Review

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CRISPR-Cas9 Gene Therapy Effects on Inherited Eye Disorders

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Abstract: Stargardt's Disease (STGD1), Retinitis pigmentosa (RP), and Leber's congenital amaurosis (LCA) inherited eye conditions that are among the main causes of blindness and visual impairment. The primary cause of these diseases, which lead to gradual vision loss, is genetic mutations affecting retinal cells. Gene-editing technologies, especially the CRISPR-Cas9 system, are emerging as promising ways to correct harmful genetic mutations. This review focuses on how effectively CRISPR-Cas9 can be used to treat certain inherited eye conditions. In experiments with patient-derived human induced pluripotent stem cells (hiPSCs), researchers were able to fix STGD1 mutations in the ABCA4 gene using CRISPR-Cas9. The corrected cells retained their ability to differentiate into different cell types and showed minimal off-target effects. This suggests the approach could offer a safe and precise way of treating these conditions. In RP, CRISPR-Cas9 was used to target the RPGR gene in mice models, successfully preserving the shape and function of the photoreceptor cells. The CEP290 mutation in LCA10 was corrected in human patients using the CRISPR-Cas9 based EDIT-101 in the BRILLIANCE clinical trial, which demonstrated a notable improvement in visual outcomes with minimal adverse effects. Even though the results so far are promising, challenges remain including long-term safety, the administration of these treatments, and the risk of unintended effects. This review emphasizes the need for further research and clinical trials to improve these therapies. It also highlights the exciting potential of CRISPR-Cas9 as a curative treatment for inherited retinal disorders.

Keywords: CRISPR-Cas9; gene editing; Stargardt's disease; retinitis pigementosa; Leber's congenital amaurosis

Introduction

The human eye is a very complex organ consisting of three major tissues: cornea, lens, and retina.[1] Damage to any of these tissues can lead to visual impairments. Retinal diseases or damage to the retina is the primary cause of visual impairments.[2] Stargardt's disease, Retinitis pigmentosa, and Leber's congenital amaurosis are inherited eye disorders caused by damage to the retina or macula.[3] Stargardt's disease is a macular dystrophy since the macula and cones are degenerated.[3] Retinitis pigmentosa and Leber's congenital amaurosis are retinal dystrophies since the rods are affected more than the cones.[3] Regardless of the dystrophy, there is progressive vision loss in all three disorders.[3]

Clustered regularly interspaced palindromic repeats (CRISPR)-Cas9 is a gene editing tool that functions to correct errors in the genome.[4] CRISPR-Cas9 turns the genes on or off to fix the mutation.[4] CRISPR-Cas9 can correct a disease mutation, thus curing the genetic disorders of the mice.[4] The two main components of CRISPR-Cas9 are guide RNA and Cas9.[4] The guide RNA matches the sequence of the target gene, and Cas9 is an endonuclease that makes a double-stranded DNA break at the specific location on the genome.[4] After the cut has been made, the cell's natural DNA repair mechanisms, homology-directed repair (HDR), and non-homologous end-joining (NHEJ) are recruited to repair the double-stranded break, which allows gene editing.[5]

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Gene editing has advanced greatly within the last decade.[6] However, gene editing and gene therapy are in their early stages of development.[6] This focused review aims to study the advancement of gene editing and gene therapy in Stargardt's disease, Retinitis pigmentosa, and Leber's congenital amaurosis.

Stargardt's Disease

Stargardt's disease (STGD1), also known as juvenile macular dystrophy, is an inherited autosomal recessive eye disorder.[7] The disease causes vision loss and retinal degeneration in adults and children.[7] Early onset of STGD1 is between 1 to 10 years of age.[8] The range of late-onset STGD1 is 45 to 72 years of age.[9] The prevalence of the disease is 1 in 8,000 to 10,000 individuals.[10] STGD1 causes retinal degeneration and vision loss.[7] Such eye impairments are caused by a biallelic mutation on the ABCA4 gene.[11] The ABCA4 gene plays a vital role in recycling retinoid byproducts.[12] However, a mutation in the ABCA4 gene can prevent the transportation of byproducts, thus leading to toxic buildup in the retina, which causes visual impairments.[13]

