Chapter 7
Stem Cell Strategies for Diseases of the Outer Retina

Alex W. Hewitt and Kathryn C. Davidson

Contents

A.W. Hewitt
Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital,
University of Melbourne, East Melbourne, VIC, Australia

Centre for Ophthalmology and Vision Science,
University of Western Australia and the Lions Eye Institute,
Perth, WA, Australia

K.C. Davidson (✉)
Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital,
University of Melbourne, East Melbourne, VIC, Australia
e-mail: kdavidson@unimelb.edu.au

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Abbreviations

ABCA4	ATP-binding cassette, subfamily A, member 4
AMD	Age-related macular degeneration
BEST1	Bestrophin
CHM	Choroideremia
CRD	Cone-rod dystrophy
CRX	Cone-rod homeobox
EFEMP1	Epidermal growth factor-containing fibulin-like extracellular matrix protein 1
ERG	Electroretinography
hESC	Human embryonic stem cells
iPSC	Induced pluripotent stem cells
LCA	Leber congenital amaurosis
MAK	Male germ-associated kinase
OAT	Ornithine aminotransferase
PR	Photoreceptor
PRPH2	Peripherin 2
PSC	Pluripotent stem cell
RCS	Royal College of Surgeon
REP1	Rab escort protein-1
RHO	Rhodopsin
RP	Retinitis pigmentosa
RP1	Retinitis pigmentosa 1
RP9	Retinitis pigmentosa 9
RPE	Retinal pigmented epithelium
RPE65	Retinal pigment epithelium-specific protein 65 kDa
RPGR	Retinitis pigmentosa GTPase regulator
TIMP3	Tissue inhibitor of metalloproteinases-3
USH2A	Usher syndrome 2A
VEGF	Vascular endothelial growth factor

7.1 Introduction

The retina is the light-sensing tissue that lines the inner surface at the posterior part of the eye. Light is perceived by chemical and electrical signals initiated in the retina that stimulate retinal ganglion cells to transmit signals to the visual centres of the brain via the optic nerve. Within the retina, phototransduction is initiated in photoreceptors (PRs), specialised neurons that convert light into electrical signals that are transmitted and ultimately processed by the visual centres within the brain. The health and function of PRs are critically dependent on neighbouring retinal pigmented epithelium (RPE) cells, which separate PRs from the blood supply in the choroid. RPE cells are attached to Bruch's membrane, which acts as a semi-permeable barrier between the

RPE and vasculature of the choroid. The choroid provides the blood supply to the outer retina. RPE cells perform a number of important functions that are essential to the overall homeostasis of the retina which include retinol cycling, nutrient transport, growth factor production, and phagocytosis of PR outer segments [1].

Dysfunction of PRs or RPE can lead to vision loss and often causes irreversible degeneration of other retinal supporting or downstream cells. Retinal degenerative diseases affect millions of people worldwide and have an immense impact on quality of life. Unfortunately the majority of these conditions are currently untreatable. However, through the use of pluripotent stem cells (PSCs), new strategies for studying these diseases offer profound hope of ultimately identifying novel treatments.

Stem cells are unique in that they are capable of both self-renewal and subsequent differentiation into any number of specialised cell types. Stem cells are frequently defined according to their origin and the range or extent to which they can differentiate. PSCs can differentiate into any somatic cell type of the body, whereas multipotent stem cells are somewhat more restricted in the types of cells they can become. PSCs can be derived from various sources and include human embryonic stem cells (hESCs) [2, 3] and induced pluripotent stem cells (iPSCs) [4–6]. A detailed discussion of hESCs and iPSCs can be found in Chap. 5. Together, hESCs and iPSCs (collectively PSCs) provide a novel set of tools for the study and treatment of many diseases through their application in developing cellular models and therapies. Indeed, retinal diseases are currently targeted for clinical trials using PSC-based therapies, demonstrating the exciting possibility that these strategies may in fact translate into clinical outcomes in the near future.

To understand stem cell-based approaches for treating retinal diseases, we begin with a brief summary of the pertinent clinical features of diseases that affect the outer retina (i.e. from the outer plexiform layer to the RPE). A particular focus has been made on diseases where PSCs have been used for either disease modelling or cell therapy and on diseases that are strong candidates for these stem cell strategies given the current state of the field. We then outline the potential of PSC-related therapies for outer retinal diseases.

