YALE JOURNAL OF BIOLOGY AND MEDICINE 90 (2017), pp.635-642.

MINI-REVIEW

YJBM

Potential of Gene Editing and Induced Pluripotent Stem Cells (iPSCs) in Treatment of Retinal Diseases

Katherine Chuang, Mark A. Fields*, and Lucian V. Del Priore

Yale School of Medicine, Department of Ophthalmology and Visual Science, New Haven, CT

The advent of gene editing has introduced the ability to make changes to the genome of cells, thus allowing for correction of genetic mutations in patients with monogenic diseases. Retinal diseases are particularly suitable for the application of this new technology because many retinal diseases, such as Stargardt disease, retinitis pigmentosa (RP†), and Leber congenital amaurosis (LCA), are monogenic. Moreover, gene delivery techniques such as the use of adeno-associated virus (AAV) vectors have been optimized for intraocular use, and phase III trials are well underway to treat LCA, a severe form of inherited retinal degeneration, with gene therapy. This review focuses on the use of gene editing techniques and another relatively recent advent, induced pluripotent stem cells (iPSCs), and their potential for the study and treatment of retinal disease. Investment in these technologies, including overcoming challenges such as off-target mutations and low transplanted cell integration, may allow for future treatment of many debilitating inherited retinal diseases.

INTRODUCTION

The human eye is composed of an anterior segment, which facilitates entry and refraction of light, and a posterior segment, which absorbs light and sends visual signals to the brain. The anterior segment contains the cornea, iris, ciliary body, and lens while the posterior segment consists of the vitreous humor, retina, choroid, and optic nerve (Figure 1). Damage to the posterior segment, such as degeneration of photoreceptors and retinal pigment epithelial (RPE) cells in the retina can lead to poor clinical outcomes.

Research in posterior segment eye diseases is often hindered by a lack of tissue samples. Because the mammalian retina does not regenerate after damage, biopsy of the posterior segment can lead to significant

*To whom all correspondence should be addressed: Mark A. Fields, MPH, Ph.D., 300 George Street, Suite 8100, New Haven, CT 06511, Tel: 203-737-6387, Fax: 203-785-7401, Email: mark.fields@yale.edu.

†Abbreviations: AAV, adeno-associated virus; ABCA4, ATP-binding cassette, sub-family A, member 4; AMD, age-related macular degeneration; Cas9, CRISPR associated protein 9; CEP290, centrosomal protein 290; CRISPR, clustered, regularly interspaced short palindromic repeats; DNA, deoxyribonucleic acid; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; IVS, intervening sequence; kb, kilobase; LCA, Leber congenital amaurosis; Nrl, neural retina leucine zipper; PRPH2, peripherin 2; RHO, rhodopsin; RNA, ribonucleic acid; RP, retinitis pigmentosa; RPE, retinal pigment epithelial cells; RPGR, retinitis pigmentosa GTPase regulator; TALEN, transcription activator-like effector nucleases; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; YFP, yellow fluorescent protein; ZFN, zinc-finger nuclease.

Keywords: gene editing, CRISPR/Cas9, retinal degeneration, personalized medicine, induced pluripotent stem cells

Author Contributions: Katherine Chuang, BS, prepared a draft of the manuscript; Mark Fields, MPH, PhD, and Lucian Del Priore, MD, PhD, offered guidance on the topic, outlined manuscript construction, provided mentorship during the writing process, and critical revisions of the manuscript.

