The ROYAL COLLEGE of OPHTHALMOLOGISTS

www.nature.com/eye

REVIEW ARTICLE

Check for updates

The application and progression of CRISPR/Cas9 technology in ophthalmological diseases

Xumeng Hu[1], Beibei Zhang[1], Xiaoli Li[1], Miao Li[1], Yange Wang[1], Handong Dan[1], Jiamu Zhou[1], Yuanmeng Wei[1], Keke Ge[1], Pan Li[1] and Zongming Song[1]✉

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated nuclease (Cas) system is an adaptive immune defence system that has gradually evolved in bacteria and archaea to combat invading viruses and exogenous DNA. Advances in technology have enabled researchers to enhance their understanding of the immune process in vivo and its potential for use in genome editing. Thus far, applications of CRISPR/Cas9 genome editing technology in ophthalmology have included gene therapy for corneal dystrophy, glaucoma, congenital cataract, Leber's congenital amaurosis, retinitis pigmentosa, Usher syndrome, fundus neovascular disease, proliferative vitreoretinopathy, retinoblastoma and other eye diseases. Additionally, the combination of CRISPR/Cas9 genome editing technology with adeno-associated virus vector and inducible pluripotent stem cells provides further therapeutic avenues for the treatment of eye diseases. Nonetheless, many challenges remain in the development of clinically feasible retinal genome editing therapy. This review discusses the development, as well as mechanism of CRISPR/Cas9 and its applications and challenges in gene therapy for eye diseases.

Eye (2023) 37:607–617; https://doi.org/10.1038/s41433-022-02169-1

INTRODUCTION

Developments in DNA sequencing technology and improvements in disease awareness have led to the discovery and characterization of novel disease-related gene mutations. Currently, there is no effective treatment for hereditary diseases caused by gene mutations, but recent clinical trials of gene replacement therapy have shown good efficacy and safety; thus, gene therapy has broad application prospects [1]. Importantly, the clustered regularly interspaced shot palindromic repeats (CRISPR)/CRISPR-associated nuclease (Cas) 9 system has been developed as a promising genome editing tool in many medical fields including the treatment of eye diseases. This system has a precise targeting function that allows the creation of biological models via targeted modification; it also facilitates the correction of disease mutations for therapeutic purposes. The Cas9 endonuclease is directed by a specific single guide RNA (sgRNA) to recognize a 23-bp DNA sequence, which contains a target specific 20-bp sequence plus an adjacent NGG nucleotide motif (where N is any nucleotide and G is guanine), named the protospacer-adjacent motif (PAM). DNA double-strand breaks (DSBs) induced by Cas9 nuclease are conformed and can be inserted, deleted, repaired, or replaced by two cellular repair processes: nonhomologous end joining (NHEJ) and homology-directed repair (HDR). These DSB repair mechanisms offer a common approach for genome editing. A third DSB to repair method is micro-homology-mediated end joining, which is also called alternative NHEJ.

Gene therapy has the potential to provide long-term benefits to human health; thus, there is considerable effort to make gene therapy a component of standard treatment, particularly in ophthalmology. The retina is an extension of the central nervous system; it thus provides a powerful and unique "window" for investigation of neuronal diseases. The blood-retinal barrier ensures immune privilege in ocular tissues, which allows tolerance to foreign antigens and viral vectors; it also facilitates antigen-specific inhibition of both cellular and humoral immune responses [2]. The eye is easily accessible, small, and highly compartmentalized; this facilitates specific targeting of different ocular tissues and allows small quantities of vector to be used. Additionally, the presence of two eyes in most animals allows for within-subject comparisons, where one eye receives experimental treatment and the other eye serves as a control. Because of its specific advantages as a target tissue, the eye has received considerable focus during the development of innovative gene therapies; eye diseases are thus at the forefront of translational studies that involve gene-based therapies.

Research background

Research concerning CRISPR sequences dates back to 1987. While studying an isozyme of intestinal alkaline phosphatase in *Escherichia coli*, Ishino et al. [3] discovered a 29-nucleotide repeat sequence downstream of the intestinal alkaline phosphatase gene. Unlike most repeat sequences, this 29-nucleotide repeat sequence was interspaced by five non-repeat sequences of 32 nucleotides; however, this phenomenon initially did not receive attention from researchers. In 2000, Mojica et al. discovered that this particular repeat sequence was present in >40% of tested

[1]Henan Eye Hospital, Henan Eye Institution, Henan Provincial People's Hospital, Zhengzhou University People's Hospital, Zhengzhou 450003 Henan, China.
✉email: szmeyes@126.com

Received: 15 February 2022 Revised: 7 June 2022 Accepted: 30 June 2022
Published online: 1 August 2022

bacteria and 90% of tested archaea, which was recognized as one family in many prokaryotic species. In 2002, Mojica and Jansen et al. [4] renamed this repeat sequence "clustered regularly interspaced short palindromic repeats" (CRISPR); they reported the presence of various meaningful coding sequences around this repeat sequence, which they termed Cas sequences. Their report provided the basis for the definitive CRISPR system classification (Types I–III).

