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Gene and Induced Pluripotent Stem Cell Therapy for Retinal Diseases

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Abstract

Given the importance of visual information to many daily activities, retinal degenerative diseases—which include both inherited conditions (such as retinitis pigmentosa) and acquired conditions (such as age-related macular degeneration)—can have a dramatic impact on human lives. The therapeutic options for these diseases remain limited. Since the discovery of the first causal gene for retinitis pigmentosa almost three decades ago, more than 250 genes have been identified, and gene therapies have been rapidly developed. Simultaneously, stem cell technologies such as induced pluripotent stem cell–based transplantation have advanced and have been applied to the treatment of retinal degenerative diseases. Here, we review recent progress in these expanding fields and discuss the potential for precision medicine in ophthalmic care.

INTRODUCTION

IRD: inherited retinal dystrophy

iPSC: induced pluripotent stem cell

RP: retinitis pigmentosa

AMD: age-related macular degeneration

Given that we rely on vision to collect most of the information about our environment, it is not an exaggeration to call vision the most important sense for human life, and its loss can be devastating. Light goes through the cornea, lens, and vitreous to reach the retina, the heart of vision, where light signals are converted to electrical signals. Retinal degenerative diseases, especially inherited retinal dystrophies (IRDs), have had no therapeutic options until recently, when the US Food and Drug Administration approved a gene supplementation therapy for patients with *RPE65* mutations, which cause Leber congenital amaurosis (LCA), a type of severe IRD in which retinal degeneration begins in infancy (6, 32, 36, 52). Since the introduction of induced pluripotent stem cells (iPSCs) in 2006 (85), stem cell therapies have become another important area for developing treatments, and an iPSC-based therapy for retinal degeneration in humans was reported by a Japanese group in 2017 (54). Several clinical trials are currently under way to treat retinal degenerative diseases using genetic and stem cell therapy approaches. Here, we summarize the current progress of gene and iPSC therapies for retinal degenerative diseases.

GENETIC SUSCEPTIBILITY TO RETINAL DEGENERATIVE DISEASES

Retinal degenerative disease can be devastating when the disease advances and the important visual sense is lost. There are two types of retinal degenerative diseases: inherited and acquired retinal degeneration. The most common form of IRD is retinitis pigmentosa (RP), which has a prevalence of 1 in 3,000–4,000 individuals worldwide (90). RP is a Mendelian disease that can be autosomal dominant, autosomal recessive, or X-linked. Patients with RP initially develop night blindness and visual field impairment around age 20–30 due to the loss of rod photoreceptors, which is followed by a decrease of visual acuity and loss of color vision around age 40–60 due to the degeneration of cone photoreceptors (**Figure 1**). Rod photoreceptors are sensitive to light and work in the dark; cones are responsible for sharp and color vision and are abundant in the macula, at the center of the retina.

In contrast to IRDs, age-related macular degeneration (AMD) affects millions of elderly people globally and is a leading cause of blindness in the elderly populations of developed countries. Because it impairs the central vision, patients with this disease suffer from low vision. AMD is a complex disease with multiple causes, including genetic and environmental factors, but age is the most important influence. Single-nucleotide polymorphisms in complement factor H (*CFH*) were reported to increase susceptibility to AMD in 2005 (34, 44), and since then, extensive whole-genome analyses have been conducted to elucidate the genetic influence on AMD pathogenesis. Polymorphisms in *CFH*, *ARMS2*, *HTRA1*, *APOE*, and other genes have been identified, along with the important roles of complement pathways, lipid metabolism, and oxidative stress (27).

More than 250 genes are known to cause IRDs (46), which are Mendelian diseases. The *RHO* P23H mutation was first identified in RP in 1990 (20), and this discovery opened the door for genetic analyses and gene-specific therapies for retinal diseases. Genetic analyses have become more popular since the advent of next-generation sequencing, and the American Academy of Ophthalmology recommends genetic tests for IRD patients because the results have a positive impact on patients and their family members, by providing more detailed prognostic information, possible therapeutic options, and so on (81). The American Academy of Ophthalmology also recognizes the important role of genetic counseling combined with genetic tests. Live long-distance video sessions of genetic counseling have been performed in some European nations. In Japan, this is not yet common, but genetic counselors serve in clinics to support IRD patients. The American Academy of Ophthalmology does not yet recommend genetic testing for complex diseases,