7.2 Outer Retina Diseases

7.2.1 Age-Related Macular Degeneration

Age-related macular degeneration (AMD) (OMIM #603075, reviewed in [7]) is a multifactorial disorder with both genetic and environmental risk factors and involves progressive degeneration of PRs and underlying RPE cells in the macula, the part of the eye responsible for central vision. The clinical hallmarks of AMD include the accumulation of extracellular deposits, termed drusen, beneath the RPE on Bruch's membrane and pigment abnormalities from dysfunctional RPE cells. Advanced

stages are characterised by central visual loss due to geographic atrophy of the RPE ('dry' AMD) and/or choroidal neovascularisation ('wet' AMD). AMD is the leading cause of blindness in the Western world and the most common cause of acquired visual impairment in the elderly, affecting over seven million people in the US and approximately 1 in 7 people over the age of 50 in Australia [8, 9]. The vast majority of patients have the atrophic, or 'dry', form of the disease, for which there is currently no treatment. A subset of people with atrophic AMD go on to develop exudative, or 'wet', AMD, which is currently managed if diagnosed early by serial injections of anti-angiogenic drugs that block vascular endothelial growth factor (VEGF)-induced neovascularisation [10]. This treatment often halts or slows vision loss and many patients experience restoration in vision with timely intervention.

7.2.2 Stargardt Disease

Stargardt disease (OMIM #248200, reviewed in [11]) is an autosomal recessive, juvenile-onset macular dystrophy caused by mutations in the *ATP-binding cassette, subfamily A, member 4* (*ABCA4*) gene. Clinically it is characterised by loss of visual acuity, though peripheral visual fields remain normal, and rapid progressive degeneration of the macula region of the retina. Histologically, it is characterised by subretinal deposition of lipofuscin-like material in RPE cells and PR segments. Later stages of the disease involve abnormal slowing of the rod and cone retinoid cycle and death of RPE and PRs. There are no treatments available for Stargardt disease.

7.2.3 Best Disease

Best Disease (OMIM #153700, reviewed in [12]) is an autosomal dominant, early onset macular dystrophy frequently caused by mutations in the Bestrophin (*BEST1*) gene [13]. Clinically it is characterised by the bilateral presence of bright yellow lesion containing lipofuscin-like material in the subretinal space that resemble a sunny-side-up egg, termed 'vitelliform', upon examination. In many individuals these lesions eventually rupture, giving a 'scrambled egg' appearance and leading to deposits and fluid in the affected area of the macula, pigment abnormalities, atrophy of the underlying RPE, and progressive reduction in central vision. Unfortunately there are currently no treatments for this retinal dystrophy.

7.2.4 Doyne Honeycomb Retinal Dystrophy

Doyne Honeycomb Retinal Dystrophy (OMIM #126600, reviewed in [14]) is an inherited disorder predominantly caused by mutations in the epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (*EFEMP1*) gene.

Clinically, it resembles AMD, with sub-RPE drusen developing in early adult life and a progressive irreversible loss of central vision. Build up of large drusen, which generally forms a honeycomb-like pattern within the macula, causes progression of the disease. Unfortunately, there are no means by which to definitively treat this uncommon retinal dystrophy.

7.2.5 Retinitis Pigmentosa

Retinitis pigmentosa (RP) (OMIM #268000, reviewed in [15]) is a heterogeneous group of ocular diseases which are clinically characterised by progressive loss of central or peripheral vision and night blindness, secondary to degeneration of the RPE and PRs. Most cases of RP are monogenic. To date more than 50 genes have been identified to cause RP, including rhodopsin (*RHO*), Usher syndrome 2A (*USH2A*), and retinitis pigmentosa GTPase regulator (*RPGR*), which collectively account for approximately 30 % of all cases [16, 17]. To date there is no means by which to definitively treat this blinding condition.

