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Concise Review: Human Induced Pluripotent Stem Cell Models of Retinitis Pigmentosa

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ABSTRACT

Hereditary retinal dystrophies, specifically retinitis pigmentosa (RP) are clinically and genetically heterogeneous diseases affecting primarily retinal cells and retinal pigment epithelial cells with blindness as a final outcome. Understanding the pathogenicity behind these diseases has been largely precluded by the unavailability of affected tissue from patients, large genetic heterogeneity and animal models that do not faithfully represent some human diseases. A landmark discovery of human induced pluripotent stem cells (hiPSCs) permitted the derivation of patient-specific cells. These cells have unlimited self-renewing capacity and the ability to differentiate into RP-affected cell types, allowing the studies of disease mechanism, drug discovery, and cell replacement therapies, both as individual cell types and organoid cultures. Together with precise genome editing, the patient specific hiPSC technology offers novel strategies for targeting the pathogenic mutations and design therapies toward retinal dystrophies. This study summarizes current hiPSC-based RP models and highlights key achievements and challenges of these cellular models, as well as questions that still remain unanswered. STEM CELLS *2018; 00:000–000*

SIGNIFICANCE STATEMENT

Hereditary retinal dystrophies including retinitis pigmentosa (RP) are clinically and genetically heterogeneous disease affecting primarily retinal cells and retinal pigment epithelial cells with blindness as a final outcome. Discovery of human induced pluripotent stem cells (hiPSCs) permitted the derivation of patient-specific stem cells with unlimited self-renewing capacity and ability to differentiate into RP-affected cell types allowing studies of disease mechanism, drug discovery, and cell replacement therapies both as individual cell types and organoid cultures. Together with precise genome editing in these cells, this study can correct the pathogenic mutations and design therapies to cure these diseases. This study summarizes current hiPSC-based RP models discussing the major achievements as well as challenges about these cellular models.

INTRODUCTION

Retinal dystrophies are one of the leading causes of seriously affected vision worldwide, yet no effective treatment exists up to date. Retinitis pigmentosa (RP) is the most common (1 in 3,000) form of inherited human retinal disorders, characterized by progressive photoreceptor and/or retinal pigment epithelial (RPE) cell loss [1]. Typically, the disease begins with night blindness, due to the early involvement of rod photoreceptors, and progresses to a decrease in the visual field and loss of central vision, due to the degeneration of cone photoreceptors. The RPE cells are responsible for photoreceptor homeostasis; thereby the primary degeneration in one cell type leads inevitably to the secondary degeneration in the other. Despite the high social and health care impact, no effective treatment has been developed to date. The progress in identification of causative mutations in different genes has been greatly accelerated by using exome capture methods, searching for a statistically significant association between genome variations and the disease phenotype. About 3,000 causative mutations have been identified in more than 60 genes in RP patients [2, 3] which can be inherited as autosomal dominant, autosomal recessive, or X-linked manner. The affected genes in RP are involved in diverse functions within photoreceptors or RPE cells. In some cases, cell type-specific functions

are affected such as the phototransduction signaling cascade, retinoid cycle, outer segment (OS) structure, connecting cilium trafficking, chaperone function, phagocytosis, membrane trafficking, and ion transport [1]. However, many ubiquitously expressed genes are likely to contribute to unique retinal function [4, 5]. This is demonstrated by pre-mRNA splicing factors whose mutations exhibit only retinal phenotype [6]. This demonstrates that retinal identity is produced by complex genetic and epigenetic interactions within retinal cells, and that the access to the affected cells is instrumental in studying disease mechanisms and testing therapeutic avenues.

The major impediment in understanding neurodegenerative disease involving the retina is the unavailability of affected tissue, as accessible, unaffected, tissues are not sufficiently informative of the disease. The accumulated knowledge of RP has largely relied on animal models (mainly rodents) in cases where gene variations observed in patients are faithfully mimicked in animals [7]. In addition to differences in genetic background, rodents are nocturnal animals with a higher proportion of rods and lack fovea and cone-rich regions [8]. Moreover, the scientific community is trying to reduce the use of animals in research. Heterologous cellular expression systems in which mutated genes have been overexpressed were used with limited success, as the overexpressed genes are not endogenously regulated [9, 10]. Postmortem samples are extremely rare and represent the end stage of the disease with an indistinguishable contribution of secondary events. In addition, the phenotypic variability in terms of disease progression, onset, disease pattern [11], even within members of one family, supports the approach that the patient-specific platform is the most appropriate one to study the pathogenicity of the disease.