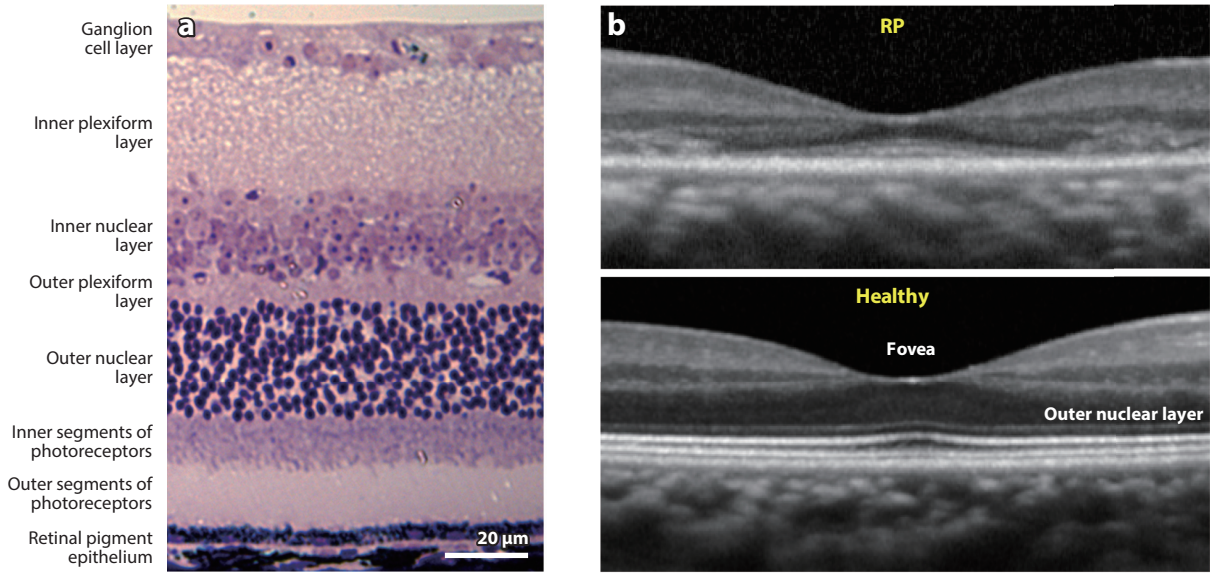

Figure 1

Retinal structure and retinal diseases. (*a*) A representative image of a mouse retina, showing the multiple layers of cells characteristic of vertebrate retinas. (*b*) Spectral domain optical coherence tomography images from a patient with retinitis pigmentosa (RP) and a healthy individual. The RP retina shows a thinning of photoreceptor-associated layers, including the outer nuclear layer.

including AMD, because the results of the genetic test cannot currently be directly connected to clinical practice. Genetic testing is becoming increasingly important because of the potential for gene-dependent therapies, which could play a critical role in the early stage of the disease (**Figure 2**). Remarkably, researchers are finding concrete solutions for incurable retinal diseases (**Figure 2**).

GENE THERAPIES

Gene Supplementation Therapy for Inherited Retinal Degeneration

In 2017, the US Food and Drug Administration approved the use of gene supplementation therapy for LCA caused by *RPE65* mutations. RPE65 catalyzes a reaction from all-*trans*-retinyl ester to 11-*cis*-retinol in the retinal pigment epithelium (RPE) (71). Impairment of RPE65 results in a deficiency of the visual chromophore 11-*cis*-retinal, which can initiate phototransduction by photoisomerization to all-*trans*-retinal in rhodopsin molecules. The success of *RPE65* gene therapy has launched the era of gene therapies and gene supplementation therapies for recessive retinal diseases (6, 36, 52). Adeno-associated virus (AAV) is the preferred vector for this type of retina treatment, and clinical trials with genes other than *RPE65* have been conducted, including *CHM/REP1* (21), which causes choroideremia, and *MERTK* (30), which causes RP (**Table 1**). Since one major limitation of AAV is its relatively small cargo space, its use presents challenges for larger genes, including *ABCA4* (3), which causes Stargardt disease, and *USH2A*, which causes Usher syndrome (43). Researchers have developed different vectors and new methodologies for delivering larger genes to the retina, including lentiviral vectors and nonviral nanoparticles (24, 84, 95). Studies in both humans and animals have also explored therapies using many other genes, including *LRAT* (8), *BEST1* (33), and *GUCY2D* (10, 56).

