



Review Article

Induced pluripotent stem cells and derivative photoreceptor precursors as therapeutic cells for retinal degenerations

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ABSTRACT

The visual impairment associated with inherited retinal degeneration and age-related degeneration of photoreceptors is causing substantial challenges in finding effective therapies. However, induced pluripotent stem cell (iPSC)-derived therapeutic cells such as photoreceptor and retinal pigment epithelium (RPE) cells provide the ultimate options in the rescue of lost photoreceptors to improve the visual function in end-stage degeneration. Retinal cells derived from iPSC are therapeutic cells that could be promising in the field of cell replacement therapy and regenerative medicine. This review presents an overview of the photoreceptor degeneration, methods of iPSC generation, iPSC in retinal disease modeling, summarizes the photoreceptor differentiation protocols, and challenges remained with photoreceptor cell replacement for the treatment of retinal diseases. Thus, the burden and increased incidence of visual impairment emphasizes the need of novel therapy, where iPSC-derived photoreceptor and RPE cells proved to be promising for curing the retinal dysfunction and act as renovation in approach to improve visual function.

KEYWORDS: *Induced pluripotent stem cells, Photoreceptors, Retinal degeneration, Retinal disease modeling, Transplantation*

INTRODUCTION

The ontogenetic of retina presents a unique structure for studying neurogenesis, cellular interactions, and gene influences. The retinal cell fates are determined by the various transcription factors and are generated from the pool of multipotent progenitor cells [1,2]. The retina is formed by the proper organization of specific neurons such as photoreceptors (rod and cone), bipolar, amacrine, horizontal, ganglion cells, and types of glial cell such as Müller glia [3]. The functional light-sensitive photoreceptor cells are responsible for the visual senses of interaction with the environments. However, vision impairment due to degeneration of photoreceptor is affecting more than 43 million people globally with an exponential increase in population with advanced age predominantly over 50 years (WHO, 2016, Visual Impairment and Blindness). The cause of visual impairment is due to the progressive loss of photoreceptors related to genetically heterogeneous disorders. The degeneration of photoreceptors associated with genetic and environmental insults leads to blindness is linked to high metabolic need in the renewal of photoreceptor outer segment discs (OSD) [4]. The photoreceptor degeneration is a complex trait altered due to a large number of variant genes, related to environmental factors and predominantly monogenic inheritance [5,6]. Numerous therapeutic strategies, such as neuroprotective agents, gene therapy, retinal prosthesis, and cell

replacement therapy have been developed, and some of them already entered the clinical trials. However, cell therapy is an option for advanced retinal diseases, and challenges persist due to the limited source of therapeutic cells, such as retinal pigment epithelium (RPE) and photoreceptors as shown in Figure 1. In this regard, induced pluripotent stem cells (iPSCs) provide a unique *in vitro* model that allows the generation of retinal progenitor cells for modeling of retinal degenerative diseases. Retinal cell derivatives generated from iPSCs are useful for drug screening for personalized medicine and effective strategies for cellular therapy in both early and end-stage retinal diseases. Furthermore, modeling of developmental disorders is particularly amenable using iPSCs and their derivatives [7].

In this review, we specially focus and summarize recent perspectives for directed differentiation of photoreceptor cells from iPSC and iPSC-derived photoreceptor transplantation in retinal disease modeling and possibilities for improving the retinal functions. All the information was obtained from the reliable literature sources.

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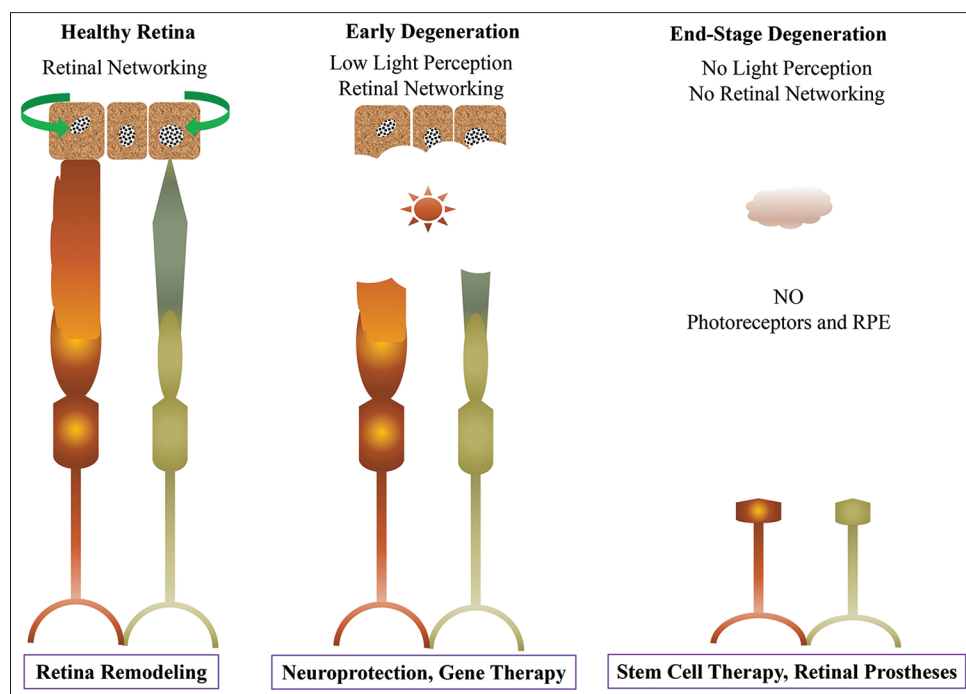


