Review Article

Taiwan J Ophthalmol 2021;11: 348-351

Access this article online

Quick Response Code:

Website:
www.e-tjo.org

DOI:
10.4103/tjo.tjo_47_21

Gene therapy for retinitis pigmentosa

Niloofar Piri*, Jacob D. Grodsky, Henry J. Kaplan

Abstract:

Rhodopsin-mediated autosomal dominant retinitis pigmentosa (RP) is the most common cause of RP in North America. There is no proven cure for the disease, and multiple approaches are being studied. Gene therapy is an evolving field in medicine and ophthalmology. In this review, we will go over the basic concept of gene therapy and the different types of gene therapy that are currently being studied to treat this disease.

Keywords:

Gene therapy, P23H mutation, retinitis pigmentosa

Introduction

Retinitis pigmentosa (RP) includes a group of heterogeneous inherited retinal degenerations that are a major cause of hereditary blindness.[1] RP affects 1 in 3000–7000 people in the United States.[2] The disease is initially characterized by night blindness and early loss of the peripheral visual field, which is the result of rod photoreceptor loss. As the disease progresses, late loss of central vision occurs, which is the result of cone photoreceptor degeneration. It is at this late stage that patients experience profound functional vision loss and blindness. Cone photoreceptor loss has been shown to be secondary to rod photoreceptor demise and is likely due to either oxidative stress or the lack of neighborhood trophic effects such as glucose transport.[1,3-5]

Rods are responsible for vision in dim light as well as peripheral vision, while cones are specifically designed for precise central acuity and color vision. It has been recently shown that the loss of rod photoreceptor outer segment contact with the retinal pigment epithelium (RPE) ultimately results in cone starvation and loss.[1]

The disease progresses slowly, and loss of photoreceptors occurs gradually and continuously. It has been shown that there can be up to 90% loss of rods before the patient noticing any visual changes, and, therefore, patients are often diagnosed only in advanced stages of the disease.[6]

RP is most frequently the result of genetic mutations in rod photoreceptors. More than 70 genes with more than 3000 mutations phenotypically present as RP.[7] Rhodopsin (RHO) was the first gene mutation identified as a cause of RP.[8] It is located on the long arm of chromosome 3 (3q22.1), has 5 exons, and is 6.7 kb[2] in length. More than 150 different RHO mutations are associated with 25% of autosomal dominant (adRP) cases. The pro23His (P23H) RHO mutation is the most common cause of adRP in North America, although it is rare in other parts of the world.[9]

Two phenotypes of RHO-mediated adRP, Class A and Class B, have been described in the literature.[10] Class A patients present early in life with severe progressive loss of rod photoreceptors resulting in early night blindness. In this group, there is severe loss of rods; therefore, any potential treatments need to be targeted toward cone rescue. Class B patients, however, demonstrate milder manifestations with

Department of Ophthalmology, School of Medicine, Saint Louis University, St. Louis, MO, USA

***Address for correspondence:**
Dr. Niloofar Piri, 1225 S Grand Blvd, Saint Louis, MO, 63104, USA.
E-mail: niloofar.piri@health.slu.edu

Submission: 07-09-2021
Accepted: 02-10-2021
Published: 19-11-2021

How to cite this article: Piri N, Grodsky JD, Kaplan HJ. Gene therapy for retinitis pigmentosa. Taiwan J Ophthalmol 2021;11:348-51.

slowly progressive disease and more well-preserved rod function throughout the disease course. In this class, potential treatments can be focused on rod preservation, which would conceivably provide the opportunity for much better outcomes given that the preservation of rods will ultimately rescue cones and prevent central vision loss. The P23H mutation results in class B disease. In this review, we will focus on the potential of gene therapy for RHO-mediated adRP.

The RHO gene transcribes the RHO protein, which is the major visual pigment in the outer segment of rod photoreceptors. It absorbs light photons at 495 nm[11] and helps with vision in dim light. RHO is a G-protein-coupled receptor that is covalently bound to 11-cis retinal (a derivative of Vitamin A) in rod outer segment discs. Light absorption results in photoisomerization of 11-cis-retinal to all-trans retinal, which activates RHO and initiates a cascade of chemical reactions that create electrical signals. These signals are transmitted to the brain where they are interpreted as vision.[12]

The RHO molecule has 3 loops; the P23H mutation is where proline is substituted by histidine in location 23.[11] There is no proven treatment for P23H RP at this time. Multiple approaches, including gene therapy, are actively being studied.