RPE: retinal pigment epithelium

AAV: adeno-associated virus

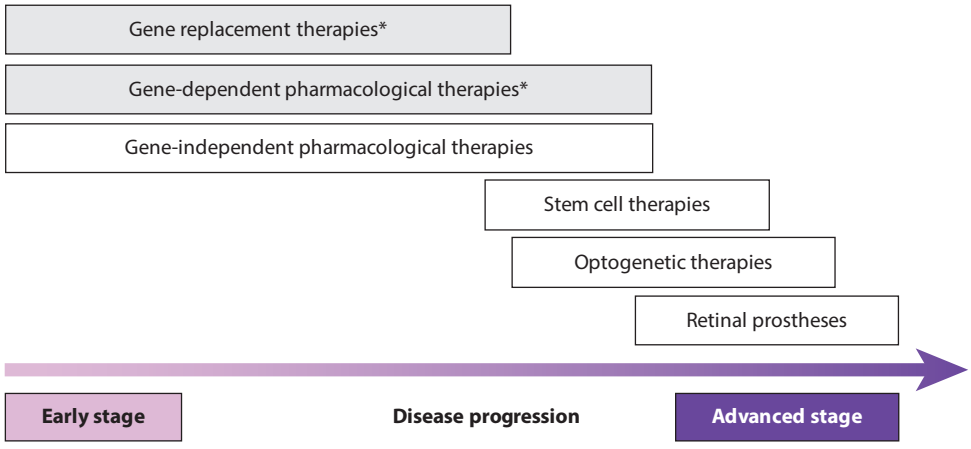

Figure 2

Therapeutic options for inherited retinal dystrophies. Gene-specific therapies in gray boxes are expected to be available in the future; asterisks indicate a requirement for molecular diagnosis. Gene replacement therapies and pharmacological therapies are effective for treating diseases at the early stage, when photoreceptors are intact or degenerating. Stem cell therapies, optogenetic therapies, and retinal prostheses can restore vision by providing photosensors to the retinas of degenerated eyes.

Trophic Factors

Several neurotrophic factors have been studied for their therapeutic effects because evidence suggests that they can protect the retina from degeneration (45), and supplementation of these factors to the retina carries great potential for mutation-independent therapies. Glial cell–derived neurotrophic factor (GDNF) (26), ciliary neurotrophic factor (CNTF) (15), brain-derived neurotrophic factor (BDNF) (63), pigment epithelium–derived factor (PEDF), and basic fibroblast growth factor (bFGF) (57) have been tested in animal models and shown to have beneficial effects

Table 1 Clinical trials for gene supplementation therapies for retinal degenerative diseases

Disease	Gene	ClinicalTrials.gov ID(s)
Leber congenital amaurosis	*RPE65*	NCT00516477, NCT01208389, NCT00999609, NCT00643747, NCT00749957, NCT01496040, NCT02781480, NCT02946879, NCT00481546
	CEP290	NCT03396042 (natural history)
Retinitis pigmentosa	*RPGR*	NCT03316560, NCT03252847, NCT03116113
	PDE6A	NCT02759952
	PDE6B	NCT03328130
	MERTK	NCT01482195
Achromatopsia	*CNGA3*	NCT02610582
	CNGB3	NCT03001310
Choroideremia	*CHM/REP1*	NCT02077361, NCT02553135, NCT01461213, NCT02407678, NCT02671539, NCT02341807
Retinoschisis	*RS1*	NCT02317887, NCT02416622
Stargardt disease	*ABCA4*	NCT01736592, NCT01367444
Usher syndrome	*MYO7A*	NCT01505062, NCT02065011
	USH2A	NCT03146078 (natural history)

that prevent the retina from degenerating. A Japanese group has been developing a gene transfer therapy with PEDF (58) that is currently in a phase I/II clinical trial. A French group has shown that rod-derived cone viability factor (RdCVF) can modulate energy metabolism to maintain cone photoreceptor health (48). Gene transfer therapy with AAV-*RdCVF* successfully delayed cone cell death in a rodent model (12), and clinical trials in human patients are being planned.

ChR: channelrhodopsin

Optogenetic Therapies

One principle of optogenetic approaches is to create new photosensors by transferring the genes of photosensitive materials to the remaining retinal circuity after the photoreceptors have degenerated. One of the benefits of this type of approach is that it utilizes existing retinal neural synapses. The critical choices for optogenetic approaches are what types of light sensors to use and what types of retinal cells to employ and target.

