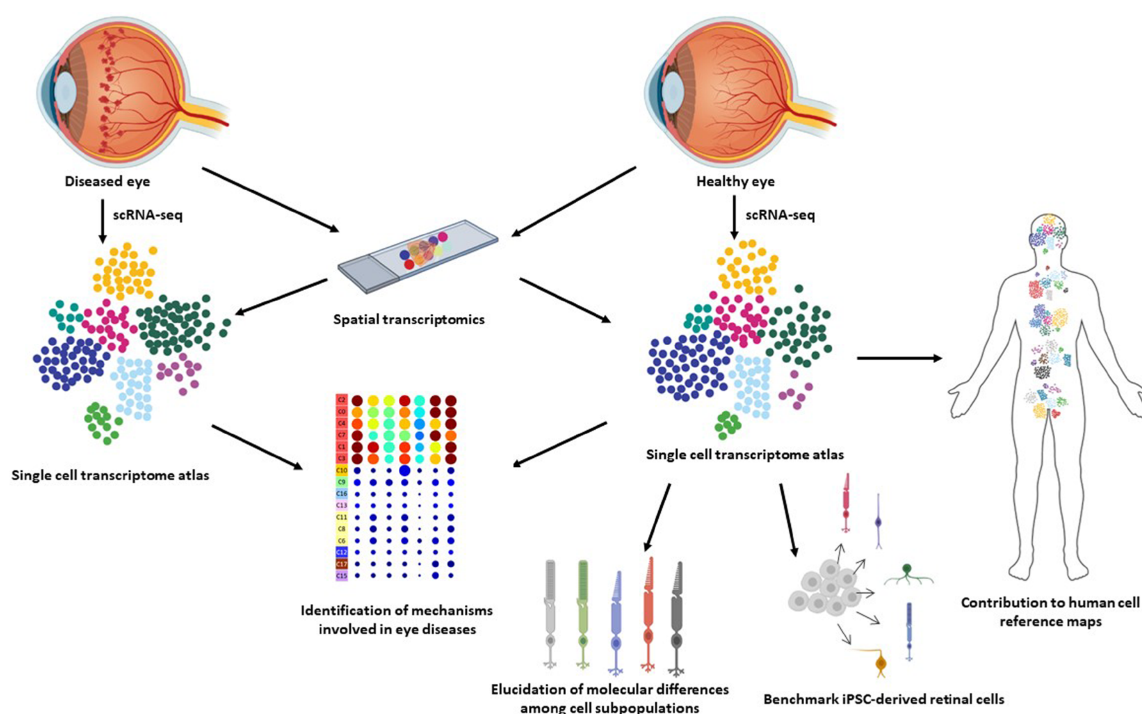

Figure 1 Potential of using the human retina transcriptome atlas to study disease mechanism, understand retinal cell subtypes and assess the quality for stem cell-derived cells.
iPSC: Induced pluripotent stem cells; scRNA-seq: single cell RNA sequencing.

strategy to advance the understanding of tissue function and the etiology of numerous diseases, as evident by global efforts to create a collection of reference maps for all human cells, including the Human Cell Atlas Project (Regev et al., 2017) and the LifeTime Initiatives (https://lifetime-fetflagship.eu/). The human retina transcriptome atlas represents a valuable resource to study the molecular signals that define retinal cell identity, cell subtypes and regional differences in humans. This transcriptome atlas can also be used as a reference map to understand the molecular basis of retinal pathologies, as well as for benchmarking to assess the quality of stem cell-derived cells.

Some vector graphs used in Figure 1 were designed by Biorender. The Centre for Eye Research Australia receives operational infrastructure support from the Victorian Government.

RCBW was supported by funding from the University of Melbourne, Centre for Eye Research Australia, Retina Australia and the National Health and Medical Research Council (APP1184076).

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***Received:** January 9, 2020*
***Peer review started:** January 16, 2020*
***Accepted:** February 22, 2020*
***Published online:** May 11, 2020*

***doi:** 10.4103/1673-5374.282253*
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***Open peer reviewer:** Kasum Azim, Heinrich-Heine-University, Germany.*
***Additional file:** Open peer review report 1.*

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P-Reviewer: Azim K; C-Editors: Zhao M, Li JY; T-Editor: Jia Y