Figure 1: Illustration showing progressive photoreceptor degeneration and potential therapeutic approaches

PHOTORECEPTOR DEGENERATION

The photoreceptors are exceptionally vulnerable cells in the retina, and progressive degeneration of these cells leads to the irreversible loss of vision. Usually, light-sensing photoreceptors (rods – dim and cones – bright) form the visual transduction cascade to perform specialized visual functions. These cells undergo complex phototransduction mechanism that interlinked with the metabolism of retinoid; thus, high metabolic rate is involved in the retinoid visual cycle at the cellular level, molecular level, and electrophysiology of photoreceptor function [8,9]. The metabolic alteration in retinoid contributes to a high level of susceptibility to genetic defects causing dysfunction or death of photoreceptors. Such anomalies lead to loss of inner retinal connection and alter the neuronal networking cascade. Fortunately, the transplanted photoreceptor precursors from the developing retina can contribute to making single and short synaptic interplay to the optical network for retinal modeling [10].

Several inherited retinal diseases are associated with dysfunction and progressive loss of photoreceptors, such as retinitis pigmentosa [11], age-related macular degenerations [12], and Leber's congenital amaurosis (LCA) [13]. Among them, retinitis pigmentosa is the leading cause of untreatable blindness that is characterized by gradual constriction of visual field. Moreover, the loss of photoreceptors in inherited retinal diseases does not have genotype–phenotype correlation due to extensive genetic heterogeneity. Inherited retinal diseases, such as macular degeneration, retinitis pigmentosa, and Usher syndrome constitute a genetically heterogeneous group with almost 293 human genetic loci and more than 256 genes identified so far (Retnet; <https://sph.uth.edu/retnet/sym-dis.htm>) [14].

PLURIPOTENT STEM CELLS AND CELLULAR REPROGRAMMING

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and iPSCs, provide a unique *in vitro* model for generating the therapeutic cells, such as photoreceptor and RPE for cell replacement therapy in retinal degenerative diseases. Here, we specifically focus on iPSCs generated from somatic cells by cellular reprogramming using defined transcription factors.

Induced pluripotent stem cells

iPSC was an innovative discovery by Takahashi and Yamanaka in 2006, where mouse embryonic/skin fibroblasts and adult human fibroblasts were converted into PSCs by the overexpression of defined transcription factors, such as Oct4, Sox2, Klf4, and c-Myc using the retroviral system [15,16]. These cells were morphologically identical and showed similar pluripotent gene expression like in ESCs system [15,16]. Furthermore, Yu *et al.* used other sets of defined factors, such as Oct4, Sox2, Nanog, and LIN28 using lentivirus to generate iPSCs from foreskin fibroblasts [17]. These iPSCs showed the expression of pluripotency genes and potential to differentiate into developmental germ layers (endoderm, mesoderm, and ectoderm) investigated using standard *in vivo* teratoma assay and alternative *in vitro* embryoid body formation [16]. iPSCs have been generated from somatic cells of different mammals, such as mice [18], human [16], monkeys [19], and pigs [20]. These iPSCs showed similar characteristic features of PSCs; however, cell reprogramming efficiency differs among different cell origin, cell types, and no consensus on the most consistent protocol for generating the reliable and safest iPSCs [21]. Still, iPSC technology has been revolutionizing the stem cell research and therapy for regenerative medicine.

Alternative methods for induced pluripotent stem cell generation

Since the discovery of iPSC technology, reprogramming protocol improvements are increasing to achieve efficient derivation and to maintain the normal genomic integrity. Recently, iPSC methods are available as commercial kit, but they do require reliability with regard to efficiency and reproducibility of reprogramming somatic cells into iPS for biomedical uses in routine work [22]. Different methods for cellular reprogramming are summarized and extensively reviewed [23] [Table 1].

Molecules for enhancing reprogramming efficiency

Generation of iPSCs is time-consuming, and the efficiency varies with cell types and without consistent protocols. Thus, for clinical application of iPSCs, it is necessary to overcome the limitations of low efficiency. Numerous lists of small molecules, growth factors, microRNAs, and siRNAs have been suggested to enhance the efficiency of cellular reprogramming based on several well-known mechanisms [36,37]. Some of the commonly used small molecules are listed in Table 2.

Molecular basis of genomic instability of induced pluripotent stem cell

Genomic instability acquired during forced reprogramming, cultural adaptation, passage-induced mutation, and viral integration has been reported [47,48]. The maintenance of genomic stability is essential to produce distinct cell lineages because differentiated cells inherit harmful effects such as chromosomal aberrations, copy number variations (CNV), and single-nucleotide variant [48]. Such effects remained significant challenges to be addressed before used in clinics.

Chromosomal aberrations occurred due to selective pressure either from parental inheritance or from forced reprogramming as shown in Figure 2. A forced expression of the proto-oncogene, such as c-Myc used in reprogramming, is associated with inactivation of p53 causing genomic aberrations in iPSCs [49]. Furthermore, the demand of high metabolic rate associated with induction of oncogenic transcription factors causes elevation of oxidative stress (ER), which leads to genomic aberrations. Such aberrations can be reduced by the use of antioxidant in growth media that significantly lower the CNVs [50]. Likewise, parental cells harboring protein-coding mutations that cause cancer, remain permanent in the iPSC cell line populations [51]. In addition, only 20% of mutations are parental inheritance, while approximately 75% of coding point mutations are associated with reprogramming and persist during the subsequent passages [52].