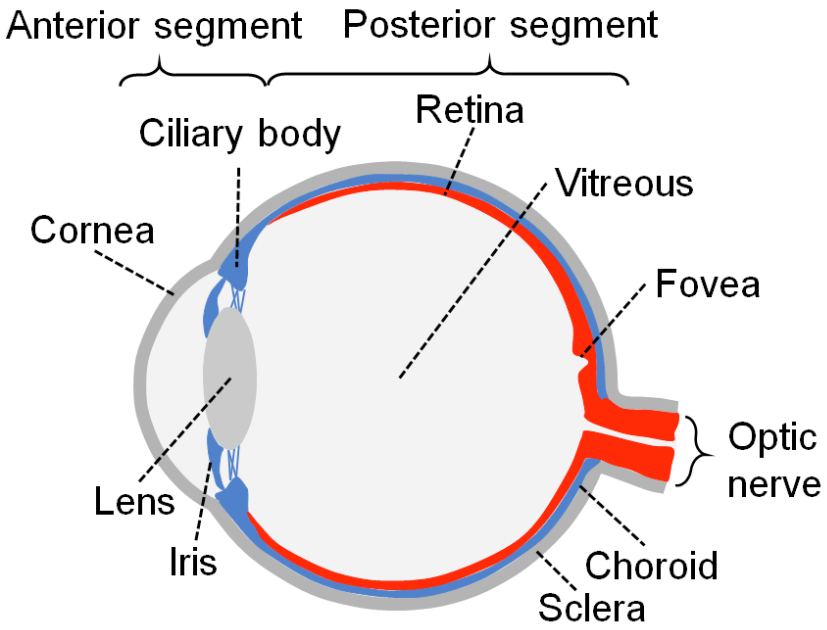

Figure 1. **Diagram of the human eye**. The anterior segment of the eye contains the cornea, iris, ciliary body, and lens. The posterior segment of the eye consists of the vitreous humor, retina, choroid, and optic nerve.

visual consequences and is avoided in living patients. Diseased eye tissues for research are often obtained postmortem at advanced stages of disease. Therefore, the ability to derive induced pluripotent stem cells (iPSCs) from donors with and without ophthalmic disease expands options for research on these diseases. When combined with gene editing, iPSCs hold enormous potential for the study of eye diseases and development of novel therapies [1].

iPSC lines derived from affected patients can be used with gene editing to study disease through disease modeling, drug screening, and regenerative medicine (Figure 2) [2,3]. iPSC lines can model pathophysiology of genetic disease when derived from affected patients [4,5]. Patient-derived cells can also be used to screen drug libraries and identify therapeutics optimized for specific patients; this is an example of "personalized medicine" [6]. In drug screening, gene editing that targets the mutated gene locus can create controls from patient-derived diseased cells, thus reducing genetic variation and other confounders that may influence phenotypic differences [7,8]. Lastly, in regenerative medicine, gene editing can repair patient-derived iPSCs to generate disease-free autologous transplants for therapeutic use.

Gene editing utilizes nucleases and deoxyribonucleic acid-binding (DNA-binding) domains to specifically target regions of DNA. These strategies include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered, regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) systems. ZFNs and TALENs target DNA through protein-DNA interactions with zinc finger proteins [9] and amino acid repeat variable di-residues [10], respectively. Both then use endonucleases to cleave DNA. CRISPR technology uses a DNA-targeting domain composed of ribonucleic acid (RNA) and a Cas9 endonuclease effector domain. Because its targeting domain relies on RNA-DNA base pairing rather than protein-DNA interactions, CRISPR/Cas9 systems are more easily customizable than ZFNs and TALENs [11,12].

RETINAL DISEASES AS TARGETS FOR GENE EDITING *IN VIVO*

Many retinal diseases are monogenic; they can be caused by mutations in single genes. Currently, over 250 genes with retinal disease-causing mutations have been identified [13]. These diseases are easier to target with gene editing because only one locus must be repaired. Thus far, research involving gene editing and iPSCs has targeted retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA) because these diseases are monogenic. RP is a genetic disorder of the retina characterized by progressive loss of rod photoreceptors resulting in night blindness, loss of peripheral vision, and blindness, that can be caused by mutations in over 90 genes implicated in disease pathogenesis [14]. LCA is an inherited retinal