Human Induced Pluripotent Stem Cells Technology and Retinal Differentiation

The groundbreaking discovery of reprograming factors that convert easily accessible differentiated cells into a pluripotent, embryonic-like state by Yamanaka and Thomson groups in 2007 [12, 13], revolutionized modern medicine, enabling the rapid production of disease-specific cellular models. Unlike primary cultures, human induced pluripotent stem cells (hiPSCs) are naturally immortalized due to a high expression of telomerase, with embryonic stem (ES) cell characteristics, and can therefore be maintained indefinitely in culture. While animal models still remain instrumental for studying the therapeutic output on the whole organism, hiPSCs are becoming a critical platform for mechanistic studies at the cellular/organoid and molecular levels, also providing an ideal source for patient-specific cell therapy as well as for drug development and toxicity assays. The multilineage differentiation capacity of human ES cells and hiPSCs offers a model platform to study RP, since their cell fate can be restricted toward clinically relevant cells in RP, RPE cells, and photoreceptors [14–17], and therefore provide an unlimited cell source to interrogate the disease at the molecular, cellular, and functional level.

Two-Dimensional Cell Type Disease Models

Creating a disease model via hiPSC technology consists of several key steps and choices, involving a selection of the patients' somatic cell type, a reprograming strategy and a choice of differentiation protocol to create affected cells mimicking the physiological conditions of the tissue. While the reported RP models use dermal fibroblasts, the choice of the somatic cell type may be important due to epigenetic memory, which may influence the hiPSC differentiation capacity toward the same germ lineage as the origin [18]. Lymphocytes [19] or keratinocytes [16] were shown to be effectively converted to hiPSC and subsequently to photoreceptors and RPE cells, however, side by side comparative studies were not performed in the context of RP (Table 1). Cell identity in culture is defined as exposure to sequences of extrinsic cues at a precise time and concentration during culturing. Therefore, the identification of combinations of patterning molecules during retinogenesis is essential for the effective generation of clinically relevant cell types in vitro. Available RP models use single cell type approach, where RPE or photoreceptors, depending on the causative gene and initial disease-causing event, are generated from patients' hiPSCs. This is typically performed in two-dimensional (2D) cultures, with inductive developmental signaling molecules added to the medium. This is then combined with inductive matrices in order to generate cells expressing mature eye markers, such as retinal pigment epithelium-specific protein 65kDa (RPE65), cellular retinaldehyde-binding protein, Mer tyrosine kinase receptor (MERTK) in RPE cells or OPSIN/RHODOPSIN, RECOVERIN in photoreceptors. The pioneer in the field was Takahashi group [17, 20], who derived hiPSC lines from RP patients carrying a mutation in *RP1*, *RP9*, *PERIPHERIN* 2 gene, and rhodopsin (*RHO)* genes. These genes mainly affect photosensitivity and OS morphogenesis of rod photoreceptors, and therefore the hiPSCs were induced to differentiate toward photoreceptors. While patient-derived photoreceptors expressed typical markers (recoverin, *RHO*, opsin), they show a decrease in rod cell viability as well as an increase of endoplasmic reticulum (ER) stress markers, strongly similar to that found in patients. These findings were corroborated in a different *RHO*-caused RP model from the patient harboring E181K mutation [21]. Furthermore, the authors successfully reverted the phenotype using a helper-dependent adenoviral vector gene transfer, to correct the mutation. Therapeutic molecules such as rapamycin, PP242, AICAR, NQDI-1, and salubrinal were shown to promote the survival of the patient's hiPSC-derived photoreceptors, leading to a decrease of ER stress markers and apoptosis [21]. This study underscores the hiPSC-based platform as a disease model and drug screening tool, however a more robust model capturing photoreceptor organization, physiology and advanced morphology is necessary in order to fully recreate the disease events.