In 2005, three research teams successively reported that the spacer DNA sequences of CRISPR originated in phages of foreign invading bacteria, rather than in prokaryotes themselves [5–7]. Subsequently, various hypotheses were proposed. In 2007, Barrangou et al. [8] screened bacteriophage-resistant strains by co-culturing Streptococcus thermophilus with different phages. DNA sequencing revealed a novel intersequence in the CRISPR locus, which was derived from invading phage DNA; this finding confirmed that the CRISPR/Cas sequences facilitated phage resistance in bacteria, thus providing experimental evidence that CRISPR/Cas is an acquired immune system in bacteria. The findings also demonstrated that Cas sequences are necessary for CRISPR functionality; the system was thus named the CRISPR-Cas system. Since then, multiple studies have been conducted regarding the CRISPR defence mechanism [9, 10]. In 2010, the basic functions and mechanisms of CRISPR were clarified, although its application remained limited. Two studies revealed the functions and mechanisms of the type II CRISPR system. First, in a study of *S. thermophilus*, Moineau et al. [11] found that Cas9 is the only enzyme in the Cas gene cluster that mediates targeted DNA cleavage. Charpentier et al. [12] identified a trans-activating CRISPR RNA (tracrRNA), which directed the maturation of CRISPR RNA (crRNA) in a manner mediated by the conserved endogenous RNase III and the CRISPR-associated Csn1 protein. In 2012, Jinek et al. [13] discovered that the mature crRNA, which defines target specificity, is base-paired to tracrRNA and forms a two-RNA structure called sgRNA; this structure greatly simplified delivery in that it could direct Cas9 to introduce DNA cleavage in vitro (Fig. 1). In 2013, multiple teams reported successful genome editing of several sites in mammals [14, 15]. Subsequently, the RNA-mediated CRISPR/Cas9 genome editing technology has been extensively used in laboratories worldwide.

CRISPR/Cas9 is a third-generation genome editing technology that rapidly developed after zinc finger nucleases and transcription activator-like effector nucleases. Compared with zinc finger nucleases and technology transcription activator-like effector nucleases, CRISPR/Cas9 is simpler and faster in terms of design, synthesis, and screening; it can also target multiple gene sites for simultaneous editing in a single cell, which has doubled genome editing efficiency and facilitated multiplexed genome editing [16]. Because of its simplicity, economical and efficient nature, and lack of species restrictions, CRISPR/Cas9 genome editing technology has been the focus of rapid development. However, the instability and off-target effect of Cas proteins have partially limited its application [17].

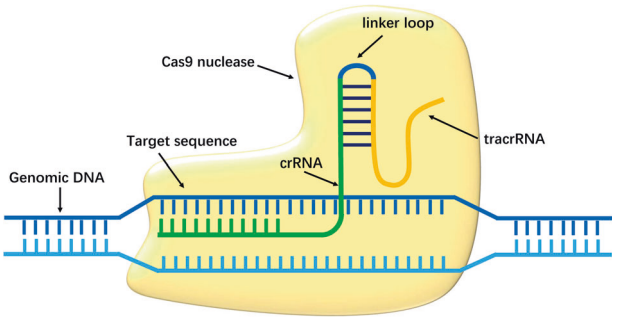

Fig. 1 Schematic representation of the Clustered-regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated (Cas) 9 system. Artificial construction of the type II CRISPR/Cas9 system enables direct chimerization of CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) to form single guide RNA (sgRNA) by linker loop. sgRNA guides Cas9-mediated cleavage at a specific site in the target gene sequence to generate double-stranded breaks, thus inducing the activation of either error-prone nonhomologous end-joining or generally error-free homology-directed repair to modify DNA in the target cell.

Mechanism

The specific mechanism of CRISPR system-mediated acquired immunity in bacteria is as follows. After a bacterium is infected by a phage, the CRISPR system recognizes the PAM sequence in the phage gene, which is a conserved sequence located at both ends of the original spacer (generally in the form of NGG); the Cas enzyme then cleaves the original spacer sequence and integrates it into the CRISPR locus. When the bacterium is later infected by the same type of phage, CRISPR locus expression is induced the locus is transcribed and post-transcriptionally processed to produce crRNA. The crRNA binds to tracrRNA and forms a complex with a Cas protein to mediate DNA cleavage, thereby resisting phage invasion [13]. Artificial construction of the type II CRISPR/Cas9 system enables direct chimerization of crRNA and tracrRNA to form sgRNA. SgRNA guides the Cas9 -mediated cleavage at a specific site in the target gene sequence to generate DSBs, thus inducing the activation of either error-prone NHEJ or relatively error-free HDR to modify DNA in the target cell (Fig. 2). In the absence of DNA repair template, DSBs will cause various mutations via NHEJ (e.g., substitution, deletion, or insertion), which can be used for gene knockout procedures. When exogenous DNA is used as a DNA repair template, accurate base replacement or insertion can be achieved via HDR, which can be used for gene insertion procedures [18]. The NHEJ mechanism is simple and does not rely on templates. NHEJ has a high probability of error via small insertions and deletions; genome editing technology utilizes the tendency of NHEJ to cause frame shift mutations that result in early stop codons. In contrast, HDR requires high sequence homology between intact donor DNA and the mutant strands; it is a less error-prone process. Therefore, with the tools currently available, NHEJ is more active and efficient than HDR; the NHEJ repair pathway is the most common approach for treatment of retinal diseases, particularly mutations caused by autosomal dominant disorders.

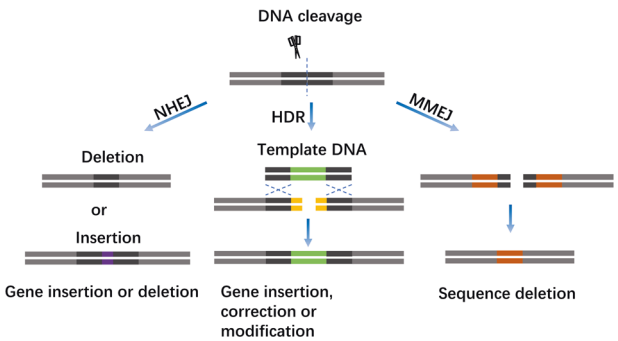

Fig. 2 Schematic representation of a double-stranded break (DSB; blue dotted line), which can be repaired through nonhomologous end-joining (NHEJ), homology-directed repair (HDR) or microhomology-mediated end joining (MMEJ) pathways. In the absence of DNA repair template, DSBs will cause various mutations via NHEJ (e.g., substitution, deletion, or insertion), which can be used for gene knockout procedures. When exogenous DNA is used as a DNA repair template, accurate base replacement or insertion can be achieved via HDR, which can be used for gene insertion procedures. The ends of the DSB are linked via microhomologous domains when MMEJ occurs, which can cause a sequence deletion at the position.