Human induced pluripotent stem cells (hiPSCs) are stem cells which can generate new cells from the human body to be studied.[14] The use of hiPSCs is relevant to studying eye-related diseases since access to brain cells is challenging.[15] However, with the use of hiPSCs from patients with neurodegenerative disease, conducting studies can be efficient.[15]

CRISPR-Cas9 and transcription activator-like effector nucleases (TALENs) are efficient tools for gene editing.[16] Both methods involve inducing DNA double-strand breaks and recruiting cell repair mechanisms for genetic change.[16] A difference between the two methods is that CRISPR relies on single-guide (sgRNA) to target specific DNA sequences, while TALENs use DNA-binding domains (DBDs) to target the specific DNA.[17] The ability to have higher cleavage efficiency makes CRISPR-Cas9 preferable over TALENs.[7]

The two main ABCA4 gene variants are c.4253+4C>T and c.3211_3212instGT.[7] These variants cause a splicing defect and generate a frameshift, respectively.[7] Even though there are no current treatments for STGD1, the investigation conducted aims to correct the two ABCA4 gene mutations using both hiPSCs and CRISPR-Cas9, along with testing the efficacy of gene editing by CRISPR-Cas9.[7] Therefore, fixing the mutation of the ABCA4 gene in STGD1 can be used as a potential therapeutic tool.[7]

Fibroblasts, patient-derived cells, were reprogrammed to hiPSC lines and collected from two patients with STGD1 with ABCA4 heterozygous mutations.[18] The pathogenicity of each variant was determined using the ENSEMBL Variant Effect Predictor (VEP).[19],[7] VEP is a software device used to determine the impact of a genetic mutation with the use of algorithms.[19],[7] Single-guide RNA and TALENS were designed, and sgRNAs were selected based on efficacy and with the least off-target effects.[7] CRISPR-Cas9 was introduced into the hiPSCs; this was done by transfecting hiPSCs with ribonucleoprotein (RNP).[7] The RNP consists of Cas9, sgRNA, and single-strand oligodeoxynucleotide (ssODN), which are necessary for gene editing.[7] The function of ssODN is to allow precise gene editing by allowing homology-directed repair (HDR) mediated modification to take place.[7],[20] PCR amplification was completed on the ABCA4 gene target region with the new modification made by CRISPR-Cas9.[7] Additionally, Sanger sequencing was carried out to determine whether the correction of the ABCA4 gene resulted in off-target alterations.[7] Lastly, RT-PCR was conducted to determine if the ABCA4 gene had been corrected and if the cells could differentiate into the three germ layers.[7]

Upon conducting the *in silico* analysis, the main two variants of the ABCA4 mutations were all missense mutations except c.514G>A, which are pathogenic.[7] Additionally, c.3211_3212inGT and c.2023G>A are the main ones causing STGD1 since these variants appear at a low frequency in the population.[7]

The cleavage efficiency for sgRNA/Cas9 was between 15% and 45%, which was higher than that of the DNA cleavage performed by TALENS.[7] Due to the low cutting made by TALENs, CRISPR-Cas9 was used to correct the STGD1 mutations in the ABCA4 gene.[7]

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The use of ssODN also plays an important role in gene editing since RNP has incorporated the ssODN into the CRISPR-Cas9 gene editing mechanism.[7] ssODNs are precise in carrying out HDR-mediated repairs, and ssODNs repair single-nucleotide substitutions.[21] HDR-mediated modifications are much more precise than non-homologous end joining (NHEJ) because NHEJ is more error-prone and more likely to generate indels when the two ends of the double-stranded breaks are re-ligated.[7] Due to this, ssODN was used in the research conducted by Siles et al.