Channelrhodopsins (ChRs) are nonspecific cation channels that depolarize upon blue light illumination and were isolated from green microalgae of the genus *Chlamydomonas* (60). Structurally, they have a seven-transmembrane region as well as rhodopsin (68), which is a G protein–coupled receptor (GPCR) in rod photoreceptors that is essential for vertebrate vision. ChR2, which triggers larger photocurrents than ChR1, was successfully used as the first optogenetic tool in 2005 and has been established as the paradigm in this field (59). *ChR2* transfection to blind rats and mice restored visual activity (9, 86), and *ChR2*-based gene therapies for humans are being developed in the United States and Europe. Although ChR2 can convert light signals to electric signals, *ChR2* gene therapies require amplifiers or light adaptors to obtain strong enough stimuli from blue light illumination to activate visual systems. To overcome this weakness of ChR2, a genetically modified ChR2 that can also receive red-shifted light has been developed (49). *Volvox*-derived ChR1 (VChR1) is a red-shifted ChR variant that was isolated from the spheroidal alga *Volvox carteri* (94). It has different spectral properties than ChR1 and ChR2, allowing action potentials to be generated at 589 nm, and its ability to be activated by yellow light illumination is useful for treating populations with retinal degeneration (77, 87).

Halorhodopsin (NpHR) (31), metabolic glutamate receptors (mGluRs) (14), and light-gated ionotropic glutamate receptor (LiGluR) (13) have also been examined as optogenetic tools. NpHR, a light-activated chloride pump that hyperpolarizes NpHR-expressing cells when they are illuminated, could be especially helpful to reactivate the inhibitory modulation of ganglion cells through expression in OFF bipolar cells.

Potential targeted retinal cells for optogenetic therapies include ganglion, bipolar, amacrine, and cone photoreceptor cells in retinas that are degenerating due to RP. Bipolar cells are considered more natural target cells for expressing photosensors; however, effective and specific promoters have not been established, especially for OFF bipolar cells, and there is also a lack of promoters for ON and OFF ganglion cells. Therefore, the currently practical method for optogenetic therapies is to use ubiquitous promoters to transfect photosensors—for example, turning all cells into ON cells with ChRs or OFF cells with NpHR.

Gene Editing

A new technique using the powerful gene-editing tool CRISPR/Cas9 has been identified (39) and developed (40) to restore retinal function in mice afflicted by a retinal degenerative disease. Since CRISPR/Cas9 can repair gene mutations, this approach can provide a complete cure when diseases are treated early. It has been tested for treating retinal degenerative diseases (19) and could play an important role in the treatment of autosomal dominant diseases with dominant negative effects

of gene variants, such as the rhodopsin *RHO* P23H mutation, which is one of the most common autosomal dominant RP variants in the United States and Europe. Since the CRISPR/Cas9 system was introduced in 2012 (40), it has revolutionized the speed and scope with which scientists can modify the DNA of living cells. This technology has great potential to treat retinal diseases by cutting out mutated genes and replacing them with the correct sequence template.

ESC: embryonic stem cell

One limitation of the CRISPR/Cas9 approach is that it is gene and sequence specific. It is not practical to develop each causal variant, because more than 70 causal genes are known for RP and more than 250 are known for IRDs, with multiple variants in each gene. Common variants that cause diseases, such as founder mutations, must be identified in order to use one therapeutic CRISPR/Cas9 tool to treat many patients. Researchers are working on this issue, and regulatory bodies are looking into opportunities to streamline the process.

In addition to the genetic approaches described above, nucleic acid therapies using RNA interference and antisense oligonucleotides have also been examined (11, 29).

STEM CELL THERAPIES FOR RETINAL DEGENERATIVE DISEASES

It has long been believed that the regenerative potency of the retina was limited to some non-mammalian species, such as amphibians and fish, whose retinal cells could be restored from retinal progenitor cells. Researchers were eager to find a clue that would open the door for retinal regeneration in mammalian retinas as well, and cells at the ciliary marginal zone or Müller cells were thought to have the capacity to act as retinal progenitors (1, 16, 25). In 2004, Ooto et al. (64) reported that, when triggered by *N*-methyl-D-aspartate (NMDA) damage, retinal Müller cells can endogenously proliferate and regenerate photoreceptor cells in vivo; in 2007, Osakada et al. (66) showed that Wnt/β-catenin signaling further promotes photoreceptor regeneration in adult mice, although functional recovery was not tested. Two more recent studies in mice genetically introduced β-catenin to induce Müller glia proliferation followed by Otx2/Crx/Nrl for rod induction, demonstrating the possibility of more efficient endogenous regeneration accompanying functional visual recovery (41, 93). These endogenous regenerative approaches would be a therapeutic option in the future.

The regenerative potency of the retina also suggests that mammalian adult retinas can regain visual function after incorporating photoreceptor precursors supplied by transplantation. Additionally, the use of in vitro biotechnology to obtain various organoids from pluripotent stem cells enables researchers to obtain large amounts of high-quality human retinal tissues or cells at any developmental stage from iPSC or embryonic stem cell (ESC) lines. This progress has expanded the possibilities for applying cell therapy to retinal degenerative diseases, and early clinical trials of these ESC/iPSC-based therapies are under way. Below, we summarize some of these early clinical trials and preclinical studies.