APPLICATION OF THE CRISPR/CAS9 SYSTEM IN THE TREATMENT OF GENETIC EYE DISEASES

The most promising application of CRISPR-Cas9 technology is gene therapy for genetic disease, which are caused by changes in genetic material; or controlled by disease-causing genes. Gene therapy for genetic eye diseases is a major research focus worldwide. Clinical trials of gene replacement therapy have been conducted for the treatment of various genetic eye diseases [1, 19–21]. Compared with the introduction of foreign genes, direct repair of mutated genes via genome editing technology is considered safer. As an efficient genome editing technology, CRISPR/Cas9 is able to simultaneously edit multiple genes in a specific and effective manner, thus offering hope for the treatment of genetic eye diseases. Additionally, combinations of CRISPR/Cas9 technology with other methods can facilitate the treatment of related eye diseases, such as adeno-associated virus (AAV) and induced pluripotent stem cell (iPSC) methods. Current CRISPR-based treatment strategies mainly include the use of NHEJ to silence disease-causing genes or delete erroneously inserted disease-causing genes, as well as the use of HDR to correct mutated disease-causing genes or introduce correct genes to replace disease-causing genes. This review will illustrate the status of CRISPR/Cas9 technology development, analyze problems in the CRISPR/Cas9 system, explore the prospects of the CRISPR/Cas9 system, and provide new ideas for animal models of human disease and gene therapy advancement; we discuss progress in applying the CRISPR/Cas9 system to eye diseases, in the order of ocular anatomy, from anterior segment to posterior segment (Table 1).

Corneal dystrophies

Corneal dystrophies are slowly progressive inherited diseases that can lead to reduced corneal transparency, altered corneal shape, and vision loss. Meesmann's epithelial corneal dystrophy (MECD) is a rare autosomal dominant corneal disease, also known as juvenile epithelial corneal dystrophy. It is characterized by small dot opacities and cysts that are symmetrically distributed on both sides of the corneal epithelium; these features can be observed by slit lamp microscopy. Although early stages of the disease are usually asymptomatic, the proliferation of cysts in adulthood can cause epithelial erosion, which lead to tearing, photophobia, and pain [22]. KRT12, which encodes keratin 12 (K12), was the first reported causative gene for MECD [23]. Subsequently, the KRT3 and KRT12 genes were found to encode K3 and K12, respectively; cornea-specific keratins K3–K12 are essential for the maintenance of corneal epithelial integrity. Twenty-three mutations in KRT12 and three mutations in KRT3 influence the severity of MECD lesions, but the mechanism that underlies the formation of KRT12-related corneal microcysts remains poorly understood [24]. Courtney et al. [25] found that a dominant-negative C395T mutation in KRT12 generated a novel PAM. They designed an sgRNA that was complementary to the sequence adjacent to this single nucleotide polymorphism (SNP)-derived PAM; in vitro analyses showed that the sgRNA could effectively inactivate or correct mutations by NHEJ, thus reducing the mRNA and protein expression of mutant KRT12. To evaluate the in vivo targeting ability of the sgRNA and to verify functional knockout of the mutant allele, Courtney et al. injected allele-specific Cas9/sgRNA expression plasmids into the corneal stroma of mice that were heterozygous for the human KRT12-L132P allele. Corneal gDNA was extracted from the stroma; the human KRT12-L132P allele was amplified, cloned, and sequenced by polymerase chain reaction. It was found that 38.5% had undergone NHEJ in vivo. The study expanded knowledge regarding the pathogenesis of KRT12 mutations; it also demonstrated the potential for CRISPR/Cas9 inclusion in gene therapy for autosomal dominant disorders. Nishino et al. [26] identified a heterozygous genetic mutation (c.394C>G, p.L132V) in the KRT12 gene in six Japanese patients

Table 1. In vivo genome editing studies via CRISPR/Cas 9 system.

Disease	Target gene	Mutation	Delivery	Animal model	Main results
MECD	KRT12	C395T	Cas9-GFP construct	Mouse model heterozygous for the human KRT12-L132P allele	Demonstrate in vivo genome editing of a heterozygous disease-causing SNP that results in a novel PAM
POAG	MYOC	/	Ad5-crMYOC	Tg-MYOC^{Y437H} mice	Lowers IOP and prevents further glaucomatous damage
CC	Crygc	A 1 bp deletion mutation in exon 3	Cas9 mRNA and sgRNA-4 were coinjected into the cytoplasm of zygotes	A mouse model of dominant cataract, zygotes that were harvested from B6D2F1 females mated with homozygous cataract males	Efficient correction of a mutant gene in a mouse disease model and successful transmission of the corrected trait to the next generation
LCA10	CEP290	IVS26	SaCas9, single AAV5 vector	Humanized CEP290 mice and NHPs	Productive editing of the NHP CEP290 gene at levels that met the target therapeutic threshold
adRP	Rho	S334ter	A single subretinal injection of SpCas9 plasmid with electroporation	Rat model carrying a RhoS334 point mutation	Extensive and robust retinal preservation was observed in gRNA-injected eyes
adRP	Rho	P23H	subretinal electroporation of the CRISPR/Cas9 plasmid expressing two sgRNAs	P23H RHO transgenic mice	Specific genome editing as well as significant reduction of the mutant RHO protein
adRP	Nrl	/	SpCas9, dual-AAV8 vectors	Nrl−/− mice	Improves rod survival and preserves cone function
arRP	Ped6b	Y347X	SpCas9, plasmid electroporation	rd1 mice	The first example of HDR-mediated gene correction in the visual system
arRP	Mertk	Intron 1 to exon 2	AAV8 or 9	RCS rats	HITI improves visual function
Wet AMD	Vegfa	/	Cas9 RNP	Adult (6 week old) male SPF C57BL/6J mice	The CNV area was significantly reduced