In another study, Tucker et al. [32], identified a retinal-specific isoform of *male germ cell associated kinase,* as the protein responsible for cilia length, in photoreceptors obtained from patient́s hiPSC.

The above-mentioned studies used patterning molecules that mediate retinogenesis, such as Dickkopf WNT signaling pathway inhibitor 1 (Dkk-1), left-right determination factor A (Lefty A), or noggin or secretase inhibitor N-[N-(3, 5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), to induce commitment toward photoreceptors.

In some forms of RP, the primary pathology occurs in RPE cells located between the neural retina and blood supply of choriocapillaris, which provide the essential support and visual function required for retinal homeostasis, through a variety of

Table 1. Actual models for RP using human induced pluripotent stem cell derived 2D or 3D models and main findings

Article	Affected gene	Cell source for reprograming	Reprograming technique	Generated affected cells	Main findings	Rescue
Jin et al. [19]	RP1, RP9, PRPH2, RHO	Fibroblasts	Retroviral transduction	2D photereceptors	Decreased number of rod cells. High expression of markers for oxidative or ER stress.	No
Jin et al. [16]	RHO	Fibroblasts	Sendai virus	2D photoreceptors	Diffused distribution of RHO protein in cytoplasm and expressions of ER stress markers in rod cells.	No
Yoshida et al. [20]	RHO	Fibroblasts	Retroviral transduction	2D photoreceptors	Reduced survival rate in the photoreceptors and increased expression of ER stress and apoptotic markers	Helper-dependent adenoviral vector gene transfer
Tucker et al. [21]	MAK	Fibroblasts	Lentiviral transduction	2D photoreceptors	Loss of retina-specific MAK isoform	No
Tucker et al. [15]	USH2A	Keratinocytes	Sendai virus	3D	Protein misfolding and ER stress	No
Li et al. [22]	MFRP	Fibroblasts	Lentiviral transduction	RPE	Defective RPE cell morphology, pigmentation and cell junctions	AAV8 vector expressing human MFRP
Lukovic et al. [23]	MERTK	Fibroblasts	Sendai virus	RPE	Defective phagocytosos of POS	No
Schwarz et al. [24]	RP2	Fibroblasts	Integration-free episomal vectors	RPE	Defects in IFT20 localization, Golgi cohesion and Gβ1 trafficking	Over-expressing GFP-tagged RP2 and sing the TRIDs
Ramsden et al. [25]	MERTK	Fibroblasts	Integration-free episomal vectors	RPE	Defective phagocytosos of POS	TRIDs
Polinati et al. [26]	HADHA	Fibroblasts	Retroviral transduction	RPE	Increased triglyceride accumulation. Irregularity in shape. Tight junctions disorganized. Pigmentation decreased.	No
Singh et al. [27]	BESTROPHIN1	Fibroblasts	Lentiviral transduction	RPE	Disrupted fluid flux and increased accrual of autofluorescent material after long-term POS feeding. Delayed RHODOPSIN degradation after POS feeding.	No
Phillips et al. [18]	VSX2	T-cells	Integration-free episomal vectors	3D optical vesicles	Growth deficit, increased production of retinal pigmented epithelium. Failure in production of bipolar cells. Delayed photoreceptor maturation.	VSX2 overexpression by lentivirus
Parfitt et al. [28]	CEP290	Fibroblasts	Integration-free episomal vectors	3D optic cups and RPE	Aberrant splicing and cilia defects.	Antisense morpholino.
Schwarz et al. [29]	RP2	Fibroblasts	Integration-free episomal vectors	3D optic cups	Reduced levels of Kif7 at cilia tips.	TRIDs
Megaw et al. [30]	RPGR	Fibroblasts	Lentiviral transduction	3D optic cups	Disruption of RPGR-gelsolin interaction.	No
Arno et al. [31]	REEP6	Fibroblasts	Episomal plasmid vectors	3D optic cups	Expression of a retina-specific isoform REEP6.1	No

Abbreviations: ER, endoplasmic reticulum; GFP, green flurescence protein; MAK, male germ cell associated kinase; MERTK, Mer tyrosine kinase receptor; MFRP, membrane frizzled-related protein; POS, photoreceptor outer segment; RP, retinitis pigmentosa; RPGR, retinitis pigmentosa GTPase regulator; TRIDs, translational read-through inducing drugs.