7.2.6 Sorsby Dystrophy

Sorsby Dystrophy (OMIM #136900, reviewed in [18]) is a fully penetrant, autosomal dominant disorder caused by missense mutations in the tissue inhibitor of metalloproteinases-3 (*TIMP3*) gene. Clinically it is characterised by bilateral loss of central vision due to subretinal neovascularisation and RPE atrophy at the macula. Similar to other retinal dystrophies currently there are no means by which to definitively treat this disease.

7.2.7 Cone-Rod dystrophy

Cone-rod dystrophy (CRD) (OMIM #120970, reviewed in [19]) is a progressive retinal degenerative disease which can be inherited in an autosomal dominant, recessive or X-linked pattern. It can be caused by mutations in a number of different genes, including cone-rod homeobox (*CRX*), *ABCA4*, and others. Clinically, it manifests by progressive vision impairment typically beginning with loss of colour vision, reduced visual acuity and sensitivity to light, followed by night blindness and loss of peripheral visual fields. Histologically CRD is characterised by degeneration, and eventually a complete loss, of outer nuclear layer PRs (generally either cones proceeding rods or vice versa). Upon examination, pigment abnormalities and atrophy of the RPE may also be observed in addition to abnormal cone function on electroretinography (ERG), a test that measures the electrical response of cells in the retina. Currently there is no treatment for CRD; however, tinted lenses and low vision aids may help with managing symptoms.

7.2.8 Leber Congenital Amaurosis

Leber congenital amaurosis (LCA) (OMIM #204000, reviewed in [20]) comprises a group of autosomal recessive early onset childhood retinal dystrophies caused by mutations in a number of different genes. Clinically, it is characterised by vision loss, nystagmus, and severe retinal dysfunction often manifesting in the early postnatal period. Progressive degeneration in the cellular structure of the retina causes ERG responses to be severely attenuated or non-recordable and may also lead to structural changes in the cornea that cause it to thin and adopt a conical shape, further distorting vision [20]. Most forms of LCA involve severe degeneration and death of PRs and have no available treatments. A rare form of LCA caused by mutations in retinal pigment epithelium-specific protein 65 kDa (*RPE65*) (OMIM #204100) results in dysfunctional, but relatively preserved, retinal cells. Mutations in this gene cause a deficiency in retinoid isomerase, which leads to a biochemical blockage of the retinoid cycle and degeneration of PRs. Gene therapy trials aimed at restoring the visual cycle in surviving PRs via adeno-associated virus delivery of *RPE65* have shown partial reversal of the dysfunction, although the reconstituted retinoid cycle is not completely normal and PR degeneration still occurs [21, 22]. Importantly though, patients who received gene therapy have shown remarkable and lasting improvements in visual function despite ongoing loss of PRs [21, 22].

7.2.9 Gyrate Atrophy

Gyrate atrophy (OMIM #258870, reviewed in [23]) is an autosomal recessive disorder characterised by slowly progressive atrophy of the choroid, RPE, and retina. Mutations in the ornithine aminotransferase (*OAT*) gene are known to cause gyrate atrophy, and dietary restriction arginine has been shown to halt visual loss [24].

7.2.10 Choroideremia

Choroideremia (OMIM #303100, reviewed in [25]) is an X-linked disease caused by mutations in the choroideremia (*CHM*) gene, which encodes Rab escort protein-1 (REP1), that lead to degeneration of the choriocapillaris, RPE, and PRs. All known *CHM* mutations produce truncated protein products, resulting in a complete loss of functional REP1 protein. In affected males, it is characterised by nyctalopia, progressive loss of peripheral and central vision as a result of complete atrophy of the choroid and retina. Heterozygous females have no visual defect, but may exhibit pigment abnormalities and atrophy around the optic disc. Unfortunately there is no effective treatment for CHM.

7.3 Induced Pluripotent Stem Cells for Retinal Disease Modelling

The extreme difficulty in obtaining ocular tissue from living people currently represents a major barrier to studying the molecular mechanisms of blinding disease. The ability to generate iPSCs from patients with specific diseases provides an extremely powerful means to investigate the underlying pathogenesis. Generating iPSCs directly from patients with a particular disease allows cells to be differentiated into specific cell types for disease modelling, drug screening, and understanding fundamental mechanisms underlying cell biology.