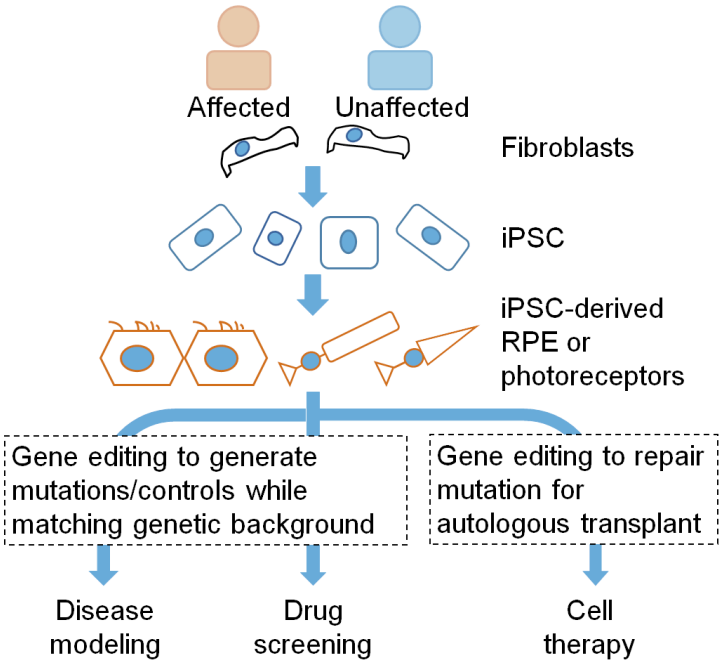

Figure 2. Potential applications of induced pluripotent stem cells (iPSCs) and gene editing in treatment of retinal diseases. iPSC-derived photoreceptor or RPE cells can be used for disease modeling, drug screening, and cell therapy. Gene editing can contribute to disease modeling and drug screening by allowing for precise mutagenesis or repair of mutations, allowing for matching genetic backgrounds in experimental and control groups. In cell therapy, gene editing can repair mutations prior to production of cells for autologous transplant.

dystrophy characterized by severe visual impairment, photophobia, nystagmus, and hyperopia caused by mutations in genes involved in the visual cycle [15]. The retina itself is a desirable target for gene-based therapies, as its response to therapy can be imaged over time. It is also inherently immune-privileged, leading to decreased host immune responses against vehicles injected into the eye to deliver gene editing components [16].

In vivo gene editing in the eye has been conducted successfully in mice. In one study, adeno-associated virus serotype 8 (AAV8) vectors delivered CRISPR/Cas9 systems causing knockdown of *neural retina leucine zipper* (*Nrl*) in mouse models of RP, including *rhodopsin* knockout mice, *RHO-P347S* rhodopsin mutant, and *phosphodiesterase 6β* hypomorphic mutation [17]. In these RP models, *Nrl*-ablated rods were resistant to degeneration and cone survival increased.

In vivo gene editing may also provide treatment for retinal neovascular diseases, which have diverse causes including proliferative diabetic retinopathy, wet age-related macular degeneration (AMD), and retinopathy of prematurity. Existing treatment for these diseases involve long-term therapy with biologics targeting vascular endothelial growth factor (VEGF) [18-20]. In a recent study, intravitreal injection of AAV with a CRISPR/Cas9 system targeting *VEGF receptor 2* (*VEGFR2*) led to decreased neovascularization and *VEGFR2* expression in murine models of oxygen-induced retinopathy and laser-induced choroidal neovascularization [21].

Use of gene editing *in vivo* in humans is also on the horizon. Editas Medicine and Allergan are hoping to target a variant of LCA, LCA10, with gene editing in clinical trials [22]. LCA10 is caused by mutations in *centrosomal protein 290* (*CEP290)*, which encodes for a centrosomal protein localized in the connecting cilium of photoreceptors [23]. The IVS26 c.2991+1655 A>G mutation in *CEP290*, which causes the insertion of a cryptic exon with a premature stop codon in intron 26, is found in about 21 percent of patients with LCA [24]. While clinical trials are still in development, past work by this group used CRISPR/Cas9 to excise the disease-causing *CEP290* mutation in primary fibroblasts derived from patients homozygous for the IVS26 mutation [25,26]. This correction led to increased amounts of wildtype CEP290 protein and correction of ciliogenesis. Treatment of visual diseases *in vivo* is a promising area to begin research on gene editing therapies.

CURRENT GENE THERAPY APPROACHES IN THE EYE

The eye has already served as a target of gene-

based approaches due to the monogenic nature of many retinal diseases and accessibility of the eye. AAV vectors [27] and lentiviral vectors [28] have delivered gene therapy to the eye in humans. AAV vectors have a size limit of 5.2 kilobases (kb), which precludes delivery of complementary DNA of larger proteins [29]. However, dual AAV therapies have been developed that capitalize on concatemerization of AAV genomes through splicing or homologous recombination to deliver longer coding sequences [30,31]. Lentiviral vectors accommodate cassettes of 8 to 10 kb, allowing for transduction of larger genes [32]. However, lentiviral vectors integrate into the genome of transduced cells, presenting the possibility of insertional mutagenesis.

Stargardt disease, an inherited macular dystrophy caused by mutations in the *ATP-binding cassette*, *sub-family A*, *member 4* (*ABCA4*) gene, has been targeted by gene therapy [33,34]. Phase I/II clinical trials to treat Stargardt disease with gene therapy are ongoing (ClinicalTrials.gov Identifier: NCT01367444, NCT01736592). These studies involve subretinal injection of SAR422459, a lentiviral vector, to deliver a functioning *ABCA4* gene to photoreceptors of patients homozygous for *ABCA4* mutations and with significant visual impairment. These studies will assess safety and delay in retinal degeneration in the short- and long-term.