The gene-edited hiPSC clones were checked to see whether differentiation occurred to form the three germ layers.[7] RT-PCR and immunofluorescence indicated that the gene-edited cells can divide into the three germ layers.[7] Thus, indicating that pluripotency is conserved.[7]

Two sgRNAs had off-target effects, sgRNA2 had seven, and sgRNA6 had 57 off-targets.[7] After conducting Sanger sequencing, it was confirmed that the editing targeted the correct location of interest and did not cause any other genomic alterations.[7]

Even though there have been no therapies to cure visual loss in STGD1 patients, there have been studies to analyze the effects of CRISPR-Cas9-mediated gene editing.[22] However, there are issues with an *in vivo* CRISPR-Cas9 system due to the off-targets and immune or inflammation responses.[22] The report indicated that two sgRNAs had off-target effects, and even though they did not cause any genomic issues, gene editing still caused off-target effects.[22]

Additionally, the hiPSCs have made it useful to examine neurodegenerative diseases such as STGD1, where the brain cells cannot be accessed.[15] RNP complexes with Cas9, sgRNA, and ssODN resulted in precise gene editing with ssODN along with a reduction in Cas9 re-cutting on the sites that have been edited.[21],[22] Delivering Cas9 via the RNP complex is considered to be more efficient for single-base substitution in comparison to plasmid-based Cas9 transfection.[20]

The use of ssODN gene editing results in the ABCA4 gene being edited without causing genomic alterations, thus indicating that CRISPR-Cas9 can be potentially used for the treatment of STGD1.[7] Studies conducted with hiPSCs and CRISPR-Cas9 have provided useful information about gene editing.[7] However, in vivo, CRISPR-Cas9 is still in the early stages, and more research needs to take place.[7] There needs to be further research on the long-term effects and side effects of using CRISPR-Cas9 for treating STGD1.[7]

Retinitis Pigmentosa

Retinitis Pigmentosa (RP) is an inherited disorder where damage to the photoreceptors; rods and cones in the eye result in progressive visual loss. It is long lasting and usually evolves over time.[33] There are two different kinds: non-syndromic or syndromic. Syndromic RP can affect other organs or tissues in the body whereas non-syndromic is when the eye condition occurs alone, and the only affected organ is the eye. Most of the RP cases are non-syndromic, about 20%-30% are syndromic.[24] In RP, the most common genes that are mutated are the ones involved in the visual cascade.[34] The visual cascades consist of the photochemical reaction when light is converted to an electrical signal in the retina.[35]

RP has many clinical manifestations; some are night blindness, dust like particles in the vitreous, posterior cataracts, and fundus damage. Night blindness is one of the symptoms that first occurs because the rods are damaged and cannot properly adjust to dim lights. Early diagnosis can be detected with dust like particles in the vitreous. The severity of the posterior cataracts associated with RP corresponds to the age of those who are affected. Lastly, the appearance of the fundus in RP also helps determine the stage of the disease when no other symptoms occur. [25] The fundus of the eye is essential to vision because it is composed of the retina, macula, optic, disc, fovea, and blood vessels. [36]

The prevalence of RP is 1 in 3,000 to 1 in 7,0000 people.[24] There are about 181 genes that are known to cause inherited RP at a chromosomal site and about 129 at sequence level, demonstrating how common RP is. [26] A specific mutation that will be discussed in more detail are mutations in the retinitis pigmentosa GTPase regulator (RPGR) which was associated with X-linked retinitis

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pigmentosa (XLRP) which seem to account for 10%-20% of all RP cases as well as one of the most severe. [27]

Currently, there is only one approved treatment for a portion of a sub-population of patients experiencing RP. [31] Otherwise, there are many treatments that help manage the condition or improve vision loss, such as retinal transplants for more severe cases, vitamin therapy to protect photoreceptors, and gene therapy.[32] Gene editing therapy has been used as a primary way to study the genetic characteristics of many diseases including RP. Gene editing for the retina is considered to be ideal because it is easily accessible and is also isolated by the blood retinal barrier.[28] CRISPR-CAS9 has shown promising treatments for RP and many other diseases. [23]

The research shows how specific gene therapies in rodents can lead to promising treatments in humans for RP. The purpose of the investigation was to test the efficacy of Short Palindromic Repeat/Cas9 mediated gene editing therapy in rodents with RPGR. Mice with Rpgr−/yCas9+/WT received subretinal injections of adenovirus vectors with the sgRNA and donor template and their therapeutic effects were examined at one, six, and 12 months. A six-month-old mouse is equivalent to a human in their 30's, a 12-month-old mouse is equivalent to a human in their mid 40's and an 18-month-old mouse is equivalent to an old, aged human.