Despite the relatively large of number diseases affecting the outer retina, to date there have only been a small number of studies describing the development and characterisation of patient-specific iPSCs (Table 7.1). This is compounded further by the relatively large degree of genetic heterogeneity amongst these diseases. Despite this, particular insight in the pathogenesis of retinitis pigmentosa 9 (*RP9*)-related RP has been made, whereby *RP9* mutations appear to cause disease, at least in part, through oxidative stress pathways [26]. Conversely, *RHO* and *USH2A* mutations are associated with an increase in endoplasmic reticulum stress [26, 27].

7.4 Pluripotent Stem Cells for Retinal Cell Replacement

Although a number of genetic mutations and variants have been identified that cause or confer risk for diseases of the outer retina, in many cases the disease mechanisms remain poorly understood. Few treatment options exist to preserve or restore vision for a majority of these diseases, and available treatments may only treat symptoms rather than the underlying disease cause. However, the cell types whose degeneration and/or dysfunction lead to vision loss in most cases are known: predominantly RPE, PRs, or a combination thereof. One potential option for treatment involves replacing the degenerative or dysfunctional cells within the outer retina with new healthy cells to restore function and, hopefully, improve vision. Transplanted cells may also protect endogenous retinal cells from further degeneration, minimising future vision loss. This approach, termed cell replacement therapy, is an attractive strategy for many retinal diseases because the population of cells that are defective or have degenerated are generally well characterised and, surgically, the eye is easily accessible. Moreover, as an immune-privileged site, the eye should have a low risk of rejecting transplanted material [28], though results from early clinical trials with allogenic foetal RPE transplants indicate that immunosuppression may still be required if the blood–retinal barrier is compromised due to disease [29–31].

For cell replacement therapy to be feasible, one needs a readily available cellular source from which to generate sufficient numbers of healthy retinal cells for transplantation. Transplant of foetal tissue has shown some promise in a clinical setting [32, 33], but this material is difficult to obtain. As described previously, PSCs can

Table 7.1 Currently described induced pluripotent stem cell lines generated for modelling diseases of the outer retina

References	Disease	Gene	Number of patients included	Disease causing variants studied	Reprogramming factors used	Differentiated cells of interest	Phenotype observed
[82]	Best Disease	*BEST1*	2	(1) A146K (2) N296H	KOSM	RPE	*BEST1* mutant RPE cells show disrupted fluid flux and increased accrual of PR outer segments compare to cells derived from unaffected siblings
[83]	Choroideremia	*CHM*	2	(1) R555stop (2) L550P	KOSM + miRNA 302/367	iPSCs	Reduced REP1-mediated enzymatic activity
[84]	Gyrate Atrophy	*OAT*	1	A226V[a]	KOSM + NANOG, LIN28, SV40 large T-antigen	iPSCs	Assessment of mutational load acquired during gene correction
[45]	Gyrate Atrophy	*OAT*	1	A226V[a]	KOSM + NANOG + LIN28	RPE	Reduced OAT enzymatic activity
[85]	LCA	–	2	–	KOSM	RPE; NSC	Expression differences identified
[35]	LCA	–	1	–	KOSM	RPE	–
[35]	RP	–	2	–	KOSM	RPE	–
[86]	RP	*MAK*	1	Alu repeat insertion in exon 9	KOSM	Retinal progenitor cells	Novel exon transcripts were identified from differentiated retinal progenitor cells
[87]	RP	*RHO*	1	G188R	KOSM	RPE, retinal progenitor cells	RHO is diffusely distributed with expression of endoplasmic reticulum stress markers
[27]	RP	*USH2A*	1	R4192H and pseudoexon IVS40	KOSM	Retinal progenitor cells	Mutations appear to cause disease through protein misfolding and endoplasmic reticulum stress
[26]	RP	*RP1*	1	721Lfs722X	KOSM	Photoreceptors	–
[26]	RP	*PRPH2*	1	W316G	KOSM	Photoreceptors	–
[26]	RP	*RHO*	1	G188R	KOSM	Photoreceptors	RHO mutation is associated with endoplasmic reticulum stress
[26]	RP	*RP9*	2	H137L	KOSM	Photoreceptors	RP9-retinitis pigmentosa is involved, at least in part, in oxidative stress pathways