In clinical applications, the genomic instability is of major concern which may induce teratoma, but the key factors that regulate genomic instability in iPSCs are unclear [47]. Furthermore, the level of genomic instability observed in iPSCs is comparable with the signs perceived in cancer cells [53]. Despite the instability concern, such transient aberrations and negligible report on genomic aberrations with malignant transformation will compromise the safety of iPSC-derived cell therapy in clinic. Thus, the appropriate technique should be applied to distinguish harmless aberrations and those causing tumor formations. For such classification of mutation pattern, high-resolution single-nucleotide polymorphism genotyping is recommended to monitor the acquired mutations to minimize the effects on cell therapy [54].

Table 1: Methods and transcription factors for the generation of induced pluripotent stem cells

Methods	Factors	Reference
Single lentiviral vectors expressing "stem cell cassette" with Cre-Lox transgene excision system	Oct4, Klf4, Sox2, cMyc	[24]
Cre-recombinase excisable DOX-inducible lentiviral vectors system	Oct4, Klf4, Sox2, cMyc	[25]
Repeated transfection of two expression plasmids without viral vectors system	(cDNA of Oct3/4, Sox2, Klf4) and cDNA of cMyc	[26]
Nonintegrating adenoviral vectors system	Oct4, Sox2, Klf4, cMyc	[27]
Nonintegrating Sendai virus-based vector system	Oct4, Sox2, Klf4, cMyc	[28]
Recombinant reprogramming proteins system (fusion of poly-arginine protein transduction domain to the C terminus of transcription factors)	Oct4, Sox2, Klf4, cMyc	[29]
Human artificial chromosomes (HACs) vectors (iHAC1 and iHAC2) system	Oct4, Sox2, Klf4, cMyc	[30]
Synthetic modified mRNA transfection system	Oct4, Sox2, Klf4, cMyc	[31]
Direct transfection of mature double-strand microRNAs (miRNAs) system	Combination of mir-200c, mir-302 s and mir-369 s	[32]
Doxycycline-inducible factors delivered by "piggyback" transposition system	Oct4, Sox2, Klf4, cMyc	[33]
Nonviral minicircle DNA vectors system	Oct4, Sox2, Nanog, Lin28	[34]
Nonintegrating episomal plasmid vectors system	Oct4, Sox2, Nanog, Lin28, Klf4, cMyc	[35]

Table 2: Small molecules and the mechanism that promotes reprogramming efficiency

Molecules	Mechanism	Reference
VPA or NaB	Inhibition of HDAC	[38,39]
BIX-01294 (BIX)	Inhibition of G9a histone methyltransferase (G9a HMTase)	[40]
SB431542 plus PD0325901	Inhibition of TGF β receptor and MEK signaling pathway	[41]
SB431542 or ALK5 (A-83-01)	Inhibition of Type 1 TGF β receptor	[42,43]
Vitamin C	Alleviates cell senescence and promotes epigenetic modifications	[44]
Y-27632	Inhibition of the ROCK pathway	[45]
PS48	Activation of PDK1	[46]

VPA: Valproic acid, NaB: Sodium butyrate, HDAC: Histone deacetylase, Rho-associated Kinase, PDK1: 3'-phosphoinositide-dependent kinase-1, MEK: MAPK/ERK Kinase

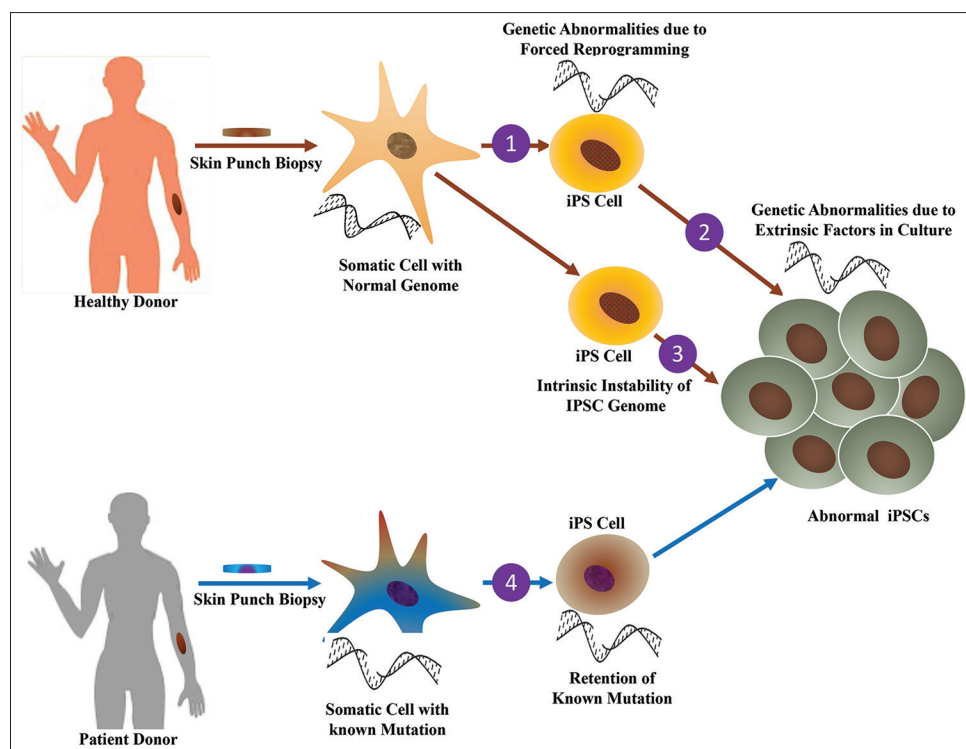


Figure 2: Illustration showing the factors associated with genomic instability in iPSC cells