Overview of Cell-Based Therapies for Retinal Degeneration

Retinal cell therapies are roughly divided into two groups: one that aims for a protective effect through the secretion of trophic factors, and one that aims to replace or restore dysfunctional or lost cells. For cell therapies aiming for rescue effects, several cell types have already reached clinical trial, such as bone marrow $CD34^+$ stem cells, cells derived from umbilical cord tissue, autologous stem cells derived from bone marrow, and fetal retinal progenitor cells.

For cell replacement or restoration therapy, candidate cell types include photoreceptors, RPE cells, and retinal ganglion cells. Photoreceptor cells are the first apparatus to respond to light and are a primary cause of many hereditary retinal degenerative diseases, including RP, in which the

loss of photoreceptors is critical and irreversible. Retinal ganglion cells are the last retinal cells to receive processed signals originating from the photoreceptors and subsequently send the signals to the brain; glaucoma is a representative disease characterized by loss of retinal ganglion cells. Compared with retinal ganglion cell regeneration, which requires restoring the long axonal pathway extending to the brain, the transplantation approach seems more feasible for photoreceptors because these cells send signals to the neighboring secondary neurons in the retina, and this technique was first tested decades ago. RPE cells are essential for photoreceptor maintenance, and RPE atrophy, which is often present in AMD, leads to photoreceptor degeneration. Because RPE cells compose a monolayer sheet structure in the eye, transplanting these cells does not require integration into the neural network for the cells to function; therefore, researchers and clinicians have naturally tried to transplant RPE cells either from others or from the patients themselves. An earlier approach to transplantation therapy used fetal retinas or RPE cells, but often the source of the graft was limited, and fetal RPE cells faced immune system rejection problems (2). The relocation of patient autologous RPE sheets from the peripheral part of the eye to the macular area was also tested, and some patients achieved good vision, but the surgical procedure was rather invasive (89).

Since the first production of ESCs by Evans & Kaufman (23) in 1981, the use of these cells to replace various cells or organs in the human body has been postulated as a future regenerative therapy. In 2002, Kawasaki et al. (42) reported the differentiation of RPE cells as a by-product of dopaminergic neurons from human ESCs (hESCs), which was followed by the functional characterization of hESC-derived RPE cells and their protective effect via the replacement of dysfunctional RPE cells in Royal College of Surgeons rats (35). The first human trials of hESC-derived RPE cells were conducted in 2012 (75). The successful production of retinal photoreceptor cells from mouse, monkey, and human ESCs was reported in 2008 (65), shedding light on the use of these ESC-derived retinal neurons for transplantation in retinal degeneration. In the meantime, a striking technology was also introduced in 2006: the production of iPSCs, which were generated from terminally differentiated adult skin cells, first from mice and then from humans (85). Another breakthrough technology was the generation of three-dimensional retinal organoids from mouse and human ESCs, reported by Eiraku et al. (22) in 2011 and Nakano et al. (61) in 2012, respectively. Researchers soon began to generate retinas from all types of human iPSCs (**Figure 3**).

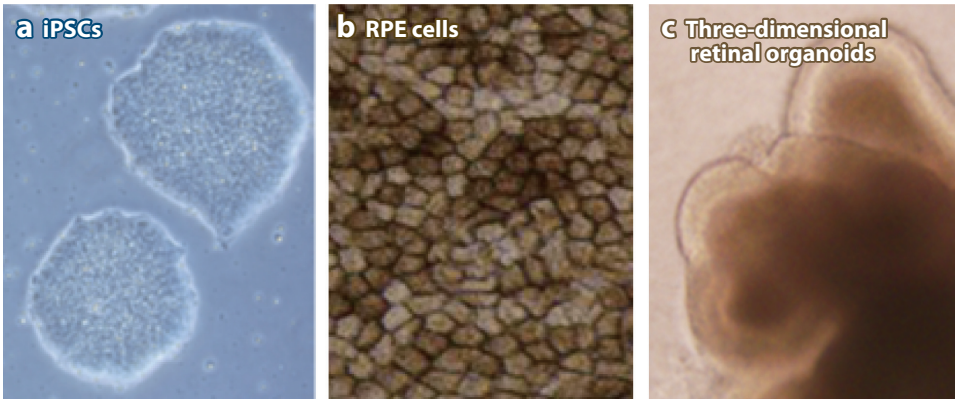

Figure 3

Differentiation of retinal cells from induced pluripotent stem cells (iPSCs), showing representative images of (*a*) human iPSCs, (*b*) iPSC-derived retinal pigment epithelium (RPE) cells, and (*c*) three-dimensional retinal organoids.