ADVANTAGES OF GENE EDITING APPROACHES

Gene editing offers several advantages over gene therapy because the corrected gene is inserted at the endogenous gene locus allowing for endogenous transcriptional control. In contrast, AAV vectors remain episomal in the nucleus. Lentiviral integration sites, while not random, are very diverse [35]. In addition, gene editing causes permanent modification of the genome, leading to stable protein expression. Gene editing can build on knowledge derived from gene therapy experiments. For example, concerns over the AAV vector size limit led to the discovery of *Staphylococcus aureus* Cas9, which is smaller in size than *Streptococcus pyogenes* Cas9 [36]. The smaller size of *S. aureus* Cas9 enables larger genes to be co-packaged with Cas9 within one AAV, leading to the decreased need for dual AAV approaches.

BEDSIDE TO BENCH: iPSCs IN HUMAN DISEASE MODELING

While gene editing shows promise for retinal disease treatment *in vivo*, gene editing can also be used with iPSCs to study disease pathophysiology *in vitro*. Patient-derived iPSCs can model diseases and serve as drug screening tools if they display a disease phenotype. Protocols for the generation of retinal pigment epithelial (RPE) cells from iPSCs have been optimized to generate well-differentiated cell lines, making patient-derived iPSCs well-suited for study of diseases affecting the RPE [37-40]. Differentiation of iPSCs into pure populations of photoreceptor-like cells has also recently been refined [41]. iPSC-derived cell lines from patients affected by variants of RP [4], LCA [5,42], Usher syndrome [43], Best disease [44], and gyrate atrophy [45] have been generated and used to understand the pathophysiology of these disorders. Because RP and LCA10 are currently targets of gene editing-based therapies, iPSC modeling of these diseases will be covered here.

iPSC-derived photoreceptor cells have helped elucidate the pathophysiology of X-linked RP caused by *retinitis pigmentosa GTPase regulator* (*RPGR*) mutations. In a recent study, iPSC-derived photoreceptor cells were generated from patients with *RPGR* mutations and X-linked RP [4]. Through co-immunoprecipitation studies, it was found that *RPGR* mutations led to disrupted binding of RPGR with gelsolin, a protein involved in filamentous actin turnover. Decreased gelsolin activity led to increased actin polymerization, mislocalization of rhodopsin, and photoreceptor degeneration. In this study, control iPSCs were derived from unaffected family members.

iPSC-derived models contribute to the study of LCA10 pathophysiology. iPSCs generated from fibroblasts of a patient homozygous for the IVS26 c.2991+1655 A>G mutation in *CEP290* were differentiated into optic cups [5]. These optic cups demonstrated decreased CEP290 production and mislocalization of RPGR leading to disrupted ciliogenesis. Splicing defects caused by the mutation were preferentially exacerbated in photoreceptor cells as compared to fibroblasts and RPE, possibly due to photoreceptor-specific splicing mechanisms. In this study, control iPSCs were generated from commercial fibroblasts from unaffected individuals.

In the above experiments seeking to describe disease phenotypes, control cell lines were derived from different patients without disease. However, this introduces confounders such as genetic background, differences in the retinal cell differentiation process, or genetic alteration introduced using iPSC reprogramming [46]. As the use of gene editing expands, future experiments could modify diseased cells to generate control cells with the same genetic background as affected cells, thus negating many of the concerns stated above.

BENCH TO BEDSIDE: USE OF iPSCs AND GENE EDITING TO DEVELOP TREATMENTS

iPSCs in Drug Screening

In theory, iPSC systems allow for generation of large numbers of diseased cells for drug screening. However, large-scale production of iPSC-derived RPE cells is still challenging due to the complex protocols involved and growth factors required for differentiation [47,48]. As such, large drug screens thus far are not widely used in the study of eye disease. Research is ongoing to develop more efficient methods to generate iPSC-derived RPE cells. For example, chetomin and nicotinamide have been identified as small-molecule inducers that allow for generation of RPE-like cells from iPSCs in a one-exposure step [49]. This protocol enables production of larger quantities of pure iPSC-derived RPE cells and monolayers for screening experiments.