A total of 72 mice were used in the study where mRNA of vitro transcribed Cas9 and sgRNA were injected into the zygotes of the mice and they were authenticated with PCR and sequencing. After receiving the injection, the mice were given 1% atropine drops and neomycin-polymyxin B-dexamethasone ophthalmic ointments. Atropine drops are a type of medication used to dilate the pupils.[28] Neomycin-polymyxin B- dexamethasone used in conjunction helps treat eye inflammation and can help prevent damage caused by foreign objects in the eye. So, these were used to prevent any other damage that could possibly occur when the subretinal injections were administered.[29] In order to determine the effects of the injection, histology and immunofluorescence, fundus photography, and DNA analyses were conducted. The immunofluorescence technique allows for the detection of antigens in different tissues.[30] The study looked at the retinal section of the WT and RPGR mice.

The photoreceptors of the mice with the engineered RPGR showed slow retinal degeneration. The result from the retina fundus imaging showed that up to three months, small yellow white spots appeared but by 12 months, the spots became sparse but there was still pigment deposition. The histological evaluation between the retinal sections of the WT and the RPGR showed that up to three months of age the retina of the RPGR mice showed subtle age-related loss of photoreceptor cells by six months of age and by 12 months, the loss of photoreceptor cells was greater. The retinal morphology of the mice who were treated with the mutant RPGR by the two different vectors showed that there was extensive and great retinal photoreceptor preservation. Nine layers of the retina had preserved photoreceptors while the untreated portion of the retina only had five. The outer nuclear layer of the treated areas was also thicker than those that were not treated.

Other gene editing therapies have been tested on RP and many other diseases. However, CRISPR-Cas9 has been shown to be a promising therapy. Some other therapies that help treat a certain disease add a functional or a partially functional copy of a gene. They also leave a dysfunctional copy of a gene. On the other hand, CRISPR-Cas9 can generate specific modifications to a locus and eliminate any defective DNA. The results from the study show that the CRISPR-Cas9 mediated RPGR gene therapy prevented any photoreceptor degeneration and that the treatment was able to preset for longer time periods. Even though the study was performed on rodents, the results are promising in translation to CRISPR-Cas9 in humans.

Leber's Congenital Amaurosis

Leber's Congenital Amaurosis (LCA) is one of the most severe retinal dystrophies associated with childhood blindness.[37] LCA is a rare inherited eye disorder prevalent in 1/30,000 people and 1/81,000 newborn babies worldwide.[38] Although rare, it is one of the most common inherited

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retinal diseases (IRDs) with a prevalence of greater than 5%.[38] The disease causes severe vision loss and degeneration in the photoreceptor cells, specifically the rods and cones, in the retina.[39]

LCA is associated with mutations in approximately 38 different genes.[40] One of the most common mutations causing the disease is in the CEP290 gene.[40] There are 18 known types of LCA, each caused by a different gene mutation.[41] The CEP290 mutation causes LCA10 which is responsible for more than 30% of all cases of LCA globally.[42] In the United States, this mutation is responsible for 77% of cases.[43] The CEP290 mutation results from a switch in a base sequence from adenine to guanine in intron 26 on chromosome 12.[43] This mutation generates an aberrant splice site, leading to the integration of a cryptic exon into the DNA sequence, resulting in a truncated CEP290 protein which leads to the symptomology associated with LCA10.[43]

Currently, there are no known cures for this mutation, but multiple clinical trials are underway to evaluate possible curative treatments.[41] The most impactful clinical trial that has been published is the BRILLIANCE trial.[44] This study specifically looked at treating the CEP290 mutation with a CRISPR-Cas9 sequence to regenerate the loss of photoreceptor cells in the retina.[44] This trial was the first human-based CRISPR-Cas9 ocular disease model and used EDIT-101, a specific CRISPR-Cas9, that contained an adeno-associated virus 5(AAV5), staphylococcus aureus Cas9 nuclease, G coupled protein kinase 1, and guide RNAs.[44] These factors are required for any CRISPR Cas9 model to help localize the cut in the DNA sequence for the specific mutations.[45],[46]

The study had fourteen participants, two of which were children aged nine and fourteen.[44] The participants were either homozygous or heterozygous in their inheritance of LCA10.[44] The study's main goals were the safety of the treatment and to look at the efficacy of the gene therapy in treating LCA.[44] The efficacy was analyzed using tests examining four main outcomes: visual acuity, retinal sensitivity, visual-related functional navigation, and vision-related quality of life.[44] All of these factors are impacted by patients with the CEP290 mutation that results in LCA.