APPLICATION OF INDUCED PLURIPOTENT STEM CELL TECHNOLOGY

iPSC technology can be directly used for developmental biology, disease modeling, drug discovery and efficacy testing, and cell transplantation. Various patient-specific iPSC lines have been generated for modeling of retinal dystrophies, such as retinitis pigmentosa, LCA, gyrate atrophy, Stargardt's macular dystrophy, bestrophinopathies, and choroideremia [55]. Furthermore, the previous study demonstrated that rod cells derived from iPSC of retinitis pigmentosa patients showed differential responsiveness to α -tocopherol (Vitamin E) [56]. The study of human organogenesis is difficult due to inaccessibility of organs which develops in early embryos. The tracking of early eye development and organoidogenesis with fully developed laminated and functional retinal structure was generated using iPSC [57]. In addition, iPSC technology has opened new possibilities for generating continuous sources of therapeutic cells, such as RPE, retinal ganglion cells, and photoreceptors. Recently, several preclinical and clinical trials have demonstrated the safety, survival, integration, and rescue effects of clinical-grade RPE cells derived from patient-specific iPSC [58,59]. Therefore, iPSCs are promising cell sources for autologous cell transplantation, which minimizes the risks of immune rejection and the development of self-organized three-dimensional tissues using iPSC open the future possibilities for next-generation organ transplantation.

PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELL-BASED EYE DISEASE MODELING

Recent advances in *in vitro* iPSC model system are helpful resources for generating enough cells to create distinctive

platforms for disease modeling, drug screening, and cell-based therapy [60]. Furthermore, human cellular models have potential benefits that overcome the limitation of the animal model, which cannot faithfully mimic human physiology. The self-renewal properties of iPSCs are promising for modeling various diseases, as other specialized primary cell lines stop proliferation after few passages in laboratory culture. Several studies used iPSCs for a wider variety of human diseases, such as genetic disorders (Down's syndrome), neurological disorders (Parkinson's disease), and inherited metabolic syndrome (juvenile diabetes) [60].

Usually, retinal biopsies are difficult to take or inaccessible to investigate the pathophysiology of inherited retinal diseases. Such pathological investigation became possible using patient-specific iPSCs to identify novel gene expression and silencing, drug screening and development, and cell transplantation in advanced retinal degeneration. iPSCs were used for exploring the structure of eye, such as limbal-like epithelium and corneal epithelial cells [61,62], lens-specific differentiation [63], trabecular meshwork [64], optic vesicle-like structures [65], retinal ganglion cells [66], photoreceptor cells [7], and retinal pigment epithelial cells [67]. Besides, patient-specific iPSCs were used for several inherited retinal diseases, such as age-related macular degeneration, retinitis pigmentosa, and glaucoma [68-70]. Human iPSCs were used to study early eye development and retinogenesis that recapitulate the *in vivo* developmental timeline using iPSC-derived retinal organoids. All retinal neurons were appropriately arranged in their proper layer, in which photoreceptors achieved sensory cilia with prominent OSD that responded the light [57]. Furthermore, RGCs were generated from iPSCs

using fibroblasts from normal tension glaucoma patient carrying a duplication of TBK1 gene [71] and multilayered optic cups from keratinocyte-derived iPSCs of patients with retinitis pigmentosa [72]. The retina precursor cells from the optic cup revealed their ability to integrate into developing mouse retina [72]. Advanced retinal diseases with severe loss of photoreceptor cells have no benefits with gene therapy, where cell transplantation remains an ultimate strategy. Therefore, the use of iPSCs-derived photoreceptors and RPE is under intense investigation to restore visual network or to arrest the loss of vision [67,72,73].

ESTABLISHMENT OF INDUCED PLURIPOTENT STEM CELL-DERIVED PHOTORECEPTOR AS THERAPEUTIC CELLS

In vitro development of light-sensitive functional photoreceptor cells is challenging, but essential therapeutic cells for retinal degenerative diseases. In early age-related macular degeneration that is associated with RPE dysfunction, transplantation of RPE cells might be beneficial for the functional recovery of native RPE; for diseases with end-stage degeneration with loss of photoreceptors, the ultimate option is photoreceptor cell transplantation. Therefore, photoreceptors derived from iPSCs using somatic cells either from patients or from healthy donor could be effective strategies for cell-based therapy. Patient-specific photoreceptor cells can also be used to investigate the role of the mutant gene and drug screening for personalized medicine. Meanwhile, iPSC-derived photoreceptor cells from a healthy donor could be promising sources for allogeneic cell transplantation. Furthermore, the sequential development of retinal neurons provides the benefit to target disease progression at different stages in retinal degeneration [74]. Numerous studies explored the potential of photoreceptor cells generated from human iPSC as listed in Table 3. iPSC-derived photoreceptor cells have been established from various cell sources such as blood, fibroblast, and keratinocytes [56,68,72,91]. However, there is no report of iPSC-derived photoreceptor cell transplantation in human trial for the vision correction due to difficulty in establishing the robust, efficient, and stable methods for generation and purification of photoreceptor cells.