KOSM KLF4, OCT4, SOX2, and c-MYC, *RPE* retinal pigmented epithelial cells, *PR* photoreceptors, *miRNA* micro-RNA, *NSC* neural stem cell
[a]Same patient sample

be expanded indefinitely in vitro and can also potentially be differentiated into any cell type in the body, including retinal cells; thus, they provide an unlimited and renewable source of cells for transplant. Furthermore, methods to differentiate PSCs to functional RPE [34–39] and PRs [40–46] are well established.

7.4.1 Moving Towards Stem Cell-Based RPE Cell Therapy

The aim of PSC-based cellular therapy is to ultimately replace degenerative retinal cells with new healthy cells that survive, integrate, and remain functionally active long term. As proof of principle, it has been shown that RPE cells can survive post-transplantation and improve visual function in rodent models of retinal degeneration [47–50]. Similarly, human PSC-derived RPE can functionally integrate and improve visual function in rodent models of retinal degenerative diseases [51, 52]. In a mouse model of RP (*Rpe65*$^{rdl2/rdl2}$), human iPSC-derived RPE cells survived long term and improved retinal function over the lifetime of the mice [52]. In the dystrophic Royal College of Surgeon (RCS) rat in which a primary defect in RPE phagocytosis leads to PR degeneration, one study found that iPSC-derived RPE did not survive beyond 13 weeks; however, long-term visual function was maintained, suggesting the effect may be due to a secondary host response [51]. In another study, hESC-derived RPE survived long term (>100 days) following subretinal injection into RCS rats and led to reduced PR degeneration and preserved visual function [53]. Whether visual improvement observed with transplanted PSC-derived RPE is due to bona fide functional cell replacement or indirect paracrine effects remains to be determined. Nonetheless, PSC-based RPE cell therapy appears very feasible.

Towards this goal, phase I/IIa clinical trials of cell replacement therapy for AMD and Stargardt disease are currently underway using allogenic hESC-derived RPE cell transplants [54] (NCT01345006, NCT01344993, NCT 01469832, Advanced Cell Technology; and NCT01674829, CHA Bio and Diostech). IPSC technology has the added advantage of allowing for generation of patient-matched cells for autologous transplant to mitigate the need for immunosuppression. Recently, the first iPSC-derived RPE clinical trials were approved for AMD in Japan (RIKEN). It is important to note that for diseases caused by specific Mendelian mutations, gene correction may be required in iPSCs from the affected patient prior to transplant.

7.4.2 Feasibility of Photoreceptor Cell Therapy

Cell replacement therapy for PRs has not yet advanced into clinical trials; however, promising results from animal studies suggest this may be feasible in the near future. Proof of principle experiments demonstrate that rod precursor cells isolated from postnatal mice can survive transplant, integrate and differentiate into mature PRs, and improve visual function in mouse models of PR dysfunction (*Gnat*$^{-/-}$) [55] and rod degeneration (*Rd1* [56] and *Rho*$^{-/-}$ [57]). HESC-derived retinal progenitor

cells also can survive transplant, differentiate to functional PRs, and improve visual responses in a mouse model of LCA (*Crx*$^{-/-}$ mice) [58]. Similarly, iPSC-derived retinal progenitor cells integrate and differentiate into PRs in vivo [43].

One complicating factor for potential PR replacement therapy in humans is that in many retinal diseases involving PR degeneration, the RPE is often implicated as well. Thus, it is likely that PR cell transplantation may need to be conducted in combination with RPE cells in a dual replacement strategy. Towards this goal, efforts to construct a two-layered patch graft of RPE and PRs are underway that utilise a thin plastic film to anchor a monolayer of PSC-derived RPE cells [59] with a second layer of PR precursor cells adhered via a biodegradable gel [60]. This research is still in the early stages of development. Other efforts to generate striated tissue constructs containing RPE and PRs from PSCs in vitro have been reported via self-assembled optic cup [61] and optic vesicle-like structures [45, 62] and retinal progenitor sheets [63].