CRISPR clustered-regularly interspaced short palindromic repeats, *Cas* CRISPR-associated, *MECD* Meesmann's epithelial corneal dystrophy, *POAG* primary open angle glaucoma, *CC* congenital cataract, *LCA* Leber congenital amaurosis, *adRP* autosomal dominant retinitis pigmentosa, *arRP* autosomal recessive retinitis pigmentosa, *AMD* age-related macular degeneration, *Sa* Staphylococcus aureus, *Sp* Streptococcus pyogenes, *RNP* ribonucleoprotein complexes, *RCS* Royal College of Surgeons, *NHP* non-human primate.

with inherited MECD; their study was the first report of that genetic mutation in Japanese MECD patients. Granular corneal dystrophy is an autosomal dominant hereditary disease, which is caused by a point mutation in the transforming growth factor-beta-induced (TGFBI) gene. At least five different mutations in TGFBI have been discovered; they cause Reis-Bucklers corneal dystrophy, Thiel-Behnke corneal dystrophy, lattice corneal dystrophy type 1, granular corneal dystrophy type 1, and granular corneal dystrophy type 2 [27]. Taketani et al. [28] designed an sgRNA that targeted the R124H mutation in TGFBI via CRISPR/Cas9. They delivered a Cas9/sgRNA expression plasmid and a single-stranded oligonucleotide HDR donor template into corneal keratocytes that had been derived from granular corneal dystrophy 2 patient. Their in vitro analysis revealed that the R124H mutation in TGFBI was effectively corrected; no off-target effects were detected. Recently, Kitamoto et al. [29] used CRISPR/Cas9 technology to develop a mouse model for TGFBI-R124C corneal dystrophy (lattice corneal dystrophy type 1) which is histologically similar to human diseases. They found that corneal epithelial wound healing was significantly delayed in homozygous TGFBI-R124C mice, indicating that the mutation is harmful to corneal homeostasis. Fuchs endothelial corneal dystrophy is a corneal endothelial disorder and the most common cause of corneal transplantation worldwide [30]. The disease has a degree of inheritance, although the underlying mechanism is unclear. Autosomal dominant inheritance has been confirmed in some cases. Common polymorphisms situated within the transcription factor 4 gene (e.g., an intronic CTG trinucleotide repeat expansion sequence in transcription factor 4) have been associated with Fuchs endothelial corneal dystrophy; such polymorphisms are suitable targets for gene therapy or editing [31]. The potential applications of CRISPR/Cas9 genome editing are rapidly expanding. Future clinical trial successes may support the use of gene therapy as a long-term treatment option for patients with corneal dystrophies.

Glaucoma

Primary open angle glaucoma is a leading cause of irreversible blindness worldwide; it typically manifests as optic disc depression and characteristic visual field defects, and high intraocular pressure (IOP) is the main risk factor. The myocilin gene (MYOC; the first disease-causing gene associated with glaucoma), is expressed in trabecular meshwork (TM) and ciliary body; the protein product may contribute to IOP elevation by obstructing the outflow of aqueous humor [32]. Mutations in MYOC reportedly cause primary open-angle glaucoma and exhibit autosomal dominant inheritance [33]. Jain et al. [34] used CRISPR/Cas9 to disrupt mutant MYOC genes in human and mouse TM cells, and in a primary open-angle glaucoma mouse model (Tg-MYOC^{Y437H}). This disruption prevented IOP elevation and lowered IOP in Tg-MYOC^{Y437H} mice by reducing the accumulation of misfolded proteins inside TM cells; it also reduced endoplasmic reticulum stress in the cells. These data suggested that TM cells can briefly tolerate increased endoplasmic reticulum stress, which is consistent with ex vivo findings concerning MYOC in human eyes. Wu et al. [35] used AAV ShH10 serotype to deliver a single vector Aquaporin 1-disrupting CRISPR/Cas9 system to mice with experimental ocular hypertension; the treatment led to IOP reduction, compared with control or non-injected eyes. Clinical translation of this approach to patients with glaucoma may enable long-term IOP reduction after a single injection.

Congenital cataract

Congenital cataract is a leading cause of visual disability in children. It is a serious blinding disease that mainly manifests as nystagmus, strabismus, photophobia, and inability to gaze. Approximately 50% of patients with congenital cataract have significant clinical and genetic heterogeneity. Congenital cataract has three distinct inheritance patterns, namely autosomal dominant, autosomal recessive, and X-linked recessive; autosomal dominant inheritance is most common [36]. More than 40 genes have been associated with the development of congenital cataract [37]. The CRYGC gene can cause autosomal dominant congenital cataract. Wu et al. [38] were the first to report a mouse model of congenital cataract caused by a specific mutation in the CRYGC; these mice carried a 1-bp deletion mutation in exon 3 of the CRYGC gene, which the translation arrest at the 76th amino acid. These researchers designed a sgRNA that targeted the mutant CRYGC gene; they injected Cas9 mRNA and sgRNA into the cytoplasm of mouse zygotes. Subsequently, 78 mice survived, 24 of which demonstrated correction of the mutation via HDR- or NHEJ-mediated genome editing; these mice exhibited normal histological features. Yuan et al. [39] constructed a rabbit model of congenital cataract with GJA8 gene knockout via co-injection of Cas9/sgRNA mRNA into rabbit zygotes. Gene mutation efficiency in the GJA8 locus reached 98.7% in embryos and 100% in pups, indicating that the Cas9/sgRNA system can achieve efficient genome editing in rabbits. The novel rabbit model of congenital cataract will facilitate the screening of new drugs for cataract prevention and treatment. Additionally, Yuan et al. [40] generated αA-crystallin gene knockout rabbits with congenital cataract by co-injection of Cas9 mRNA and sgRNA into zygotes; their results suggested that CRISPR/Cas9-mediated mutation of the αA-crystallin gene in rabbits recapitulates the congenital cataract phenotype. These data will contribute to the construction of a new animal model that can be used for the identification and pathological analysis of congenital cataract in the future. Zhao et al. [41] provided a stepwise method for evaluation of F0 zebrafish cataract candidate genes using CRISPR/Cas9 ribonucleoprotein complexes (RNPs); their method allowed simple and efficient evaluation of cataract candidates within 2 weeks.