Human iPSC-derived photoreceptor cells have been generated from various diseases, such as retinitis pigmentosa, enhanced S-cone sensitivity syndrome, and gyrate atrophy as listed in Table 3. Derivative photoreceptor cells revealed the characteristic expressions, such as cell lineage markers (OTX2, CRX, RECOVERIN, RHO, B-OPSIN, and R/G OPSIN) [7,56,72], expression of proteins (VGLUT1 and SNAP-25) responsible for neurotransmitter release [65], the presence of light-responsive sensory cilia in the outer segment disc [72], and electrophysiology responses [56]. iPSC-derived photoreceptor cells were generated using somatic cells of a patient with retinitis pigmentosa harboring different mutations (RP1, RP9, PRPH2, RHO, and USH2A). Such mutations lead to degeneration of photoreceptor cells over time due to ER-stress [56,72]. Interestingly, the different types of mutation causing retinitis pigmentosa subsequently decreased in

iPSC-derived rod photoreceptor cells [56,68]. However, mutant iPSC-derived rod cells undergo degeneration in *in vitro* culture that expressed the oxidative and ER stress marker responsible for the presence of disease phenotypes [56,68]. This evidence further suggests that the loss of photoreceptors in retinitis pigmentosa is postdevelopmental degeneration due to the ER stress and the unfolded protein response [68]. Furthermore, iPSC-derived retinal organoids carrying TRNT1 mutation cause high-level expression of oxidative stress molecules and abnormal accumulation of LC3-II, leading to defective autophagy [92]. The high expression of ER stress and apoptotic markers is associated with poor survival of photoreceptor cells with the rhodopsin mutation (E181K). The use of rapamycin, PP242, AICAR, NQDI-1, and salubrinal reduces the cellular stress enhancing the better survival of iPSC-derived photoreceptor precursors [82]. The data provide a novel approach for screening new drugs to attenuate degeneration in iPSC-derived photoreceptor cells, but it is still elusive whether such drugs could recover the light-responsive function of photoreceptor cells.

The *in vitro* degeneration of retinal cells and ER stress demonstrate its utility for disease modeling that recapitulates the disease phenotypes. Derivative retinal cells can be corrected for functional defect either using gene correction or pharmacological means. The previous study revealed the restoration of ornithine- δ -aminotransferase (OAT) activity in (A226V) OAT iPSC-RPE by supplementation of 600 μ M Vitamin B6 and gene-corrected hiPSC-RPE cells [81]. The patient-specific iPSC carrying rhodopsin mutation (E181K) corrected using adenoviral gene transfer, which showed the ability to differentiate into rod photoreceptors [82]. Furthermore, patient-specific photoreceptor precursor cells showed the ability to integrate into the dystrophic mouse retina and develop into mature photoreceptor cells [72]. Thus, the combination of iPSCs and gene therapy approach could be used in treating various degenerative diseases causing blindness.

INDUCED PLURIPOTENT STEM CELL-DERIVED PHOTORECEPTOR CELL TRANSPLANTATION

Effective transplantation of derivative photoreceptor cells should meet basic criteria for high-quality clinical-grade manufacturing, safety, and efficacy. Worthwhile rapid, efficient, and consistent differentiation protocol is necessary for replacement therapy in visually challenged patients. The acquisition of mature photoreceptors in iPSC-derived retinal organoids and strategic approaches for retinal cell transplantation is shown in Figure 3a-d. However, the use of animal-derived growth factors, viral vectors during reprogramming, genomic instability and prolonged time for differentiation limit their clinical application. The use of small molecules to overcome the cross-species antigenic contamination has been suggested [77]. Furthermore, numerous studies revealed the improved differentiation protocols using chemically defined optimal culture condition for the development of retinal cell lineages and photoreceptor precursors that showed sequential expression of retinal cell markers [68,78]. Although the efficiency of differentiation was better, there was no consistency in defined molecules for induction of photoreceptors

Table 3: Differentiation factors, sequential expression of induced pluripotent stem cell-derived photoreceptor markers, and transplantation outcomes