Progress in biotechnology has allowed for the use of substantial amounts of high-quality retinal neurons derived from hESCs or iPSCs at the desired developmental stage.

Human Embryonic Stem Cell–Derived Retinal Pigment Epithelium Transplantation in Patients with Stargardt Disease and Age-Related Macular Degeneration

Schwartz et al. (76) reported the first clinical trial (phase I/II studies) using a hESC-based therapy to treat Stargardt disease (a juvenile form of macular dystrophy) and AMD. The studies demonstrated that this treatment is safe and has some efficacy in restoring visual function compared with untreated contralateral eyes. Since pluripotent cells have enormous proliferative capability as well as plasticity, one of the concerns about using hESC-derived cells was tumor formation. In this study, 18 patients were transplanted with 5,000–15,000 hESC-derived RPE cells, and 13 of the patients exhibited patches of subretinal pigmentation that suggested the stable survival of graft cells. There were no adverse events directly related to the transplanted grafts, including tumorigenicity, during a median observation period of 22 months, but the required intake of immunosuppressants seemed to burden the patients. Another phase I/II open-label trial using hESC-derived RPE cells to treat Stargardt disease also reported that there was no uncontrollable proliferation or inflammation, with borderline improvements in the best-corrected visual acuity of 4 out of 12 patients but no short-term benefit to retinal sensitivity (55).

More recently, da Cruz et al. (17) conducted a safety study in which they transplanted 6 × 3 mm hESC-derived RPE patches prepared on a vitronectin-coated synthetic membrane into wet-AMD patients with massive acute subretinal hemorrhages and rapid vision loss. The large patches were safely and stably placed in the submacular area and led to vision recovery.

The studies described above demonstrate the safety and practicality of ESC-based therapies. Patient selection or transplantation strategies (e.g., using a sheet or cell suspension) may need further refinement or optimization to obtain substantial efficacy.

Induced Pluripotent Stem Cell–Derived Retinal Pigment Epithelium Transplantation for Wet Age-Related Macular Degeneration

Following the first hESC-derived RPE clinical trial, the Takahashi group (54) conducted the first human clinical trial of an autologous iPSC-derived RPE sheet. One of the major reasons for using an autologous cell sheet was to test the immune competency of a self-derived cell sheet. After the removal of the choroidal neovascularization that had resulted from the patient's polypoidal choroidal vasculopathy, a subtype of wet AMD, a 3 × 1.3 mm RPE cell sheet was prepared from the patient's skin and transplanted. The patient's vision had been deteriorating despite repeated treatments with anti–vascular endothelial growth factor (VEGF) therapy, which is currently the first-choice approach for wet AMD. After the transplant was complete, the exudative change was resolved, and the patient's vision stabilized with no evidence of background disease recurrence for four years. The pigmented area expanded to approximately 1.5 times the original sheet size, with no sign of rejection even without immunosuppression and no sign of tumor formation. This clinical study ended as a one-case trial, and more cases are required to confirm the safety and efficacy of the procedure, but the trial demonstrated the therapy's practicality. Graft preparation could be a major problem with this approach, however: The process of preparing the graft sheets from the patient's skin was expensive and took 10 months.

A more practical approach to iPSC-based therapy would be to use human leukocyte antigen (HLA)–matched iPSC-derived RPE cells. The Takahashi group (82, 83) reported some evidence

that these cells cause less proliferation of HLA-matched lymphocytes and repress rejection in HLA-matched transplantation. In these studies, RPE cell stocks were prepared from an iPSC line that had homologous alleles on the six major HLA loci for the most frequent allele combination in the Japanese population; approximately 17% of the Japanese population has at least one allele match on each of these six loci. A second clinical trial is currently under way in which five HLA-matched patients were recruited to test immune system acceptance of allografts of iPSC-derived RPE cell suspensions without using immunosuppressants. The hope is that this study will provide insight into the immunogenic properties of iPSC-derived RPE cells, as the need to take immunosuppressants following RPE transplantation can be burdensome for elderly patients with AMD, and immune inflammatory responses can increase disease recurrence. This approach also requires optimizing the delivery procedures for the iPSC-derived RPE cells by using either cell suspensions or cell sheets, depending on the disease type and pathology.

