Chapter 20
Modeling Retinal Dystrophies Using Patient-Derived Induced Pluripotent Stem Cells

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Abstract *Retinal* degenerative disease involving photoreceptor (PR) cell loss results in permanent vision loss and often blindness. Generation of induced pluripotent stem cell (iPSC)-derived retinal cells and tissues from individuals with retinal dystrophies is a relatively new and promising method for studying retinal degeneration mechanisms in vitro. Recent advancements in strategies to differentiate human iPSCs (hiPSCs) into 3D retinal eyecups with a strong resemblance to the mature retina raise the possibility that this system could offer a reliable model for translational drug studies. However, despite the potential benefits, there are challenges that remain to be overcome before stem-cell-derived retinal eyecups can be routinely used to model human retinal diseases. This chapter will discuss both the potential of these 3D eyecup approaches and the nature of some of the challenges that remain.

Keywords Retinal degeneration · Patient · Dystrophy · Stem cell · hiPSC · Pluripotent · Eyecup

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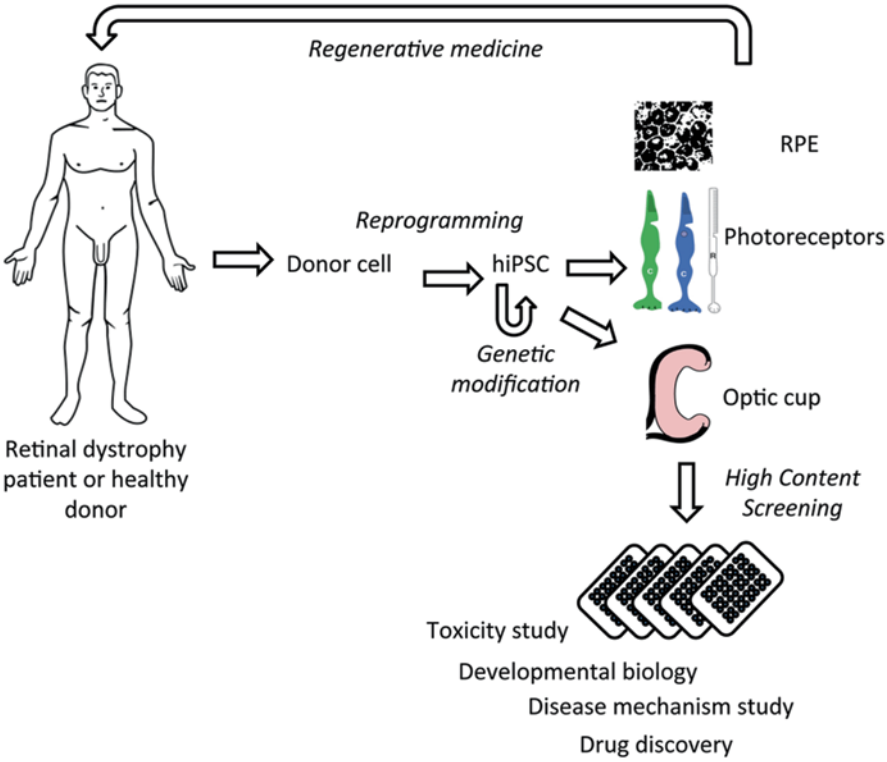

Fig. 20.1 A strategy to utilize human-induced pluripotent stem cell (*hiPSC*)-derived PR or retinal pigment epithelial (*RPE*) cells for cell replacement therapies or to generate retinal eyecups to be used to model human eye disease and for high-content drug screening

20.1 Introduction

Retinal degenerative diseases include Mendelian orphan diseases such as retinitis pigmentosa (RP) and the complex genetics disease age-related macular degeneration (AMD), which is the second most common cause of irreversible blindness in the western world. Vision loss in these diseases results from the dysfunction and death of photoreceptor (PR) cells and/or adjacent retinal pigment epithelial (RPE) cells. While both environmental and genetic causes have been implicated in retinal degeneration, in many cases the particular gene(s) and/or risk factor(s) involved are unknown, making it difficult to develop targeted therapies [1]. Even when the mutation is known, FDA-approved therapies are in general not yet available. Pluripotent stem cells (PSCs) of human origin offer exciting new approaches to study human "diseases in a dish," offering a new means to explore mechanisms of retinal disease and opening the possibility of novel cell-based therapeutic approaches (Fig. 20.1). PSC-derived retinal cells could also facilitate high-content screening of human disease-based cells for neuroprotective and other therapeutic compounds.

One approach for such studies is to use dissociated cell cultures. Another approach, which has the advantage of better modeling the in vivo situation, is the use of 3D retinal cultures in the form of retinal eyecups that preserve the stereotypical laminar pattern of the mature neural retina, complete with a thin RPE layer [2].