Gene therapy is a therapeutic technique designed to either replace a gene of interest within a cell that lacks it or correct an abnormal gene of interest.[13] Over the years, gene therapy has shifted to deliver therapeutic genes to a group of target cells rather than individual cells.[13] Among ocular diseases, routes of delivery include intravitreal, subretinal, and/or suprachoroidal inoculations depending on the target cells. The intravitreal route is generally used to treat the inner retina, while the subretinal and suprachoroidal routes are generally used to treat the outer retina and RPE.[13,14]

There have been recent advances in gene therapy for the treatment of Leber's Congenital Amaurosis (LCA) type 2 (LCA2; NCT00999609), which is the result of a null mutation or biallelic loss of function in the RPE65 gene. The treatment has been promising and is easily studied, as it functions to replace the null mutation (i.e., a nonexisting gene) via subretinal injection of an adeno-associated virus (AAV) vector carrying a human RPE65 gene. This resulted in the first and only Food and Drug Administration (FDA) approved gene therapy for retinal degeneration to date (voretigene neparvovec-rzyl: Luxturna).[15]

In contrast to a disease with a null mutation such as LCA, autosomal dominant P23H RP requires a more complex approach to treat. It is the result of the patient having one copy of a wild type (WT) or functional gene and one copy of a mutated gene, which results in a misfolded protein and ultimately leads to rod photoreceptor degeneration.

There are two proposed methods of treatment for P23H RP - a targeted approach and nonselective inhibition. The concept behind the targeted approach is to inhibit the expression of the mutant RHO. However, since there are more than 150 mutations of RHO, it is difficult to selectively target and inhibit each mutation. On the other hand, nonselective inhibition of RHO involves two steps. The first, is to simultaneously downregulate both the WT and mutant RHO variants; the second, similar to the method described above, an exogenous WT or functional RHO gene is employed to restore the RHO WT gene function.[13,16] In this technique, AAV is used to initially deliver an RNA interference (RNAi)-based gene suppressor to knock down and inactivate the target mutated gene; then a separate AAV-vector, which is resistant to the RNAi, is used to deliver the functional, replacement gene.

Other methods that are being studied to achieve the above goal, are summarized below.

Anti-sense oligonucleotide-based therapy

Anti-sense oligonucleotide (ASOs) are single-stranded DNA molecules complementary to targeted mRNAs. These ASOs bind to mutated mRNA resulting in their degradation by recruiting a ribonuclease.[2,17] ASO remains intact and can inhibit additional targeted mRNAs.

P1123 is an ASO that targets the P23H mutation in the human RHO gene. This ASO knocks down the mutant gene without affecting the functional allele.[2] Experimental studies in mice and rats were promising and demonstrated a 40% decrease in the expression of the mutant gene without affecting WT gene expression after intravitreal injection.[18] One advantage to P1123 is that it can be delivered intravitreally, rather than subretinally. The small size of the molecule allows it to reach the outer retina when administered intravitreally.

P1123 received orphan drug and fast track designation for P23H-adRP by the FDA in 2019. ProQR therapeutics has sponsored a Phase I/II clinical trial with 35 participants and is estimated to be completed in October 2021 (ClinicalTrials.gov: NCT04123626).

Short hairpin RNA-based therapy

Short hairpin RNA (shRNAs) are small RNA molecules that form hairpin structures within the cell. They are processed within the cell by RNase to active small interference RNAs (siRNA). siRNA then binds to the

target mRNA, as explained above, and degrades it by recruiting a ribonuclease.

This has been tested in dual AAV suppression and replacement therapy of experimental mice models of P23H adRP in 2011.[16] An AAV-shRNA targeted RHO in an independent location from the mutation resulted in cleavage of both mutant and WT RHO. An AAV-shRNA-resistant RHO was then replaced by another vector, which resulted in expression of WT RHO. Both variants were delivered via subretinal injection. About 68% reduction in mutant variant expression was observed. Rod electroretinogram (ERG) b-wave amplitudes were also shown to significantly improve.