Leber's congenital amaurosis

Leber's congenital amaurosis (LCA) is a serious form of retinal dystrophy that causes congenital blindness. It is also the earliest and most serious hereditary retinopathy; it is present in approximately 5% of patients with hereditary retinal degeneration. LCA is characterized by substantial genetic and allelic heterogeneity [42]. Affected patients demonstrate vision loss at birth or shortly after birth; they may also have symptoms such as sensory nystagmus, amaurotic pupils, absence of retinal electrical activity (as measured by electroretinography), and photophobia. Most patients exhibit an autosomal recessive inheritance pattern. Eighteen genes have been associated with LCA [43]. Mutations in the human KCNJ13 gene may be causative factors for early-onset blindness in LCA. To test this hypothesis, Zhong et al. [44] injected a Cas9/sgRNA plasmid into mouse zygotes, which yielded mice with Kcnj13 gene mutations that simulated human KCNJ13-related LCA disease. Retinal pigment epithelium (RPE) cells without the KCNJ13 protein survived, while the overlying photoreceptors exhibited degeneration; these findings suggested that KCNJ13 gene expression in RPE cells is necessary for photoreceptor survival. Jo et al. [45] performed CRISPR/Cas9-mediated therapeutic correction of a disease-associated nonsense mutation in the Rpe65 in rd12 mice (a model of human LCA); the results provided new insights for the development of LCA therapies. The earliest clinical trial of gene replacement therapy for RPE65-induced LCA achieved good results [46]. Young and old adult patients with severe advanced disease received subretinal injections of AAV vector carrying the RPE65 gene in the eye with worse vision; the results demonstrated the feasibility and safety of this treatment approach [47]. Encouraging results from various animal models indicated that AAV-mediated RPE65 expression could slow or reverse vision loss; this supported its use in human clinical treatment [48]. The United States Food and Drug Administration approved gene therapy for LCA patients with

biallelic mutations in the RPE65 gene [49]. Mutations in the CEP290 gene are among the most common causes of LCA identified thus far. LCA10 is caused by mutations in the CEP290 gene, which encode a centrosomal protein; this is the most common LCA subtype in western people, CEP290 mutations involve splice defects caused by intronic mutations, which lead to an early stop codon in the coding gene encoding in half of the RPE cells [50]. Ruan et al. [51] introduced the splice mutation into 293FT cells and generated a cellular model of LCA10. They found that the use of sgRNA/SpCas9 could effectively remove the splice mutation and restore expression of the wild-type CEP290 gene in 293FT cells. Maeder et al. [52] developed EDIT-101, a candidate genome editing therapy to remove abnormal splice donors generated by the IVS26 mutation in the CEP290 gene; this was expected to restore normal expression of CEP290. Subretinal delivery of EDIT-101 to humanized CEP290 mice led to rapid and sustained genome editing. An alternative non-human primate vector also achieved efficient editing of the non-human primate CEP290 gene and met the target therapeutic threshold of 10%; this finding demonstrated that CRISPR/Cas9 could edit somatic primate cells in vivo. These results provided evidence to support further research concerning the use of CRISPR/Cas9-based gene therapy strategies in the treatment of LCA10 patients. The next important milestone in LCA treatment is the ongoing phase I/II clinical trial, which enrolled the first patient in 2020.

Retinitis pigmentosa

Retinitis pigmentosa (RP) constitutes a large group of inherited retinal dystrophies. RP is characterized by the progressive degeneration of rod photoreceptors and RPE; it can eventually lead to vision loss. The main manifestations are night blindness, progressive visual field defects, fundus bone spicule pigmentation, and electroretinogram abnormalities. RP has three patterns of inheritance: autosomal recessive (50–60%), autosomal dominant (30–40%), and X-linked recessive (5–15%) [53]. Thus far, 24 autosomal dominant RP pathogenic genes and 1 linkage region (RP63) have been identified, among which rhodopsin (RHO) genes are involved in 30–40% of autosomal dominant RP cases [54]. According to The Human Gene Mutation Database, there are 161 RHO gene mutations. The first RHO gene mutation identified was P23H, a high-risk mutation that affects 12% of autosomal dominant RP patients in the United States. Bakondi et al. [55] established an autosomal dominant RP rat model carrying a RhoS334 point mutation; the model demonstrated sustained photoreceptor loss and corresponding vision decline. The rats received a single subretinal injection of a sgRNA/Cas9 plasmid, immediately followed by electroporation to stimulate plasmid absorption by photoreceptors. The results showed that the use of CRISPR/Cas9 could selectively generate allele-specific disruption of RhoS334; this approach corrected retinal dystrophy, increased light sensitivity, and improved visual function in the rats. Latella et al. [56] delivered Cas9 and two sgRNAs in a plasmid, electroporated the plasmid in the mouse model carrying the human P23H mutant alleles, and electroporation was performed after subretinal injection. They found that knockdown mutant RHO expression could be achieved by targeting the Rho gene that carried the P23H dominant mutation. Using a similar ablate-and-replace treatment strategy, Tsai et al. [57] were able to ameliorate disease progression in knock-in mouse models with P23H and D190N mutations. Lv et al. [58] utilized the CRISPR/Cas9 system to knock out the Rp9 gene and knock in the RP-specific Rp9 mutant in mouse 661 W retinal photoreceptor cells in vitro; this approach disrupted the proliferation and migration of mutant retinal photoreceptor cells. Therefore, the mutant Rp9 allele is presumably associated with autosomal dominant RP. Lv et al. also demonstrated the significance of using CRISPR/Cas9 technology in mammalian cells for in vitro analyses of human genetic diseases. Giannelli et al. [59] performed intravitreal injection of Cas9/sgRNA with AAV9 into the retinas of Rho P23H mutant mice; this treatment slowed photoreceptor degeneration and improved retinal function. These results have demonstrated the potential applications of CRISPR/Cas9 technology for the treatment of dominant hereditary retinopathy.