20.2 Retinal Dystrophies Can Arise from Mutations in Genes Predominantly Expressed in Photoreceptors or the RPE

The Retinal Information Network, or Retnet (https://sph.uth.edu/RetNet/), which maintains a curated list of genes causing retinal disease, lists well over 200 retinal disease-associated genes and loci. Rhodopsin gene mutations are the most common causes of autosomal dominant RP (adRP), with the P23H mutation being the most common opsin mutation. Rhodopsin mutations can result in protein mis-folding and trafficking defects, and eventually result in PR death [3]. Defects in cells other than PRs can also result in PR degeneration. For example, defects in the Mer tyrosine kinase (*MERTK*) can result in a childhood onset rod-cone dystrophy by altering the normal phagocytic functions of RPE cells, which disrupts the normal cycling of visual pigments that are shed on a daily basis from PR outer segments [4]. Similarly, mutations in the *RPE-65* gene, which encodes a retinoid isomerase that is predominantly expressed in the RPE, can result in Leber congenital amaurosis (LCA), a severe early onset form of childhood retinal degeneration [5].

20.3 Existing Models of Retinal Degeneration Using hiPSCs

Although mouse models of human *RP* exist and are being used for mechanism and drug development purposes, mice and humans are not equivalent, and species-specific differences have been an issue in translational studies. In a mouse model of Usher 3 syndrome, a form of RP with hearing loss, the causal mutation in *clarin-1* expressed in mice, leads to auditory deficits but no detectable retinal degeneration phenotypes [6]. It is hoped that by generating models of human eye disease in a dish through the use of human-induced pluripotent stem cells (hiPSCs) some of these species-related issues can be avoided.

Several studies of human retinal disease have used hiPSCs to study *RP* and gyrate atrophy (GA) [7, 8]. GA of the retina can arise from a defect in the metabolism of ornithine-δ-aminotransferase (OAT) and begins in childhood, often resulting in total vision loss in 30–40-year olds [9, 10]. From an individual with a form of GA that is responsive to dietary supplementation with pyridoxine (vitamin B6), hiPSCs were generated by Meyers et al. who then directed them to become RPE cells, a cell type affected by this disease [8]. Restoration of OAT activity was observed upon treatment with high doses of vitamin B6. Furthermore, showing the power of hiPSC

technology, it was shown that mutant cells could be restored to normal function by genetically correcting the OAT gene mutation via bacterial artificial chromosome (BAC)-mediated homologous recombination.

In another recent report, whole exome sequencing of stem-cell-derived retinal cells from a single patient with RP was used to identify a homozygous Alu insertion in exon 9 of Male germ cell Associated kinase (MAK) [11]. In hiPSCs from healthy unaffected donors, a transcript-lacking exon9 was found in undifferentiated cells, whereas a transcript-bearing exon 9 and a previously unrecognized exon 12 were seen in differentiated retinal precursors. In the RP-based hiPSCs with the Alu insertion, this splicing switch never occurred, suggesting a defect in alternative splicing. For retinal disorders in which the basis for disease is not yet known, this strategy demonstrates the usefulness of exome sequencing to uncover differences in altered gene expression and/or alternatively spliced variants due to retinal degeneration.

Another important study involved a model for RP in which Jin et al. generated hiPSCs from RP patients with early (RP9) or late onset (RP1, PRPH2, or RHO) retinal degeneration [7]. Compared with unaffected controls, stem-cell-derived rod PRs from disease backgrounds decreased over time, and markers for oxidative and/or ER stress became elevated. These observations bode well for retinal disease modeling—a major concern has been that since many retinal dystrophies occur only after many decades of life, they would not demonstrate a detectable phenotype in relatively short-term cell culture. There is mounting evidence that some neurodegenerative disorders with later-stage onset can also be modeled in vitro [12].

20.4 Generating hiPSCs and PRs

Lineage-specific variation can affect hiPSC generation, maintenance, and PR differentiation. Numerous studies suggest that not all stem cell lines are created equal; stem cells created by different labs, by different reprogramming methods, and even different cell lines derived from the same patient with the same methods can show variable differentiation potential [13]. To reduce this inherent variation, it is important that microenvironmental, epigenetic, and other factors that contribute to retinal differentiation be better understood.