Disorder	Affected gene	Cell Source	Method	Differentiation factor	Cell types and markers	Drug testing/transplantation	Functional assessment	Reference
Retinitis Pigmentosa	USH2A variant (Arg4192His)	Keratinocytes	Sendai virus OCT4, SOX2, KLF4, c-MYC	Noggin, Dkk-1, IGF-1, bFGF, DAPT, aFGF, B27, N2	Multilayer eyecup-like structures and retinal rosettes (recovertin, rhodopsin, B-opsin, R/G-opsin) Protein misfolding and ER stress in differentiated cells	Subretinal space; P4 immune compromised <i>Rag1-/- x C7b1-/-</i> mice	Extensive cellular integration and differentiated to photoreceptor cells Axonal projections toward the inner plexiform layer and the RPE layers	[72]
Retinitis Pigmentosa	Mutations (RP1, RP9, PRPH 2 or RHO gene)	Fibroblasts	Retroviral transduction OCT3/4, SOX2, KLF4, c-MYC	Lefty-A, Dkk-1, RA, Taurine, N2	Immature photoreceptors (CRX, recovertin); Rod cells (rhodopsin); cone cells (opsin). ER stress in rod cells	Drug screening; Anti-oxidant vitamins (alpha-tocopherol, ascorbic acid, beta-carotene)	α -tocopherol is beneficial to RP9- mutated rod cells survival. No effect of ascorbic acid and β -carotene	[56]
Retinitis Pigmentosa	<i>RHO</i> -G188R mutation	Fibroblasts	Sendai virus OCT3/4, SOX2, KLF4, c-MYC	Lefty-A, Dkk-1, RA, Taurine	Photoreceptors precursors (recovertin, CRX); Rod cells (rhodopsin)	Not performed	N/A	[68]
Undefined	Normal	Fibroblasts	Lentiviral transduction OCT4, NANOG, LIN28, SOX2	Noggin, Dkk-1, IGF-1, N2, B27	DNA damage-inducible transcript (BiP and CHOP) expressed Photoreceptor precursors (Otx2, Crx, recovertin, AIP1); Rod cells (rhodopsin); cone cells (S-opsin)	Subretinal Space; Adult wild-type mice along with Cyclosporine A (10 mg/kg/day)	FACS purified photoreceptor cells survived and integrated into the host retina after transplantation	[7]
Enhanced S-cone sensitivity syndrome (ESCS)	NR2E3 mutations	Fibroblasts	Sendai virus OCT4, SOX2, KLF4, c-MYC	IWR1e, CHIR, SAG, N2, DAPT	Photoreceptor precursors (OTX2, CRX, recovertin) Rod cells (NRL, NR2E3, ROR β , SW opsin)	Subretinal injection; Immune compromised SCID mice.	cGMP grade photoreceptor precursors cells were safe, and no intraocular tumor formed	[75]
MAK-associated Retinitis Pigmentosa	Homozygous for <i>Alu</i> insertion in <i>MAK</i> lacking exon 9 transcript	Fibroblasts	Lentiviral transduction OCT4, SOX2, KLF4, c-MYC	B27, N2, Noggin, Dkk-1, IGF1, bFGF, DAPT, aFGF	Photoreceptor precursors (OTX2, recovertin)	Not performed	MAK exon 12 is retina-specific, but not MAK exon 9	[76]
Undefined	N/A	Fibroblasts	Retroviral transduction OCT4, SOX2, KLF4, MYC	CKI-7, SB-431542, N2, RA, Taurine	Photoreceptor precursors (CRX, NRL recovertin) Rod cells (rhodopsin)	Not performed	Point mutations in exon 9 and 12 are not a common cause	[77]
Undefined	N/A	Fibroblasts	Retroviral transduction OCT4, SOX2, KLF4, MYC	Dkk-1, Lefty A, N2, RA, Taurine	Photoreceptor precursors (CRX, recovertin) Rod cells (rhodopsin)	Not performed	N/A	[78]
Undefined	N/A	Fibroblasts	Retroviral transduction OCT4, SOX2, KLF4, MYC	B27, N2, Noggin, Dkk-1, Lefty A, IGF1, SHH, T3, RA, Taurine	Photoreceptor precursors (CRX, recovertin, arrestin 3) Rod cells (Rhodopsin); cone-specific opsin blue and opsin red/green	Not performed	N/A	[79]

Contd...

Table 3: Contd...

Disorder	Affected gene	Cell Source	Method	Differentiation factor	Cell types and markers	Drug testing/transplantation	Functional assessment	Reference
Undefined	N/A	Corneal fibroblast	STEMCAA lentivirus OCT4, SOX2, KLF4, MYC	B27, N2, RA, Taurine, bFGF, aFGF, SHH	Photoreceptor precursors (CRX, recoverin) Rod cells (NRL, NR2E3, RORβ, SW opsin)	Not Performed	N/A	[80]
Undefined	N/A	hiPSC lines; fibroblasts, cord blood, keratinocytes	Lentivirus SOX2, OCT4, NANOG, LIN28. Episomes SOX2, OCT4, KLF4, MYC, NANOG, LIN28, SV40T	Heparin, N2, B27, Taurine, RA	Photoreceptor precursors (Otx2, recoverin) Rod cells (rhodopsin); cone cells (S-opsin, L/M-opsin)	Not Performed	Retinal organoids acquired mature photoreceptors with functional domains containing an outer segment disc that were responsive to lights	[57]
Healthy donor	N/A	Blood (activated T cells)	Retroviral transduction: 3 bicistronic MMLV constructs: (OCT4, SOX2), (c-MYC, KLF4), and (NANOG, LIN28)	Heparin, N2, B27	Photoreceptor precursors (CRX, recoverin) Rod cells (rhodopsin); cone cells (S-opsin)	Not performed	<i>In vitro</i> synaptogenesis was recapitulated in hiPSC-derived optic vesicles. CX36 required for electrical synapses were upregulated in optic vesicles	[65]
Gyrate atrophy	N/A	hiPSC lines, gyrate atrophy fibroblasts	Lentiviral transduction OCT4, SOX2, NANOG, LIN28, c-MYC, KLF4	Noggin, Dkk-1 B27, N2	Photoreceptor precursors (OTX2, CRX, NRL recoverin) Phototransduction (CNGA1, A3, B1 and B3, RETGC, ODE6B, arrestin). Photoreceptor-like cells showed electrophysiological responses	Not Performed	Gene-corrected hiPSC-RPE showed normal level of ornithine-δ-aminotransferase (OAT) activity	[81]
Retinitis pigmentosa	Rhodopsin mutation (E181K)	Fibroblasts	Retroviral transduction OCT3/4, SOX2, KLF4, c-MYC	N2, Noggin, Dkk-1, IGF-1	NRL-positive rod cells DNA damage-inducible transcript (BiP and CHOP) expressed, Rhodopsin E181K mutation was responsible for the loss of rod cells	Drug Screening: mTOR inhibitors (rapamycin and PP242), AMPK activators (AICAR), ASK1 inhibitors (NQDI-1), and inhibitors for eIF2α phosphatase and protein synthesis (salubrinal)	Suppression of E181K mutant rhodopsin-related cell loss by mTOR inhibition, AMPK activation, ASK1 inhibition, or the suppression of protein synthesis	[82]
Undefined	N/A	hiPS cell lines (IMR90 clone 4)	N/A	N2, Heparin, B27, BMP4, Wnt3A, SU5402	Photoreceptor precursors (CRX, recoverin) Cone-specific opsin	Not performed	N/A	[83]
Undefined	N/A	hiPS cell lines	mRNA reprogramming Kit, Episomal vectors	B27, N2, Insulin, Noggin, BDNF, CNTF, RA, DAPT	Photoreceptor progenitors (CRX, NRL, NR2E3), Rod cells (rhodopsin, recoverin, and phosphodiesterase 6 alpha)	Subretinal space; Rdl mice with end-stage retinal degeneration	Differentiated into cell photoreceptors, formed cell layer, interact with host retina and visual functions was partially restored	[74]