CRISPR/Cas9 has been proposed to achieve a wide range of additional targeted in situ gene regulatory functions through the inhibition or activation of gene transcription in vivo. Yu et al. [60] hypothesized that the loss of cone photoreceptors leads to blindness in RP patients, presumably as a secondary change after rod photoreceptor degeneration. The Nrl gene, which encodes a neural retina-specific leucine zipper protein, plays a decisive role in rod photoreceptor development. Yu et al. used AAV-delivered CRISPR/Cas9 to disrupt Nrl; they found that rod photoreceptors acquired some features of cones and demonstrated greater survival, which prevented secondary cone degeneration. Importantly, CRISPR/Nrl treatment significantly increased rod cell survival and preserved cone cell function in three different mouse models of RP. Moreno et al. [61] engineered targeted repression of Nrl by using AAV-delivered CRISPR/Cas9, which yielded a potentially reversible cone-sparing intervention. Their results supported a potential treatment strategy to delay the degeneration of rod and cone function by ablating the Nrl gene, regardless of the pattern of RP inheritance. The above two studies also demonstrated the feasibility of a gene independent strategy using CRISPR/Cas9 tools.

The rd1 mouse is a model with two homozygous mutations in the Ped6b gene: a point mutation (Y347X) and an intronic insertion of a leukemia virus (Xmv-28). Wu et al. [62] performed genome editing using CRISPR/Cas9 to repair the nonsense mutation (Y347X) in the Ped6b gene of rd1 mice. Despite low repair frequencies, the mice showed significant improvement in retinal structure and function, as well as preservation of neurological function; the offspring showed more robust disease amelioration, indicating that the Y347X mutation site plays a key role in RP and CRISPR/Cas9 genome editing technology can effectively relieve clinical symptoms in rd1 mice. Because of limitations concerning efficiency and effectiveness in the HDR and NHEJ, processes, Suzuki et al. [63] developed a homology-independent targeted integration strategy using CRISPR/Cas9 technology; this approach could knock out DNA in dividing and non-dividing cells, both in vitro and in vivo. Additionally, a vector carrying a copy of Mertk exon 2 was inserted into rats with a 1.9-kb deletion mutation from intron 1 to exon 2 of the Mertk gene. The rats demonstrated improvement in rod-cone response and visual function, suggesting that homology-independent targeted integration may further expand the scope of retinal diseases that can be corrected by CRISPR; this method has potential for gene therapy. There has been considerable progress in the general understanding of disease genetics and mechanisms, gene delivery vectors, and genome editing systems; this progress is driving substantial development from laboratory work to clinical applications. Gene therapy is emerging as a clinically applicable therapeutic approach. The greatest benefits of gene therapy presumably can be achieved by replacing mutated genes with healthy copies or correcting potential mutations prior to retinal cell degeneration.

Usher syndrome

Usher syndrome (USH), also known as hereditary deafness-retinitis pigmentosa syndrome, is a rare autosomal recessive genetic disease that is characterized by congenital sensorineural hearing loss, visual field defect, and progressive vision loss; it exhibits clinical and genetic heterogeneity. Thus far, 16 genes have been associated with USH; mutations in the USH2A gene are the most prevalent [64]. Fuster-García et al. [65] used CRISPR/Cas9 technology to introduce c.2299delG and c.2276G>T mutations in HEK293 cells by means of HDR. Furthermore, they introduced

sgRNA/Cas9 targeting the c.2299delG mutation to correct the mutation in fibroblasts from an USH patient who carried a homozygous form of that mutation. Although the genome editing efficiency was lower in fibroblasts than in HEK293 cells, the results illustrated the potential for CRISPR/Cas9 application to larger genes. Additionally, Liu et al. [66] used the recombinant Cas9 and synthetic gRNA RNPs approach to correct USH2A c.2299delG in patient-derived iPSCs without additional genetic effects; this approach is suitable for therapeutic genome editing.