7.4.3 Bioengineered Substrates for Cell Transplants

Native RPE exist as a polarised monolayer, and this cellular architecture is critical to their function. Previous studies in animals have demonstrated that sheets of retinal cells survive better following transplantation than dissociated cells [64]. Furthermore, RPE may fail to survive or function on damaged Bruch's membrane, which is a common feature of ageing and some retinal diseases such as AMD [65, 66]. Given these concerns, artificial substrates on which to seed RPE cells are being developed to facilitate transplant of intact, polarised sheets of cells. These include polyester membranes [67], ultrathin parylene films [59], plasma polymers [68], and polyimide membranes [69]. Current clinical trials deliver hESC-derived RPE cells as suspensions via subretinal injection, but a clinical trial application has been submitted to transplant hESC-derived RPE immobilised on a polyester membrane to address this potential issue (NCT01691261) [70].

7.4.4 Pluripotent Stem Cells Recapitulate Retinal Ontogeny

One further advantage of using PSC-derived retinal cells for transplantation is the ability to generate cells at various ontogenetic stages of development. This is important because studies have shown that human foetal RPE and early postnatal mouse PRs function significantly better in vivo than the same respective cells isolated from older tissue [56, 57, 71]. HESC- and iPSC-derived PRs behave similar to early postnatal mouse PRs when transplanted into mice [43, 58]. Likewise, hESC-derived RPE resemble human foetal RPE in vitro and in vivo [36, 72–75], albeit with some differences in growth factor expression and attachment to Bruch's membrane [66]. In both cases however, published results demonstrate the feasibility of generating PSC-derived retinal cells that functionally resemble early developmental stages most useful for transplantation.

7.4.5 *Stem Cell Transplants for Trophic Support*

It is conceivable that transplanted cells could produce trophic factors that provide a neuroprotective effect in the retina without functional integration [76]. This strategy of transplanting cells to provide paracrine support has shown improved visual outcomes in animal models of retinal degeneration using mesenchymal stem cells [77] and umbilical tissue-derived stem cells [78]. Both of these types of stem cells are not pluripotent, meaning they are restricted in the range of cell types they can generate and may not be capable of becoming retinal cells. Mesenchymal stem cells can be obtained from various adult tissues and have an innate ability to home to a site of injury and mitigate endogenous tissue repair in part through modulation of the immune response (reviewed in [79]). However, their ability to differentiate into functional, mature retinal cells remains questionable [80, 81]. Thus, these non-PCSs may be ineffective for replacement therapy. However, transplanting cells to provide trophic support to the retina is a practical treatment strategy, and there are currently several clinical trials underway using cells isolated from bone marrow (NCT01531348) and umbilical tissue (NCT01226628) for RP and atrophic AMD, respectively.

7.4.6 *Receptivity of the Diseased Retina*

A final requirement for cell replacement therapy is that the diseased environment must allow for integration and function of transplanted cells in the retina. One particular concern for retinal diseases with complex or unknown genetic influences (such as some types of RP) or with strong environmental influences (such as AMD) is that degeneration may be an indirect effect of complex or yet unknown disease processes rather than an intrinsic defect in the retinal cells themselves. If this is the case, then it is conceivable that transplanted retinal cells may also succumb to the diseased environment and eventually die along with endogenous cells if the underlying cause of the disease is not addressed. Nevertheless, if cell therapy significantly delays this degenerative process then it will serve as a valuable treatment option.

7.5 Concluding Remarks

In summary, the development of stem cell strategies to treat retinal diseases offers exciting possibilities for the future. PSC-derived RPE cells have now progressed into clinical trials, while the ability to create in vitro human models using iPSCs has revolutionised the field by providing a platform to study disease pathogenesis and to screen therapeutic compounds. There are still many unanswered questions, including whether multigenic diseases or those with unknown genetic, strong environmental or epigenetic influences can be modelled effectively with iPSCs. It also remains to be

determined whether improvements from cell therapies in animal models will translate to human conditions and whether the diseased retina will facilitate long-term function of cell transplants. Regardless of these uncertainties, stem cell approaches provide hope for new insight and treatments for a large number of retinal diseases.

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