Contd...

Table 3: Contd...

Disorder	Affected gene	Cell Source	Method	Differentiation factor	Cell types and markers	Drug testing/transplantation	Functional assessment	Reference
Undefined	N/A	Fibroblasts	OrtP/EBNA1-based episomal vectors pEP4EO2SEN2K, pEP4EO2SET2K, and pCEP4-M2L	N2, FGF2, DAPT	Photoreceptor precursors (OTX2, CRX, NRL recoverin) Rod cells (rhodopsin); cone cells (blue opsin, R/G-opsin, cone arrestin)	Not performed	Notch pathway inhibition accelerated the generation of photoreceptor precursors	[84]
None	N/A	hiPS cell line (NCL1), cord blood-derived CD34+	Episomal plasmids	N1, Dkk-1, Noggin, IGF1, IWR1, SB431542, LDN193189	Photoreceptor precursors (OTX2, CRX, BLIMP1, AIPL1, recoverin) Rod cells (NRL); cone cells (RXR γ)	Subretinal space; IL2r γ -/- mice (B6.129S4-Il2rgtm1Wjl/J)	Photoreceptor precursor cells integrated into the host retina with synaptic end-feet to plexiform layer	[85]
None	N/A	hiPS cell line (IMR90-4)	N/A	B27, Taurine, RA, N2	Photoreceptor precursors (Recoverin)	Not performed	Bioreactor culture conditions improved retinal lamination and increased the yield of photoreceptors	[86]
None	N/A	hiPS cell line (H9, H1, IMR90-4)	N/A	N2, B27, Taurine, RA	Rod cells (CD133/CD73, Rhodopsin); cone cells (S-opsin, L/M-opsin, cone arrestin) Photoreceptor precursors (OTX2, CRX, NRL recoverin)	Subretinal space; Cone cells transplantation into Nrl $^{-/-}$, and Aipl1 $^{-/-}$ mice	Cone precursors incorporated into host cone-rich retina. Also, cones have the potential to replace lost photoreceptors in end-stage retinal degeneration	[87]
None	N/A	hiPS cell line (hiPSC-2 clone), fibroblasts	N/A	N2, B27, FGF2	Photoreceptor precursors (CRX, Recoverin, CD73)	Trans-vitreous injection; hemizygous P23H rats along Cyclosporine A (210mg/L)	Transplantation of CD73+ cells showed no significant functional improvement	[88]
Healthy donor	N/A	hiPS cell lines (UE017, UE022, and UC005), urine cells	N/A	B27, N2, Taurine	Rod cells (rhodopsin); cone cells (blue opsin, R/G-opsin) Photoreceptor precursors (OTX2) Rod cells (rhodopsin); cone cells (S-opsin, L/M-opsin)	Not performed	Matured rod and cone cells acquisition in retinal organoids without the addition of RA	[89]
None	N/A	NIHEK primary cell line	Sendai virus OCT4, SOX2, KLF4, c-MYC	B27, N2, DAPT, Taurine, RA	Photoreceptor precursors (OTX2, CRX, recoverin) Rods cells (rhodopsin); cone cells (B-opsin, R/G-opsin)	Not performed	Aberrant-hiPSC differentiated into retinal organoids that acquired matured photoreceptors	[90]