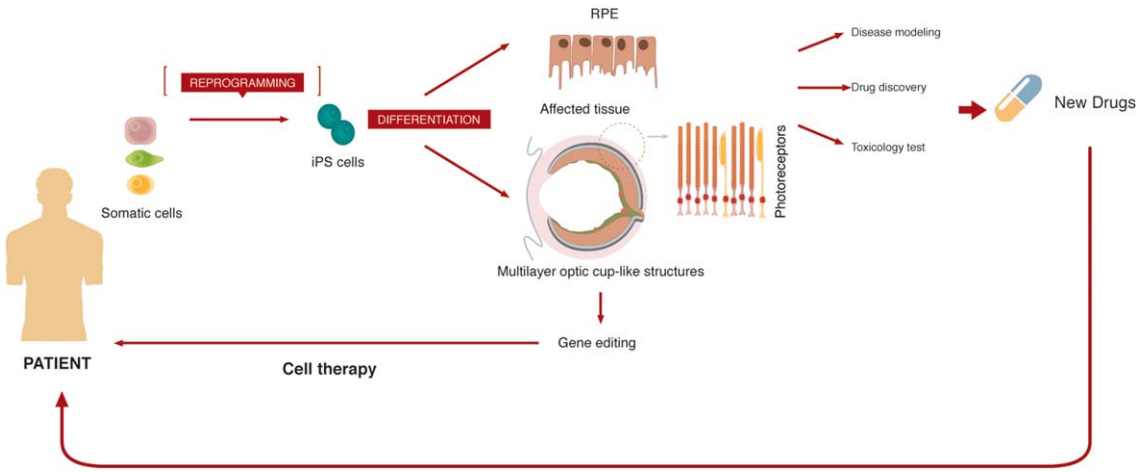

Figure 1. Schematic overview of current human induced pluripotent stem cells (hiPSCs) disease modeling of retinitis pigmentosa. Somatic cells from patients are reprogramed toward hiPSC. Disease affected cells such as RPE cells and optic cups derived from hiPSC, serve as cell sources for further mechanistic studies of disease and drug screening for future developments of new therapies in patients. Abbreviation: RPE, retinal pigment epithelial.

functions. These include visual pigment recycling, daily phagocytosis of photoreceptor outer segments, polarized secretion of growth factors, vectorial fluid flow, and formation of tight junctions that enable the functioning of the retina [23]. Mutations in visual cycle proteins: RPE-specific retinaldehyde-binding protein-1 (RALB), RPE65, lecithin retinol acyltransferase (phosphatidylcholine-retinol O-acyltransferase, LRAT) and photoreceptor phagocytosis such as MERTK do not lead to degeneration of RPE cells themselves, but instead abrogate RPE cell functions related to photoreceptor OS turn over. This function is fully captured by introducing xeno-derived purified photoreceptor outer segments [33] or rodent derived neural retina, as for example in [22]. In contrast, mutations in membrane frizzled-related protein (MFRP), a type II transmembrane protein, induce defective RPE cell morphology, pigmentation, and cell junctions, and the disease can be recapitulated in the hiPSC-RPE cell monolayer [34].

RPE cells are more readily obtained in vitro from pluripotent cells compared to photoreceptors. This is partly due to the default differentiation of pluripotent cells toward ectoderm upon fibroblast growth factor (bFGF) withdrawal, which induces the formation of pigmented patches that can be enriched by manual excision and enzymatic expansion [15, 33, 35]. Others use exogenously added growth and transcription factors involved in retinogenesis such as transforming growth factor beta (TGF-β) family protein Activin A, nicotinamide [36], in order to increase the target cell population purity.