In one study of iPSC-derived RPE cells from patients with AMD, screening of several antioxidant drugs revealed curcumin to be protective of RPE cells from oxidative stress-induced cell death [50]. In another study, iPSC-derived rod photoreceptors from RP patients with mutations in *retinitis pigmentosa 1 (RP1), retinitis pigmentosa 9 (RP9), peripherin 2 (PRPH2),* or *rhodopsin (RHO)* genes, were used to screen the vitamins alpha-tocopherol, ascorbic acid, and beta-carotene for protective effects. Alpha-tocopherol was found to be protective of rod photoreceptor cells from patients with *RP9* mutation, but not in those from patients with other mutations [51].

In these studies, control cells were not matched using cells from the same patient, nor were the controls age-matched. However, by repairing mutations in cells from affected patients, gene editing can generate control cells that differ only in one gene. This technology provides the most rigorous comparison of control and diseased cells.

iPSCs in Ex Vivo *Gene Editing*

iPSCs can potentially create specific cells for autologous transplantation. Other than one clinical trial using iPSC-derived RPE cells, clinical trials have used only human embryonic stem cell (hESC)-derived cells [52,53]. hESC lines are derived from the inner cell mass of unused preimplantation embryos from *in vitro* fertilization [54]. hESC-derived RPE cells have been utilized in several clinical studies [55,56]. hESC use is more prevalent than iPSC use because deleterious mutations can occur during the extensive culture process in which somatic cells are reprogramed into iPSCs and differentiated into RPE cells. This was recently highlighted in a clinical trial where autologous iPSC-derived RPE cells were transplanted into human retinas. While one transplanted subject had stable vision a year after surgery, the second subject's iPSC-derived RPE cells were not transplanted because they developed copy-number variants and single nucleotide variations, the most concerning of which was found in an oncogene [57,58].

In experimental systems seeking to generate autologous transplants, gene editing could be used to repair gene mutations prior to transplant. Therapeutically, autologous transplants are advantageous due to the lower risk of immune rejection. Gene editing with CRISPR/Cas9 has repaired *RPGR* gene mutations, a cause of X-linked RP, in iPSCs derived from affected patients [59]. As of the writing of this review, these repaired iPSCs have not yet been differentiated into RPE cells to assess function.

Transplantation of disease-free cells into human retina is well-tolerated. In phase I/II clinical trials, hESC-derived RPE cells were transplanted into one eye of nine patients with Stargardt disease and nine patients with AMD [55,56]. Transplanted cells were well-tolerated for up to 37 months after transplantation.

CHALLENGES OF GENE EDITING IN TREATMENT OF RETINAL DISEASE

While gene editing holds promise for the treatment and study of genetic disease, challenges remain in the implementation of this technology. Off-target mutations are possible, creating mutations at unintended regions of the genome [60]. In a recent study, two mice underwent CRISPR/Cas9 repair of a *phosphodiesterase 6b* mutation and developed off-target mutations [61]. Whole-genome sequencing revealed 117 insertion-deletion mutations and 1,397 single-nucleotide variants in both corrected mice that were not found in the uncorrected mouse. Furthermore, mutations were unpredictable because DNA surrounding these regions had poor homology with the targeting RNA.

Gene editing in regenerative medicine may also be limited by efficiency of transplanted cell integration. While early studies showed integration of transplanted photoreceptor-like cells into the retina [62], more recent studies conclude that transfer of intracellular contents between donor and host cells, rather than cell integration, is more prevalent after subretinal photoreceptor transplantation [63-65]. These studies suggest that rather than direct photoreceptor replacement, future approaches to transplantation must capitalize on the transfer of intracellular contents to deliver protein or nucleic acid to host photoreceptors.

CONCLUSIONS AND OUTLOOK

Gene editing is contributing to the understanding

of and therapeutic development for retinal diseases. Gene editing has treated retinitis pigmentosa and retinal neovascularization in mice, offering optimistic outlooks on gene editing *in vivo*. Clinical trials for gene editing of LCA10 are in development. In addition to *in vivo* applications, gene editing may be applied to personalized medicine when used with iPSCs. These approaches include generating controls for disease modeling and drug screening, and *ex vivo* repair of gene mutations in regenerative medicine. While gene editing and iPSCs show promise, further work is required to ensure safety regarding off-target mutations from gene editing and mutagenesis that may occur during the derivation and differentiation of iPSCs. Despite these challenges, gene editing technology has made rapid advances and is a valuable tool in understanding and treating retinal diseases.