This dual vector shRNA suppression and replacement therapeutic strategy for P23H-adRP was named RhoNova and received orphan drug designation in Europe in 2010 and in the US in 2013. Roche has owned RhoNova since December 2019 with no further clinical updates.[2]

More recently, a gene therapy candidate for RHO-adRP composed of a single AAV2/5 vector expressing both an shRNA targeting human RHO and a healthy copy of the RHO gene-modified to be resistant to the shRNA has been developed. Iveric Bio has the license to commercialize this product candidate for the treatment of RHO-adRP, now named IC-100. The start of Phase I/II currently is delayed as they are evaluating the preclinical safety results further.

Correction of the Mutant Allele or Genome-Editing Gene Therapy

This more direct approach couples genome-editing technologies with homologous recombination to specifically correct mutations at the DNA level.[19] The latest and most promising tool for genome editing is the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated gene (Cas) system.[20] The innovative CRISPR technology has the ability to "cut" and remove the target cell's mutant gene and simultaneously add the desired functional gene with precision, affordability, and relative ease.[13,21]

CRISPR technology has the potential advantage of correcting the mutant genome permanently in non-dividing cells *in vivo*.[2] Promising experimental studies have recently inspired several clinical trials to utilize CRISPR to target a variety of retinal diseases, including CEP290-associated LCA (LCA10). Editas Medicine has started a Phase I/II nonrandomized clinical trial (ClinicalTrials.gov NCT03872479) for LCA10. It is planned to enroll 18 participants and to be completed in 2024.

Mega-Nuclease Gene-Editing Techniques

Meganucleases are "molecular DNA scissors" that can be used to replace, eliminate, or modify sequences in a highly targeted way. This genome editing technology, named ARCUS by Precision BioSciences, has since been applied in many preclinical studies of different liver diseases.[2] This approach requires that at least one copy of the WT gene is expressed in the targeted cell so that removal of the mutant protein is replaced by WT protein – i.e., a cell with a biallelic mutation would not be corrected by mega-nuclease gene editing *per se*. McCall *et al*. recently reported the therapeutic efficacy of ARCUS meganuclease in a transgenic mini-swine model of human P23H RHO-adRP.[22] A single subretinal injection of an AAV5 vector expressing P23H RHO-targeting ARCUS meganuclease was performed in unilateral eyes. Sustained reduction in mutant RHO expression, modest improvement in rod ERG response compared to untreated control eyes, as well as preserved histopathology of the outer retina were observed.

Optogenetics

Optogenetics is a novel gene therapy approach to address the limitations of traditional gene therapy options. Unlike most other retinal gene therapies, this approach can be used in diseases with significant photoreceptor loss, as seen in the early clinical trial using channelrhodopsin-2 to treat advanced RP.[23] In this technology, light-activating "optogenes" that convert the remaining bipolar cells and retinal ganglion cells into photosensitive cells are delivered, essentially behaving as artificial photoreceptors. Furthermore, this approach is gene independent, and can, therefore, be used for a variety of retinal degenerations. RST-001 Phase I/II Trial for Advanced RP has enrolled 14 participants (ClinicalTrials.gov NCT02556736) and is estimated to be completed in 2035. Safety data reported in June 2021 have not shown significant concerns.

Conclusion

The field of gene therapy has made extensive progress in medicine, including ophthalmology, in recent years. Gene therapy strategies are searching for the option that is simple, does not need to be repeated frequently, has the best efficacy results, least safety concerns, and least financial burden on patients and healthcare systems. Physicians should remain up to date on underlying disease pathophysiology and current developments in gene therapy, as it may very well be the answer to a once-incurable, blinding RP.

Financial support and sponsorship
Nil.

Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

References

1. Kaplan HJ, Wang W, Dean DC. Restoration of cone photoreceptor function in retinitis pigmentosa. Transl Vis Sci Technol 2017;6:5.
2. Meng D, Ragi SD, Tsang SH. Therapy in rhodopsin-mediated autosomal dominant retinitis pigmentosa. Mol Ther 2020;28:2139-49.
3. Wang W, Lee SJ, Scott PA, Lu X, Emery D, Liu Y, *et al.* Two-step reactivation of dormant cones in retinitis pigmentosa. Cell Rep 2016;15:372-85.
4. Narayan DS, Wood JP, Chidlow G, Casson RJ. A review of the mechanisms of cone degeneration in retinitis pigmentosa. Acta Ophthalmol 2016;94:748-54.
5. Caruso S, Ryu J, Quinn PM, Tsang SH. Precision metabolome reprogramming for imprecision therapeutics in retinitis pigmentosa. J Clin Invest 2020;130:3971-3.
6. Malanson KM, Lem J. Rhodopsin-mediated retinitis pigmentosa. Prog Mol Biol Transl Sci 2009;88:1-31.
7. Dias MF, Joo K, Kemp JA, Fialho SL, da Silva Cunha A Jr., Woo SJ, *et al.* Molecular genetics and emerging therapies for retinitis pigmentosa: Basic research and clinical perspectives. Prog Retin Eye Res 2018;63:107-31.
8. Dryja TP, McGee TL, Hahn LB, Cowley GS, Olsson JE, Reichel E, *et al.* Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. N Engl J Med 1990;323:1302-7.
9. Jacobson SG, Kemp CM, Sung CH, Nathans J. Retinal function and rhodopsin levels in autosomal dominant retinitis pigmentosa with rhodopsin mutations. Am J Ophthalmol 1991;112:256-71.
10. Cideciyan AV, Hood DC, Huang Y, Banin E, Li ZY, Stone EM, *et al.* Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. Proc Natl Acad Sci U S A 1998;95:7103-8.
11. Gorbatyuk MS, Knox T, LaVail MM, Gorbatyuk OS, Noorwez SM, Hauswirth WW, *et al.* Restoration of visual function in P23H rhodopsin transgenic rats by gene delivery of BiP/Grp78. Proc Natl Acad Sci U S A 2010;107:5961-6.
12. Sakmar TP, Huber T. Rhodopsin. In: Squire LR, editor. Encyclopedia of Neuroscience. Oxford: Academic Press; 2009. p. 365-72.
13. Prado DA, Acosta-Acero M, Maldonado RS. Gene therapy beyond luxturna: A new horizon of the treatment for inherited retinal disease. Curr Opin Ophthalmol 2020;31:147-54.
14. Planul A, Dalkara D. Vectors and gene delivery to the retina. Annu Rev Vis Sci 2017;3:121-40.
15. Maguire AM, Russell S, Wellman JA, Chung DC, Yu ZF, Tillman A, *et al.* Efficacy, safety, and durability of voretigene Neparvovec-rzyl in RPE65 mutation-associated inherited retinal dystrophy: Results of phase 1 and 3 trials. Ophthalmology 2019;126:1273-85.
16. Millington-Ward S, Chadderton N, O'Reilly M, Palfi A, Goldmann T, Kilty C, *et al.* Suppression and replacement gene therapy for autosomal dominant disease in a murine model of dominant retinitis pigmentosa. Mol Ther 2011;19:642-9.
17. Crooke ST. Molecular mechanisms of antisense oligonucleotides. Nucleic Acid Ther 2017;27:70-7.
18. Murray SF, Jazayeri A, Matthes MT, Yasumura D, Yang H, Peralta R, *et al.* Allele-specific inhibition of rhodopsin with an antisense oligonucleotide slows photoreceptor cell degeneration. Invest Ophthalmol Vis Sci 2015;56:6362-75.
19. Diakatou M, Manes G, Bocquet B, Meunier I, Kalatzis V. Genome editing as a treatment for the most prevalent causative genes of autosomal dominant retinitis pigmentosa. Int J Mol Sci 2019;20:2542.
20. Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 2014;346:1258096.
21. Peddle CF, MacLaren RE. The application of CRISPR/Cas9 for the treatment of retinal diseases. Yale J Biol Med 2017;90:533-41.
22. McCall MA, Jalligampala A, Fransen J, Noel J, Wang W, Fleissig E, *et al.* Therapeutic Efficacy of ARCUS Meganuclease Gene Editing-Arrest of Rod Degeneration and Restoration of Rod Function in a Transgenic Pig Model of Autosomal Dominant Retinitis Pigmentosa. Paper Presented at: Molecular Therapy; 2020.
23. Simunovic MP, Shen W, Lin JY, Protti DA, Lisowski L, Gillies MC. Optogenetic approaches to vision restoration. Exp Eye Res 2019;178:15-26.