The changes in participants' baseline visual acuity were looked at using the Early Treatment Diabetic Retinopathy Study (ETDRS) and the Berkley Rudimentary Vision Test (BRVT).[44] The ETDRS is a chart that contains multiple rows of equally spaced five-letter groupings that get increasingly smaller as you move downward.[47] The participants who were determined to have reduced visual acuity based on the ETDRS chart used the BRVT to find their visual acuity.[44] The BRVT is a simple and effective test used to look at visual acuity in individuals determined to have significantly reduced visual acuity.[48] Retina sensitivity to red, blue, and white light was measured with a full field stimulus test (FST).[44] Visual-related functional navigation was measured using the Ora-Visual Navigation Challenge (VNC), a mobility test requiring participants to navigate obstacles under different light conditions.[49] Finally, the participant's vision-related quality of life was measured in children using the Children's Visual Function Questionnaire (CVFQ) and in adults using the National Eye Institute Visual Function Questionnaire–25 (NEI VFQ-25).[44]

EDIT-101 was administered as a single-dose subretinal injection after participants underwent a pars plana vitrectomy and three days of oral prednisone.[44] The purpose of the pars plana vitrectomy is to remove some part of the vitreous humor in the eye to allow easier access to the posterior part of the eyeball for the subretinal injection.[50] The EDIT-101 was given in either a low dose for cohort 1, an intermediate dose for cohort 2, or a high dose for cohort 3; cohort 4 consisted of the two children participants who received the intermediate dose.[44] After the EDIT-101 injection, oral prednisone was continued for three weeks in all cohorts.[44]

After two years of monitoring the participants for varying amounts of time, the results showed significant improvements due to the insertion of the EDIT-101 CRISPR-Cas9 treatment.[44] The treatment was safe, with 22 adverse side effects reported.[44] Most of these side effects were predicted and expected.[44] The treatment was also effective, with eleven out of the fourteen participants finding support in at least one of the four main efficacy outcomes of visual acuity, retinal sensitivity, visual-related functional navigation, and vision-related quality of life.[44] Six out of the fourteen participants found improvement in two or more efficacy outcomes.[44]

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This study supports future research involving CRISPR-Cas9 as a potential curative treatment for the genetic mutation of CEP290. Most participants showed efficacy of inserting EDIT-101, a CRISPR-Cas9 gene therapy, with minimal adverse effects. It also shows support for conducting more research on other mutations responsible for LCA, such as CRB1, GUCY2D, and RPE65.[51] The BRILLIANCE clinical trial opens the door for future human studies to be done, as the trial showed gene editing as a possible treatment for mutations that cause other IRDs like LCA.

Overall, the BRILLIANCE clinical trial was a pivotal study in showing genetic therapies' impact on human inherited diseases. Further study in CRISPR-Cas9 models may show the potential to generate treatments for diseases caused by mutations with previously unknown cures.

Conclusion

CRISPR-Cas9, an important tool for gene editing, has advanced tremendously within the last decade.[6] This covered the three eye disorders STDG1, RP, and LCA to determine the effects of CRISPR and whether the treatment can be used as gene therapy.

The study with STDG1 showed promising gene editing in hiPSCs and CRISPR-Cas9.[7] However, further studies need to be conducted to do in vivo gene editing with CRISPR-Cas9.[7] The study with RP in mice indicated that CRISPR-Cas9 mediated with Rpgr gene therapy prevents photoreceptor degeneration. However, further studies need to be conducted to see the effects of CRISPR-Cas9 on humans.[27] The study with EDIT-101 and its efficacy with the CEP290 mutation was effective in the human participants with LCA10 and showed promise for further in vivo studies on humans to correct inherited genetic mutations.[44] The findings in these studies demonstrate the effects of CRISPR-Cas9 on STDG1, RP, and LCA and provide strong support for future research into genetic therapy as a possible curative treatment for eye diseases caused by mutations in the human genome.

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