RPE dysfunction was modeled in several RP patient-specific cells. Dysfunctional RPE in MERTK-associated RP was successfully modeled using hiPSCs [33]. MERTK, known to mediate OS phagocytosis, is disrupted in royal college of surgeons (RCS) rats [37, 38], a classic model for retinal degeneration inherited as an autosomal recessive trait, and found to cause early-onset RP in patients [4, 33]. As expected, the generated RPE cells from the affected patient failed to phagocytize the shed OS material; a circadian activity performed by RPE cells in contrast to healthy control's cells, highlighting the capacity of hiPSCs to mimic the disease.

A similar approach was used by Schwarz et al. [24], in which the authors generated hiPSC-derived RPE from an RP patient carrying the most common mutation in RP2 protein (R120X). This ubiquitously expressed protein exhibits only retinal phenotype when mutated. RP2 is thought to be involved in cilia trafficking as GTPase activating protein for Arl3 [39]. The levels of RP2 were partially recovered by using translational read-through inducing drugs (TRIDs), that suppress premature termination codons by insertion of a near-cognate transfer RNA (tRNA) codon at the stop codon [40]. The authors were able to reestablish about 20% of full-length protein expression and partially restore the normal phenotype in hiPSC-derived RPE cells [41]. The same approach was used to restore the expression of MERTK, followed by reestablishment of phagocytosis after treatment with TRIDs [25]. Further investigation needs to be done in order to elucidate whether a similar approach is effective in vivo, and determine whether it is dependent on mutation sequence.

Mutations in MFRP, the RPE-specific membrane receptor of unknown function, cause autosomal recessive RP. Disease model described by Li et al. [34] revealed that increased β-actin is involved in the disease. Patient's hiPSC-RPE cells had an abnormal shape, with lower levels and mislocalized pigment distribution, loss of clear cellular boundaries and cell-to-cell contact. By introducing the healthy copy of *MFRP* with adeno-associated virus vector, actin organization was restored, together with normal pigmentation and transepithelial resistance. In parallel, gene therapy in $Mrfp^{rd6}/Mrfp^{rd6}$ mice using AAV, showed restoration of vision. This study confirms that hiPSC-based disease models are faithful and versatile gene therapy platforms.

RPE degeneration was also modeled in non-RP diseases such as long–chain 3-hydroxyacyl-CoA dehydrogenase caused retinopathy, and early pathogenic changes were revealed [26]. Best disease, caused by mutations in RPE-specific protein BESTROPHIN1 (BEST1), was reported by Singh et al. [27]. Age related macular degeneration (AMD), a multigenic disease, was modeled in vitro by accelerating RPE cell senescence through accumulating A2E, lipofuscin fluorophore, which accumulates with age, and blue light [42]. This study reveals the decreased antioxidant capacity in AMD-associated (ARMS/HTRA1) risk haplotypes.

The obvious advantage of the 2D approach is that the molecular events occurring along the disease evolution in target

cells can be delineated by studying a homogeneous cell population, as is the case of RPE cell, which can be manually selected, excised, and selectively enriched. In the case of retinal cells, the caveat is that generated monolayer cell culture normally includes a mixture of the different cell types [14, 16, 43], not organized in proper nuclear layers and therefore unable to form retinal microcircuitry necessary for signal propagation.

Three-Dimensional Culture Disease Models

The self-organizing capacity of pluripotent cells into organoids was first described by Sasai and colleagues, who showed that mice and human ES cells could self-organize in vitro into stratified structures of the developing eye [44]. These structures resemble their normal in vivo counterparts and provide unprecedented experimental systems for studying developing human tissue. The approach was recapitulated by several groups, and slightly different experimental strategies [19, 45, 46] in some cases reaching the stage of the generation of photoreceptor outer segments by Zhong et al. [46–48]. It is particularly interesting that unpatterned homogenous aggregates of hiPSCs spontaneously differentiate into highly ordered 3D retinal organoids that spatially and temporally capture retinogenesis with properly layered retinal cells: horizontal, amacrine, bipolar, Müller cells, ganglion cells, and photoreceptors. These organoids present not only a powerful tool to study retinal development and disease evolution, but potentially a source for organ and cell replacement. Retinal organoids can be generated from hiPSCs in xeno-free and feeder-free conditions, and can be easily cryopreserved, retaining their phenotypic characteristics [49]. The challenge with 3D approaches is to monitor a particular cell population within the organoid. This can be resolved by introducing fluorescent protein transgenes under cell-type specific promoters by viral vectors randomly in the genome or by gene editing approach. In this regard, postmitotic photoreceptor precursor (*CRX*$^{+}$) reporter was inserted in Adeno associate integration site 1 (AAVS1) "safe harbor," widely used transcriptionally active locus, in human embryonic stem cells (hESC) line [50] which allowed transcriptional profiling of the photoreceptors along the differentiation process. Similarly, *venus* cDNA was knockedin into the first exon of *RAX* and *CRX* in order to track early and postmitotic photoreceptors, respectively, in the optic cup [44]. This approach has yet to be explored in modeling RP, and can be exploited to create 3D retinal cultures from patients' hiPSCs and allow cellular monitoring under dynamic conditions.