Acknowledgments: This work has been supported in part by an unrestricted/challenge award to Yale Eye Center from the Research to Prevent Blindness (RPB), Inc., New York, NY, USA; and the Foundation Fighting Blindness, Columbia, MD, USA.

REFERENCES

1. Zheng A, Li Y, Tsang SH. Personalized therapeutic strategies for patients with retinitis pigmentosa. Expert Opin Biol Ther. 2015;15(3):391-402.
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861-72.
3. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;318(5858):1917-20.
4. Megaw R, Abu-Arafeh H, Jungnickel M, Mellough C, Gurniak C, Witke W, et al. Gelsolin dysfunction causes photoreceptor loss in induced pluripotent cell and animal retinitis pigmentosa models. Nat Commun. 2017;8(1):271.
5. Parfitt David A, Lane A, Ramsden Conor M, Carr A-Jayne F, Munro Peter M, Jovanovic K, et al. Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. Cell Stem Cell. 2016;18(6):769-81.
6. Egawa N, Kitaoka S, Tsukita K, Naitoh M, Takahashi K, Yamamoto T, et al. Drug screening for ALS using patient-specific induced pluripotent stem cells. Sci Transl Med. 2012;4(145):145ra04-ra04.
7. Wang Y, Liang P, Lan F, Wu H, Lisowski L, Gu M, et al. Genome editing of isogenic human induced pluripotent stem cells recapitulates long QT phenotype for drug testing. J Am Coll Cardiol. 2014;64(5):451-9.
8. Reinhardt P, Schmid B, Burbulla LF, Schöndorf DC, Wagner L, Glatza M, et al. Genetic Correction of a LRRK2 Mutation in Human iPSCs Links Parkinsonian Neurodegeneration to ERK-Dependent Changes in Gene Expression. Cell Stem Cell. 2013;12(3):354-367.
9. Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci U S A. 1996;93(3):1156-60.
10. Streubel J, Blucher C, Landgraf A, Boch J. TAL effector RVD specificities and efficiencies. Nat Biotech. 2012;30(7):593-5.
11. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol. 2013;31(3):227-9.
12. Wang H, La Russa M, Qi LS. CRISPR/Cas9 in genome editing and beyond. Annu Rev Biochem. 2016;85(1):227-64.
13. Bennett J. Taking stock of retinal gene therapy: looking back and moving forward. Mol Ther. 2017;25(5):1076-94.
14. Daiger SP, Bowne SJ, Sullivan LS. Perspective on genes and mutations causing retinitis pigmentosa. Arch Ophthalmol. 2007;125(2):151-8.
15. den Hollander AI, Roepman R, Koenekoop RK, Cremers FP. Leber congenital amaurosis: genes, proteins and disease mechanisms. Prog Retin Eye Res. 2008;27(4):391-419.
16. Zhou R, Caspi RR. Ocular immune privilege. F1000 Biology Reports. 2010;2:3.
17. Yu W, Mookherjee S, Chaitankar V, Hiriyanna S, Kim JW, Brooks M, et al. Nrl knockdown by AAV-delivered CRISPR/Cas9 prevents retinal degeneration in mice. Nat Commun. 2017;8:14716.
18. VanderVeen DK, Melia M, Yang MB, Hutchinson AK, Wilson LB, Lambert SR. Anti-Vascular Endothelial Growth Factor Therapy for Primary Treatment of Type 1 Retinopathy of Prematurity. Ophthalmology. 2017;124(5):619-33.
19. Avery RL, Pearlman J, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, et al. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. Ophthalmology. 2006;113(10):1695 e1-15.
20. Ip MS, Scott IU, Brown GC, Brown MM, Ho AC, Huang SS, et al. Anti Vascular Endothelial Growth Factor Pharmacotherapy for Age-Related Macular Degeneration. Ophthalmology. 2008;115(10):1837-46.
21. Huang X, Zhou G, Wu W, Duan Y, Ma G, Song J, et al. Genome editing abrogates angiogenesis in vivo. Nature Communications. 2017;8(1):112.
22. Allergan and Editas Medicine enter into strategic R&D alliance to discover and develop CRISPR genome editing medicines for eye diseases [Internet]. Allergan Press Releases; 2017; March 14, 2017. Available from: https://www.allergan.com/news/news/thomson-reuters/allergan-and-editas-medicine-enter-into-strategic.
23. Drivas TG, Holzbaur EL, Bennett J. Disruption of CEP290 microtubule/membrane-binding domains causes retinal degeneration. J Clin Invest. 2013;123(10):4525-39.
24. den Hollander AI, Koenekoop RK, Yzer S, Lopez I, Arends ML, Voesenek KE, et al. Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. Am J Hum Genet. 2006;79(3):556-61.
25. Maeder ML, Shen S, Burnight ER, Gloskowski S, Mepani R, Friedland AE, et al. 687. Therapeutic correction of an LCA-causing splice defect in the CEP290 gene by CRISPR/Cas-mediated genome editing. Mol Ther. 2015;23:S273-S4.