N/A: Not available

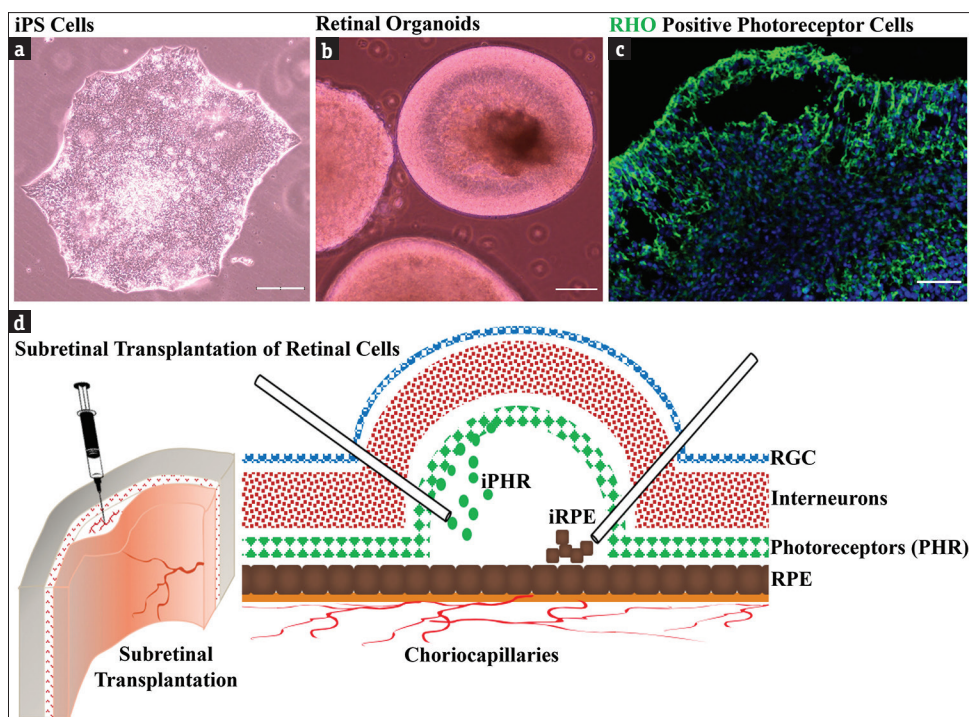


Figure 3: Generation of induced pluripotent stem cell-derived retinal organoids with the acquisition of photoreceptor cells. (a) Induced pluripotent stem cell colony with tightly packed cells with defined boundaries, (b) induced pluripotent stem cell-derived retinal organoids, (c) Retinal organoids with RHO-positive photoreceptor cells at apical part and (d) representative illustration showing subretinal transplantation of induced pluripotent stem cell-derived RPE and photoreceptor cells. Scale bars: 150 μm (c), 200 μm (a and b)

from iPSC. Thus, the optimization of minimal culture conditions which endogenously upregulate the specific transcription factors of retinal cells is necessary. The key factors such as B27, N2, DKK1, and Noggin enhance the early stage differentiation of iPSC into retinal and photoreceptor precursors [79]. However, with improvement in the methodology for iPSC generation without viral vectors [Table 1], feeder-free culture conditions and chemically defined directed differentiation approaches for efficient generation of photoreceptor precursor cells have offered the platform for modeling human diseases.

The transplantation of postnatal photoreceptor precursors and PSC-derived photoreceptor precursors showed the proof of its potential benefit for the restoration of visual functions [7,93,94]. Transplantation of photoreceptor cells derived from iPSC in *rdl* mouse model of end-stage retinitis pigmentosa revealed the robust cell survival, developed a mature outer nuclear layer (ONL), and connected to interneurons improving the visual function [74]. However, the functional restoration after transplantation could be associated with RNA and/or protein transfer between graft and host photoreceptors; instead of transplanted photoreceptors migrating and integrating into the photoreceptor layer of recipients [95,96], these observations raise the possibility of material transfer as an alternative strategy for the treatment of retinal disorders. Surprisingly, CD73+ photoreceptor precursors derived from human iPSC transplanted into rat eyes revealed the capacity to survive and mature in proximity to host inner retina without significant functional improvement by electroretinography [88]. They suggested that more suitable transplantation strategies and closely related species (such as nonhuman

primates) may improve the functionality of CD73+ cells after transplantation [88]. Further, the purification of transplantable photoreceptors using fluorescence-activated cell sorting and magnetic-activated cell sorting can be effective strategies for obtaining approximately 90% of pure cells, avoiding the possible risk of teratoma formation [7,88]. In addition, PSC-derived retinal organoids in three-dimensional culture systems offer the accessibility to the human cells such as photoreceptors and RPE for future clinical applications in degenerative diseases [97,98]. Furthermore, transplantable photoreceptor cells could be isolated from heterogeneous cells layered in the retinal organoids using novel label-free sorting approaches based on the inherent characteristics, such as mechanical and morphological properties [97]. Moreover, transplantation studies have revealed that photoreceptor precursors can differentiate into mature photoreceptor cells, and migrate to host ONL, in which donor cells acquire synaptogenic potential and initiate retinal synaptic circuitry with naïve photoreceptors via cellular integration [97,98]. While sensory photoreceptor cell transplantation has been the center of focus, it is essential to include RPE cells for successful and sustainable recovery of vision in certain diseases [99]. Thus, cotransplantation of RPE and photoreceptors provides a favorable environment for incorporation of donor cells as shown in Figure 3d.

CONCLUSIONS

iPSC-derived photoreceptor cells are promising and future hope for effective cell-based therapy in end-stage retinal diseases. However, based on numerous publications, the hype has been created in the scientific community and to the public

indicating the success of photoreceptor cell transplantation. Till date, there is no evidence on human studies showing successful and effective photoreceptor transplantation that reversed the loss of vision. Furthermore, similar data on transplantation, such as cell survival, integration, and trophic support for improving visual functions, were interpreted differently. More preclinical studies are required to claim the science behind the success of iPSC derivative transplantation.

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Conflicts of interest

There are no conflicts of interest.

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