The early work of Phillips et al. [19] modeled Visual System Homeobox 2 (*VXS2*) mutation-caused microphthalmia, by comparing the optic vesicles from a healthy and microphthalmia diagnosed patient, and provides a paradigm for elucidating transcription factors and signaling pathways triggered by this early development active gene.

As a step toward clinical application, hiPSCs from large RP patient's cohort were created using Current Good Manufacturing Practice (CGMP) [51], by establishing common criteria for cellular identity and sterility. All hiPSC lines successfully differentiated toward the 3D retina and were not tumorigenic upon injection into immune compromised severe combined immunodeficiency (SCID) mice.

Leber congenital amaurosis model caused by defective CEP290, a protein involved in ciliogenesis was modeled by Parfitt et al. [28]. By blocking the aberrant splicing by an antisense morpholino, the expression of full-length CEP290 and normal cilia-based protein trafficking was established, revealing potential therapeutic strategies for this disease. Cheetham and colleagues [29], showed that KiF7 protein is significantly reduced in photoreceptors from 3D optic cups with mutant RP2 compared to healthy cells. Normal expression of this protein is rescued by TRIDs, clearly providing proof-of-concept for therapy of premature stop mutation caused disease.

X-linked RP (XLRP), caused by a mutation in the RP GTPase regulator (RPGR) gene was modeled using iPSC-derived retinal organoids [30]. Confirming the findings in mice, RPGR, involved in rhodopsin trafficking, interacts and activates the actin-severing protein Gelsolin, directly involved in cilia formation. Increased actin polymerization, as well as significant photoreceptor loss was observed in the patient's optic cups, revealing disruption of cell signaling pathways in mutant photoreceptors directly related to Gelsolin malfunction.

Chen and colleagues [31], used 3D retinal organoids to track the expression levels along retinal differentiation and confinement to rod inner segment and cell body compartments of the Receptor Expression Enhancer Protein 6, involved in the autosomal-recessive type of RP.

The Faithfulness of hiPSC Based Disease Models

The likeliness of faithfully recapitulating the disease in vitro depends on the robustness and reproducibility of cell fate manipulation and the clinical phenotype severity. In addition, earlier disease onset is predictively more successfully modeled in vitro than late onset phenotypes, as these require accumulation of other genetic, environmental, or aging factors to trigger degeneration. It is widely known that reprograming erases age-related molecular markers such as loss of nuclear LAP2α, heterochromatin markers tri-methylated H3K9 (H3K9me3) and heterochromatin protein 1 gamma (H3K9me3, HP1γ), as well as reactive oxygen species (ROS) accumulation [52, 53], and these need to be induced de novo in cell culture. Possibly, direct differentiation between somatic cells as shown recently [54, 55] could preserve age related markers, but full photoreceptor cellular identity has yet to be achieved from somatic sources.

Conclusion and Challenges

The impact of vision loss is devastating for patients, their families and society as a whole. Despite the great efforts used in unveiling the genetic complexity of RP, and biochemical pathways involved in photoreceptor functioning, there are currently no therapeutic interventions that may halt the evolution of the disease or restore the lost visual function. The patient-specific hiPSCs represent a new paradigm, enabling clinically relevant disease models to elucidate the role of human mutations associated with RP, therapeutic screening, and replacement therapies. The need for a patient-specific genetic background is further supported by the high phenotypic variability of a single mutation, even within the same family affected by RP.