Fundus neovascular disease

Fundus neovascularization is a common pathological change that occurs in many eye diseases related to retinal and choroidal blood circulation, such as age-related macular degeneration (AMD) and proliferative diabetic retinopathy [67]. Exudation, hemorrhage, and hyperplasia can seriously damage eye structure and function, leading to varying degrees of visual impairment in affected patients. Neovascularization is a complex process that involves various interacting cytokines. Multiple studies have shown that neovascularization is associated with an imbalance of proangiogenic and antiangiogenic factors. Numerous growth factors have been shown to act on vascular endothelial cells, among which the cytokine vascular endothelial growth factor (VEGF) has the greatest effects on intraocular neovascularization [68]. AMD is a leading cause of blindness among older adults in developed countries; it seriously affects their quality of life. AMD is a multifactorial disease caused by a combination of environmental and genetic risk factors. Choroidal neovascularization (CNV) is a characteristic pathological change in wet AMD; it is mainly caused by the overexpression of VEGFA [69]. In a mouse model of CNV, Kim et al. [70] induced targeted mutations by subretinal injection of Vegfa gene-specific Cas9/sgRNA RNPs delivered through an AAV vector. Within 3 days after injection, the mutation frequency in RPE cells was 25% ± 3%. Furthermore, Cas9 RNPs effectively reduced the area of laser-induced CNV. The findings in that study demonstrated the potential for application of CRISPR/Cas9-mediated in vivo genome editing in the treatment of nongenetic degenerative diseases; they also extend the application scope of gene therapy to new dimensions. Ling et al. [71] corrected targeted mutations by subretinal injection of Cas9 mRNA along with expression cassettes that encoded an sgRNA targeting Vegfa, both of which were delivered through an engineered lentiviral vector in a mouse model of Vegfa- induced wet AMD. The results showed that subretinal injection of engineered lentivirus knocked out 44% of Vegfa in the RPE and reduced the area of CNV by 63%. Thus, lentiviruses engineered for the transient expression of nucleases may serve as the basis of new treatments for fundus neovascular diseases. VEGFA has become a common target for ocular neovascularization inhibitors; another potential target is VEGFA receptor (VEGFR2) [68]. Huang et al. [72] used CRISPR/Cas9 technology to edit exon 3 of the VEGF2 gene in human retinal microvascular endothelial cells. The results showed that mutation of the VEGFR2 gene significantly reduced the VEGF stimulation-induced proliferation of vascular endothelial cells. Moreover, the response of mutant VEGFR2 cells to VEGF was inhibited; the degree of inhibition was greater than the inhibition achieved by treatment with aflibercept and ranibizumab. This work established a strong foundation for CRISPR/Cas9 as a strategy to treat intraocular neovascular diseases. Research supporting the involvement of complement in AMD began in the 1990s with the discovery of complement activation in histological analyses of AMD-related drusen [73]. In 2005, a series of reports linking the development of AMD with common SNPs in the complement factor H (CFH) gene led to further research concerning the role of complement in the AMD pathogenesis [74]. Factor I is a major negative regulator of the complement system. There is evidence of complement overactivation within the Bruch's membrane in early AMD [75]. Mutations in the genes encoding complement C3, CFH, complement factor B (CFB), and (especially) complement factor I (CFI) are reportedly associated with the development of AMD [76]. Four research groups identified a common SNP in CFH that significantly contributes to the development of AMD [77]. Early targeted sequencing studies identified a common SNP in the CFH gene (rs1061170) that is associated with an increased risk of AMD. This SNP replaces a tyrosine residue with a histidine residue at position 402 (Y402H). The rs1061170 coding variant in exon 9 is the most common CFH polymorphism associated with AMD [74] The rs800292 mutation in exon 2 is also a CFH polymorphism that alters the amino acid sequence of factor H (FH), which is presumably protective against AMD in some populations [78]. Carriers of both the intronic SNP rs1410996 and the Y402H risk variant in the CFH gene locus have a 15-fold greater risk of AMD; this is associated with peripheral retinal drusen and altered reticular pigment [79]. Reticular pseudo-drusen have been associated with rare complement factor I, C3, and C9 gene variants, suggesting a role for alternative pathways(AP, one of the complement system) dysregulation in an undefined mechanism of pathogenesis [80]. A systematic review and meta-analysis suggested that the polymorphism rs1061170/Y402H might be a genetic predictor of treatment response to anti-VEGF therapy in AMD patients [81]. Complement activation and polymorphisms interfering with FH function, an inhibitor of the complement AP, are associated with an increased risk of AMD. Schnabolk et al. tested an alternative approaches of AAV5-VMD2-CR2-fH or AAV5-VMD2-mCherry using subretinal delivery in C57BL/6J mice; one month later, CNV was induced by argon laser photocoagulation. CNV was reduced by administration of AAV5-VMD2-CR2-fH [82]. CRISPR/Cas9 can be used for gene editing to correct SNPs at loci that confer high risks of AMD, such as the CFH locus [83].

Thioredoxin interacting protein (TXNIP) is a pro-oxidant, pro-inflammatory, and pro-apoptotic protein that is strongly induced under sustained exposure to high glucose; it is involved in the progression of proliferative diabetic retinopathy [84]. However, the mechanism by which TXNIP removes damaged mitochondria through mitophagy, a type of macroautophagy, has not been determined. To investigate the cellular and molecular mechanisms associated with mitophagy in retinal cells under diabetic conditions, Devi et al. [85] maintained a rat Müller cell line under high glucose (25 mM) or low glucose (5.5 mM) conditions for 5 days; they found that high glucose exposure led to TXNIP upregulation in the cytosol and mitochondria, which caused mitochondrial oxidative stress and mitochondrial dysfunction. Conversely, knockout of TXNIP by CRISPR/Cas9 and TXNIP sgRNA prevented high glucose-induced mitochondrial damage and mitophagy in the rat Müller cell line. Heterozygous mutations of pancreatic and duodenal homeobox 1 (Pdx1) are considered risk factors for type 2 diabetes. Wiggenhauser et al. [86] used CRISPR/Cas9 knockout of the transcription factor pdx1 to generate a novel diabetic pdx1$^{-/-}$ zebrafish mutant as a model for diabetic retinopathy; it exhibited disturbed pancreatic development and hyperglycemia. Their findings demonstrated that the PDX1$^{-/-}$ zebrafish mutant could serve as a novel model for analyses of hyperglycemia-induced blood vessel alterations. The above studies have demonstrated the potential for using Cas9/gRNA to treat diabetic retinopathy and other microvascular complications of diabetes.