26. Maeder ML, Mepani R, Gloskowski S, Skor M, Collins M, Shen S, et al. 124. Therapeutic correction of an LCA-causing splice defect in the CEP290 gene by CRISPR/Cas-mediated gene editing. Molecular Therapy. 2016;24:S51-S2.
27. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008;358(21):2231-9.
28. Parker MA, Choi D, Erker LR, Pennesi ME, Yang P, Chegarnov EN, et al. Test-retest variability of functional and structural parameters in patients with Stargardt disease participating in the SAR422459 gene therapy trial. Transl Vis Sci Technol. 2016;5(5):10.
29. Wu Z, Yang H, Colosi P. Effect of genome size on AAV vector packaging. Mol Ther. 2010;18(1):80-6.
30. Trapani I, Colella P, Sommella A, Iodice C, Cesi G, de Simone S, et al. Effective delivery of large genes to the retina by dual AAV vectors. EMBO Mol Med. 2014;6(2):194-211.
31. Trapani I, Toriello E, de Simone S, Colella P, Iodice C, Polishchuk EV, et al. Improved dual AAV vectors with reduced expression of truncated proteins are safe and effective in the retina of a mouse model of Stargardt disease. Hum Mol Genet. 2015;24(23):6811-25.
32. Balaggan KS, Ali RR. Ocular gene delivery using lentiviral vectors. Gene Ther. 2012;19(2):145-53.
33. Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet. 1997;15(3):236-46.
34. Binley K, Widdowson P, Loader J, Kelleher M, Iqball S, Ferrige G, et al. Transduction of photoreceptors with equine infectious anemia virus lentiviral vectors: safety and biodistribution of StarGen for Stargardt disease. Invest Ophthalmol Vis Sci. 2013;54(6):4061-71.
35. Schroder AR, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. Cell. 2002;110(4):521-9.
36. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, et al. In vivo genome editing using Staphylococcus aureus Cas9. Nature. 2015;520(7546):186-91.
37. Sonoda S, Spee C, Barron E, Ryan SJ, Kannan R, Hinton DR. A protocol for the culture and differentiation of highly polarized human retinal pigment epithelial cells. Nat Protoc. 2009;4(5):662-73.
38. Idelson M, Alper R, Obolensky A, Ben-Shushan E, Hemo I, Yachimovich-Cohen N, et al. Directed differentiation of human embryonic stem cells into functional retinal pigment epithelium cells. Cell Stem Cell. 2009;5(4):396-408.
39. Osakada F, Jin ZB, Hirami Y, Ikeda H, Danjyo T, Watanabe K, et al. In vitro differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. J Cell Sci. 2009;122(Pt 17):3169-79.
40. Kamao H, Mandai M, Okamoto S, Sakai N, Suga A, Sugita S, et al. Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. Stem Cell Reports. 2014;2(2):205-18.
41. Barnea-Cramer AO, Wang W, Lu SJ, Singh MS, Luo C, Huo H, et al. Function of human pluripotent stem cell-derived photoreceptor progenitors in blind mice. Sci Rep. 2016;6:29784.
42. Lustremant C, Habeler W, Plancheron A, Goureau O, Grenot L, de la Grange P, et al. Human induced pluripotent stem cells as a tool to model a form of Leber congenital amaurosis. Cell Reprogram. 2013;15(3):233-46.
43. Tucker BA, Mullins RF, Streb LM, Anfinson K, Eyestone ME, Kaalberg E, et al. Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. Elife. 2013;2:e00824.
44. Singh R, Shen W, Kuai D, Martin JM, Guo X, Smith MA, et al. iPS cell modeling of Best disease: insights into the pathophysiology of an inherited macular degeneration. Hum Mol Genet. 2013;22(3):593-607.
45. Meyer JS, Howden SE, Wallace KA, Verhoeven AD, Wright LS, Capowski EE, et al. Optic vesicle-like structures derived from human pluripotent stem cells facilitate a customized approach to retinal disease treatment. Stem Cells. 2011;29(8):1206-18.
46. Chen FK, McLenachan S, Edel M, Da Cruz L, Coffey PJ, Mackey DA. iPS cells for modelling and treatment of retinal diseases. J Clin Med. 2014;3(4):1511-41.
47. Buchholz DE, Pennington BO, Croze RH, Hinman CR, Coffey PJ, Clegg DO. Rapid and efficient directed differentiation of human pluripotent stem cells into retinal pigmented epithelium. Stem Cells Transl Med. 2013;2(5):384-93.
48. Zahabi A, Shahbazi E, Ahmadieh H, Hassani SN, Totonchi M, Taei A, et al. A new efficient protocol for directed differentiation of retinal pigmented epithelial cells from normal and retinal disease induced pluripotent stem cells. Stem Cells Dev. 2012;21(12):2262-72.
49. Maruotti J, Sripathi SR, Bharti K, Fuller J, Wahlin KJ, Ranganathan V, et al. Small-molecule-directed, efficient generation of retinal pigment epithelium from human pluripotent stem cells. Proc Natl Acad Sci U S A. 2015;112(35):10950-5.
50. Chang YC, Chang WC, Hung KH, Yang DM, Cheng YH, Liao YW, et al. The generation of induced pluripotent stem cells for macular degeneration as a drug screening platform: identification of curcumin as a protective agent for retinal pigment epithelial cells against oxidative stress. Front Aging Neurosci. 2014;6:191.
51. Jin ZB, Okamoto S, Osakada F, Homma K, Assawachananont J, Hirami Y, et al. Modeling retinal degeneration using patient-specific induced pluripotent stem cells. PLoS One. 2011;6(2):e17084.
52. Kimbrel EA, Lanza R. Current status of pluripotent stem cells: moving the first therapies to the clinic. Nat Rev Drug Discov. 2015;14(10):681-92.
53. Reardon S, Cyranoski D. Japan stem-cell trial stirs envy. Nature. 2014;513(7518):287-8.
54. Pera MF, Reubinoff B, Trounson A. Human embryonic stem cells. J Cell Sci. 2000;113 (Pt 1):5-10.
55. Schwartz SD, Regillo CD, Lam BL, Eliott D, Rosenfeld PJ, Gregori NZ, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. Lancet.