Combined with gene editing methods, patients' hiPSCs thus enable autologous therapeutic approaches, or allow

specific cell population tracking in 3D models (Figure 1). Both technologies are evolving rapidly toward more defined and precise approaches, such as non-integrative approaches, in introducing reprograming factors or mutation targeting, by avoiding off-target cleavage by synthetic nucleases [56].

In the case of hereditary dystrophies, it is necessary to correct the causative mutation prior to the transplantation of autologous cells (Figure 1). The directed genome manipulation ensures the appropriate temporal and tissue-specific expression of the affected gene under the regulation of endogenous elements, and has traditionally relied on homologous recombination (HR). The discovery that double strand break increases the efficiency of HR by several orders of magnitude has fostered the design of site-specific nucleases as a strategy of choice for in situ gene editing. Precise mutation correction using technology adapted from the bacterial clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 system [57] revolutionized modern medicine. The advantage of CRISPR is that its specificity depends largely on a guide RNA that can be designed to target different genomic loci. RP pathogenic mutation correction by CRISPR was outlined recently by Bassuk et al. [58]. The authors generated hiPSCs from a patient with a point mutation in the *RPGR* gene, which causes an aggressive, X-linked variant of RP (XLRP). They showed that CRISPR technology was able to precisely edit the pathogenic mutation and produce gene-corrected hiPSCs for eventual application in autologous transplantation for RP.

The challenges of retinal organoids are manifold. The photoreceptors do not exhibit fully mature OS. Possibly this is due to the absence of direct contact with RPE cells, since the studies in vivo revealed that aberrant RPE is associated with lack of OS morphogenesis [59]. The production process is several months long and largely dependent on manual manipulation and subjective selection criteria at initial stages. Additionally, there is functional and phenotypic variability among different hiPSC lines [60] and between hiPSC and hESC [61]. As a consequence, the variability in differentiation efficiencies, maturity at particular stage measured by expression of specific cell markers and structural features are high. Moreover, the homogeneity of specific cell marker distribution within a single 3D retina remains to be demonstrated. Commonly only a portion of the histological section of the entire laminated retina is represented with the assumption that the later is representative of the entire 3D retina. Therefore, in order to have major translational impact, this model will require standardized, robust production protocols and large-scale analyses. The lack of quantitative methods for analyzing 3D organoids is an emerging issue. Recently published study describes a fluorescence quantification-based approach using microplate reader [62]. Other imaging technologies include live imaging of 3D retinas [63], although it remains to be explored if this approach is feasible in high throughput format with sufficient resolution and low phototoxicity.

Possibly, the greatest challenge in disease modeling is the aging effect, as reprogrammed cells rejuvenate independently of the donor age. Age related markers such as telomerase length, ROS, nuclear organization proteins, and heterochromatin markers as well as mitochondrial metabolism, have been shown to be lost in aged fibroblast upon reversion to a pluripotent state [64]. Despite recent advances [54], direct conversion of aged somatic cells into photoreceptors, which would preserve the aged related markers, has not yet been achieved. Efforts to evoke an age-related effect have been described in neurodegenerative diseases such as Parkinson [64, 65] by overexpression of progerin, a protein causing premature aging, in hiPSC-derived fibroblasts and neurons, accumulating lipofuscin fluorophore in RPE cells [42] or prolonged cell cultures as was described in Sánchez-Danés et al. [65].

Undoubtedly, the possibility to differentiate hiPSC toward the patient's affected cells, especially 3D optic cup-like structures, has revolutionized the modern ophthalmology offering a unique in vitro model of the human eye. Further investigations are required to determine whether we need more sophisticated organoid structures, including direct contact between RPE and photoreceptors, as well as interactions between heterogeneous cell populations inside and outside the retina.

Further challenges in the creation of human eye models should include vascularization; to bring nutrients to the cells to create more humanized retinal models in which to investigate the molecular mechanisms that underlie retinal diseases, and develop new treatment options.

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Author Contributions

AAC; manuscript writing, data collection; DL, manuscript writing, data collection, final approval of manuscript. PJ; final approval of manuscript. S.E.: conception and design, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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