Studies of stem-cell-derived retinal cells have traditionally been carried out with mouse PSCs [14–19]. In the past few years, however, newer protocols for generating retinal and RPE cells of human origin have also been developed. These new methodologies include improvements in microenvironment, extracellular matrices, timing, and cell purity [2, 8, 20–23]. One of the most noteworthy improvements has focused on self-organized pseudostratified optic cup structures bearing all the retinal cell types in their proper orientation (Fig. 20.2, unpublished data) [2, 8]. Rax positive neural retina can be distinguished after 2–3 weeks while early PR markers can be distinguished by 30–45 days in vitro. With a brief treatment with the small molecule Wnt agonist, CHIR99021, these cultures can even be coaxed to develop a thin layer of pigmented RPE cells. The advanced morphology and mature gene expression in 3D systems demonstrate the importance of cell–cell interactions; such

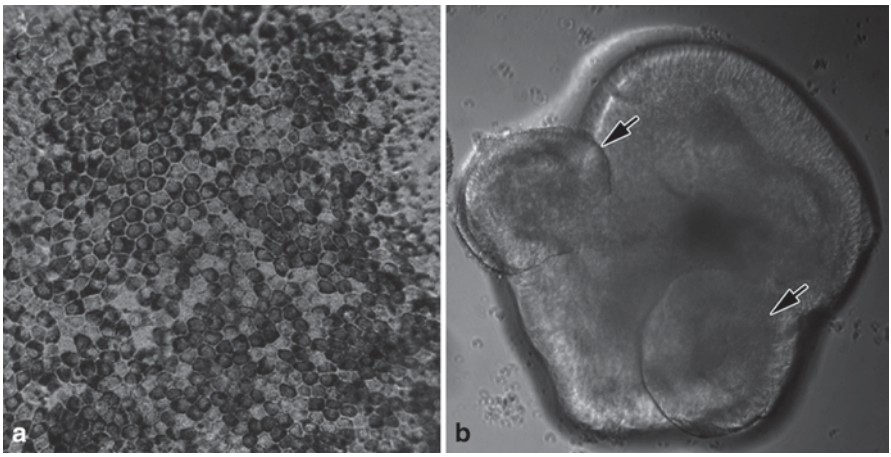

Fig. 20.2 A representative RPE monolayer (**a**) and pseudo-stratified optic cup-like structure (b) from human iPS cells. *Arrows* indicate retinal eyecup structures in 18-day-old human stem-cell-derived retinas

retinal progenitors injected in vivo have been observed to have elevated expression of visual pigment and other PR-specific genes and bear outer segment-like structures that do not normally develop in simple monolayer cultures in vitro [2, 18, 21]. Collectively, these features should make it easier to develop in vitro assays for studying retinal degenerative disorders.

20.5 Limitations to Using hiPSC for Modeling Human Disease

When a disease source can be isolated to a single primary cell with a single gene mutation identified, the disease can be studied rather successfully, as exemplified in the case of the defective RPE cells in *GA*. When a disease affects PRs directly, purified PR progenitors can be grown in monolayer culture or as more complex optic cup structures. Unfortunately many retinal diseases result from a complex interplay between distinct cell and tissue types. Additionally, systemic factors can play a role, and these complex interactions can be difficult or impossible to adequately model in vitro.

There are also technical reasons why a disease phenotype might not be easily observed, especially when dealing with a subtle disease phenotype. Human iPSC-derived embryoid bodies and neurospheres can vary considerably in terms of cell type composition, number and size of optic cups, and demonstrate differences in the timing of differentiation (Wahlin et al., unpublished observations). Parameters such as oxygen tension, cell medium composition, and feeding frequency can also adversely affect cell culture variability and can act as additional sources of experimental variability. As stem cell and retinal differentiation protocols become better refined, such issues will hopefully become less problematic.

20.6 Exciting New Tools to Study Disease-Specific Cell Lines

Major sources of variation are the stem cells themselves, and this poses challenging problems when studying diseases with subtle phenotypes [13]. Variation in hiPSC-derived retinal cells from different individuals, which could result from genetic and epigenetic differences, might mask any retinal degeneration phenotype induced by an actual disease-causing mutation. Using new genome editing tools including zinc fingers nucleases (ZFNs), TALE effector nucleases (TALENs), or the type II bacterial CRISPR RNA-guided nucleases, one can now generate mutant cell lines with congenic controls rather than relying on genetically unmatched controls [24–26]. Due to decreased variability, these controls could greatly simplify interpretation of experimental findings. This technology could also be used to repair genetic mutations for the purpose of replacing defective retinal cells by transplantation.

20.7 Small Molecule Screens for Neuroprotective Compounds

Platforms for identifying neuroprotective small molecule chemicals are being investigated for many neurodegenerative models and have proven successful in some cases. For instance, a screen for proneurogenic compounds has uncovered molecules that protect against animal models of ALS and Parkinson's disease [27, 28]. For retinal survival assays, there are ongoing small molecule screens to protect primary rodent retinal ganglion and PR cell cultures (unpublished data). The recently developed eyecup methods should offer a new and powerful tool for investigating PR development and neuroprotection in a more in vivo-like environment.

Although much work remains, hiPSCs show great potential to contribute to the development of personalized medicine approaches and will likely help in the study of diseases for which no working model exists. Future studies should shed light on a plethora of retinal diseases, leading to greater understanding of disease mechanisms and hopefully also to advances in therapy.

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