Cell Therapy for Hereditary Retinal Degeneration

RP is primarily a group of genetic diseases that cause rod photoreceptor degeneration. More than 70 causal genes have now been reported, and even with comprehensive screening, the identification rate for causal genes remains less than 50% in Japan (4, 62). Gene supplementation therapy at an early stage would recover visual function and slow or stop disease progress in patients whose disease type results from haploinsufficiency. However, treatment of advanced cases with severe photoreceptor loss in both rods and cones would require different approaches, including retina transplantation (**Figure 2**).

In the 1990s, several groups studied fetal retina transplantation using animal models and found some evidence of the reconstruction of the host–graft neural network, as shown by acquired light responses in the superior colliculus (73, 92). However, this approach often led to double inner nuclear layers (one from the host and one from the graft), suggesting inefficient synapse contact between the host bipolar cells and graft photoreceptors. In 1999, Das et al. (18) transplanted human fetal retinas into the eyes of RP and AMD patients, demonstrating the safety of the therapy and the possible effect after transplantation.

The second boom for retinal cell transplantation therapy came with reports from MacLaren et al. (50), in which the transplanted photoreceptor precursors were beautifully integrated into the outer nuclear layer of host cells, and Pearson et al. (69), who showed that photoreceptor precursor transplantation in *Gnat*$^{-/-}$ mice led to the functional recovery of rod-derived vision. Although these and subsequent studies had seemed to confirm the functional synaptic reconstruction of host bipolar cells and graft photoreceptors, several later reports overturned this idea and reinterpreted the observed phenomenon as a cytoplasmic transfer, in which some proteins or materials are transferred from the graft photoreceptors to the host photoreceptors, thereby restoring function (70, 74, 79). Since then, the construction of a host–graft neural network has been further tested only in end-stage retinal degeneration models with few remaining photoreceptors.

Around the same time, retinal organoids became available from ESC and iPSC cell lines, which enabled the Takahashi group to test the transplantation of retinal sheets prepared in differentiating culture. An end-stage retinal degeneration model (*rd1*) was used in which the remaining photoreceptors had almost disappeared, and the results showed that embryonic-stage retinal tissues derived from mouse ESCs or iPSCs could form a photoreceptor layer, including the outer segments (5). Furthermore, these photoreceptor cells contacted host bipolar dendrite terminals in an organized structure in which the researchers could observe the presence of pre- and postsynaptic marker proteins (53) (**Figure 4**). Ex vivo electroretinograms of isolated retinas on multiple electrode arrays showed the activities of host and graft retinal cells as well as those from host