2015;385(9967):509-16.
56. Schwartz SD, Tan G, Hosseini H, Nagiel A. Subretinal transplantation of embryonic stem cell-derived retinal pigment epithelium for the treatment of macular degeneration: an assessment at 4 years. Invest Ophthalmol Vis Sci. 2016;57(5):ORSFc1-9.
57. Mandai M, Watanabe A, Kurimoto Y, Hirami Y, Morinaga C, Daimon T, et al. Autologous induced stem-cell–derived retinal cells for macular degeneration. N Engl J Med. 2017;376(11):1038-46.
58. Garber K. RIKEN suspends first clinical trial involving induced pluripotent stem cells. Nat Biotech. 2015;33(9):890-1.
59. Bassuk AG, Zheng A, Li Y, Tsang SH, Mahajan VB. Precision medicine: genetic repair of retinitis pigmentosa in patient-derived stem cells. Sci Rep. 2016;6:19969.
60. Cho SW, Kim S, Kim Y, Kweon J, Kim HS, Bae S, et al. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. Genome Res. 2014;24(1):132-41.
61. Schaefer KA, Wu W-H, Colgan DF, Tsang SH, Bassuk AG, Mahajan VB. Unexpected mutations after CRISPR-Cas9 editing in vivo. Nat Meth. 2017;14(6):547-8.
62. Gust J, Reh TA. Adult donor rod photoreceptors integrate into the mature mouse retina. Invest Ophthalmol Vis Sci. 2011;52(8):5266-72.
63. Singh MS, Balmer J, Barnard AR, Aslam SA, Moralli D, Green CM, et al. Transplanted photoreceptor precursors transfer proteins to host photoreceptors by a mechanism of cytoplasmic fusion. Nat Commun. 2016;7:13537.
64. Santos-Ferreira T, Llonch S, Borsch O, Postel K, Haas J, Ader M. Retinal transplantation of photoreceptors results in donor-host cytoplasmic exchange. Nat Commun. 2016;7:13028.
65. Pearson RA, Gonzalez-Cordero A, West EL, Ribeiro JR, Aghaizu N, Goh D, et al. Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. Nat Commun. 2016;7:13029.