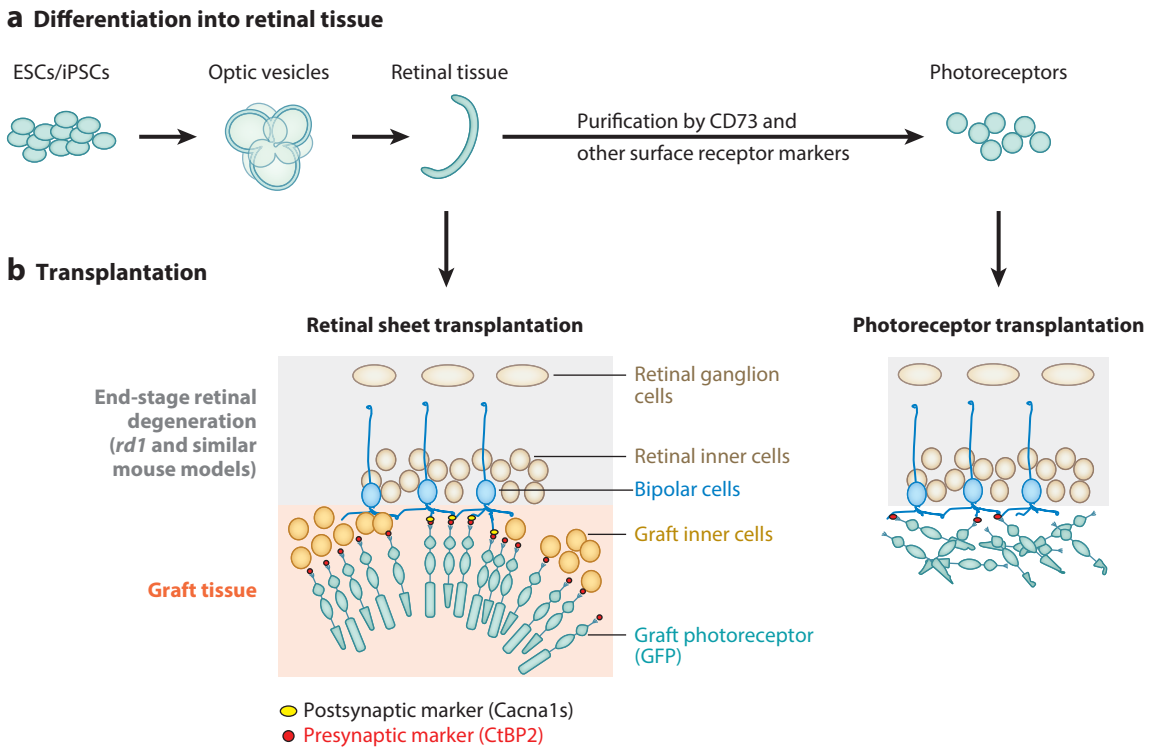

Figure 4

Therapeutic strategy for the transplantation of retinal tissues and cells derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). The retinal optic vesicle structures are differentiated from ESCs or iPSCs, and graft sheets or enriched photoreceptor cells are prepared from them (panel *a*). Recent studies have preferred to use end-stage animal models with minimal remaining photoreceptor cells (such as *rd1* mice; panel *b*) to avoid the transfer of cytoplasmic materials between the host and graft photoreceptor cells. The retinal sheet has an organized structure that includes unnecessary inner cells, whereas cell transplantation allows for direct contact between the host bipolar cells and graft photoreceptors but in a disorganized structure. Some of the transplanted photoreceptors, either in grafted retinal tissue or in cell suspensions, were reported to contact host bipolar dendrites, as indicated by the presentation of synaptic markers, including CtBP2. The Takahashi group (53) demonstrated contact between GFP-labeled host bipolar cells in the *rd1* host retina and CtBP2-tdTomato in graft photoreceptors with the presence of the postsynaptic marker Cacna1s.

ganglion cells. These responses were not observed in the untransplanted *rd1* retinas of the same age or in transplanted retinas blocked by the mGluR6 blocker L-2-amino-4-phosphonobutyric acid (L-AP4), indicating that transplanted photoreceptors were the sources of the retinal electrical activity. Likewise, retinal tissues derived from hESCs and human iPSCs also matured and formed photoreceptor layers after they were transplanted into mice or rats with end-stage retinal degeneration, contributing to possible synaptic contact with host bipolar cells and eliciting some light responses recorded from the host retina (38, 78, 88). An additional safety study of iPSC-derived retina transplantation is currently being prepared.

Another major approach for ESC/iPSC-derived cell therapy is to use photoreceptors or cones purified with surface photoreceptor markers such as CD73 (28, 47) (**Figure 4**). Studies have also shown that the transplantation of hESC/iPSC-derived retinal cells can lead to synapse formation of host and graft cells, with some restoration of light responses (7, 80). These reports, together with the studies from the Takahashi group, suggest that it is possible to reconstruct functional

synapses between graft photoreceptors and graft bipolar cells. However, further studies are needed to achieve the robust host–graft contact that would lead to substantial vision restoration.

CHALLENGES FOR FUTURE APPLICATIONS

Retinal degenerative diseases, especially IRDs, remain incurable, and therapeutic options are badly needed. In addition to the further development of gene and cell therapies, many steps are required to provide fruitful care for individuals with these diseases. Ideally, each affected patient should receive a molecular diagnosis based on the more than 250 causal genes for IRDs. Genetic information allows clinicians to predict the prognosis of the disease and provide more specific genetic counseling, and gene-dependent therapies cannot be provided if the patient's causal gene is undetermined. Gene sequencing in clinical practice has become more popular thanks to recent developments in sequencing technology. These advances enable analyses of many genes (including whole genomes and exomes) at a reasonable price and speed, but interpreting the resulting data becomes more complicated; there is also a risk of revealing unexpected genetic concerns, such as mutations in cancer-associated genes. In addition, although genetic counseling can be important for patient care, the number of trained counselors remains limited. It is also important to gather information from patients and their family members about what services are helpful to them, including low vision care and social support.

There is also a need to establish therapeutic evaluation methods. Ophthalmology has made great advances in in vivo imaging, such as adaptive optics (72, 91) and multiphoton imaging (37, 51, 67). These methods allow each individual photoreceptor to be distinguished, and researchers have begun to take advantage of them to evaluate cell functions in vivo. Retinal degenerative diseases progress slowly, and it can take decades for patients' symptoms to worsen. Therefore, it is critical to determine how to evaluate retinal structure and function when conducting clinical trials, developing precision medicine, and providing individualized care to treat these previously untreatable diseases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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