Proliferative vitreoretinopathy

Proliferative vitreoretinopathy (PVR) is a common blinding disease that manifests as abnormal repair of damaged vitreous and retina. PVR often occurs after rhegmatogenous retinal detachment, ocular trauma, or intraocular surgery. During the process of repairing the damaged blood-retinal barrier, RPE cells, glial cells, and fibroblasts proliferate and migrate to form a fibroproliferative membrane that is attached to the retina. Extensive contraction and stretching of the fibroproliferative membrane results in retinal

detachment and permanent loss of vision. The epithelial mesenchymal transition (EMT) of the RPE to the formation of a myofibroblast phenotype, followed by attachment, diffusion, proliferation, and migration to the retinal surface, is a key event in the pathogenesis of PVR [87]. Priglinger et al. [88] used SpCas9 and sgRNA to knock out and knock down the β1,6 N-acetylglucosaminyltransferase V (Mgat5) gene in the RPE. The silencing of Mgat5 by siRNA and CRISPR-Cas9 genome editing in the RPE in vitro led to reduced galectin-3 binding capacity, thus inhibiting the attachment and spreading of human RPE cells to Mgat5 modified N-glycans; this confirmed the relationship between Mgat5 and EMT, while providing a theoretical basis for the use of galectin-3 as a future target for PVR treatment. Additionally, mouse double minute 2 (MDM2) plays an important role in transforming growth factor-beta 2 (TGF-β2)-induced EMT in human RPE. Liu et al. [89] found that CRISPR/dCas9 (i.e., catalytically dead Cas9) could block the TGF-β2-induced expression of mdm2 and EMT biomarkers, offering a new approach for PVR treatment.

Retinoblastoma

Retinoblastoma (RB) is the most common intraocular malignant tumor in childhood, with leukocoria as the primary clinical sign. Autosomal dominant inheritance contributes to 6% of RB cases, while sporadic inheritance contributes to 94% of such case. RB is caused by biallelic inactivation of the RB1 gene locus on chromosome 13 in germ cells or somatic cells [90]. Although the cure rate for RB has increased in recent years, no targeted molecular therapy techniques have been available thus far, partly because of the lack of highly penetrant and rapid RB animal models. Naert et al. [91] used CRISPR/Cas9 to inactivate the Rb1 and Retinoblastoma-like 1 (Rbl1) genes in *Xenopus tropicalis*. Neither Rb1 nor Rbl1 single mosaic mutant *X*. tropicalis developed tumor, while Rb1/Rbl1 double mosaic mutant tadpoles rapidly developed RB. Their work demonstrated the first CRISPR/Cas9-mediated *X*. tropicalis model of cancer and the first hereditary non-mammalian RB model; this model could eventually be used in preclinical studies and may help to identify novel drug targets. Zheng et al. [92] generated retinal organoids from CRISPR/Cas9-derived RB1-null human embryonic stem cells; they found that the loss of RB1 promoted S-phase entry and resulted in widespread apoptosis. This research suggested that, at least in human retinal organoids, RB1 deletion alone is not sufficient to cause tumor development.

The gene delivery vector-AAV. In vivo approaches are designed to directly treat mutations in affected cells in situ (Fig. 3). Vehicles for in vivo gene transfer exist in form of virus-based vectors, such as AAV. Today, AAV is the vector of choice for retinal gene delivery because of its high tropism for outer retinal cells and its good safety profile; most clinical trials use this vector for gene delivery. As a small non-pathogenic single-stranded DNA virus, AAV belongs to the Parvoviridae family. AAV is a safe gene vector with long-term effectiveness during gene therapy for hereditary retinal degeneration; numerous AAV serotypes can infect various retinal cells [93]. In the past, gene replacement therapy delivered foreign genes to the retina in a manner mediated by AAV. With the development of CRISPR/Cas9 technology, combined administration of AAV and CRISPR has yielded new areas of research. AAV2 was the first serotype used for gene transfer applications; it has been used in most AAV clinical trials thus far. Thy1-YFP transgenic mice express yellow fluorescent protein (YFP) in all retinal ganglion cells, amacrine cells, and bipolar cells in the retina; this expression feature can be used in analyses of optic nerve injury-related disease [94]. Hung et al. [95] used AAV2 to deliver Cas9 and sgRNA to the retina in Thy1-YFP transgenic mice; they targeted YFP and found that their method effectively reduced YFP-positive cells without affecting retinal function, confirming

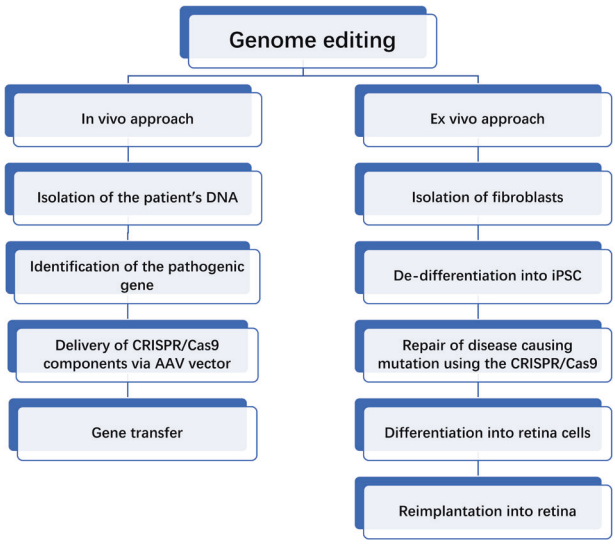

Fig. 3 The protocol for genome editing in the retina includes in vivo and ex vivo approaches. In vivo approaches are designed to directly treat mutations in affected cells in situ. Clustered-regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 can be combined with iPSCs to correct gene mutations in vitro; cells with these corrections can then be transplanted into diseased retina.

the feasibility of combining AAV and CRISPR/Cas9. In addition to the need for greater understanding of serotype-specific differences, the development of AAV-mediated human gene therapy requires greater understanding of species-specific differences in retinal tropism and transduction, which is important for translating preclinical data from animal models to human clinical trials [96].

iPSCs. As a genome editing tool, CRISPR/Cas9 can be combined with iPSCs to correct gene mutations in vitro; cells with these corrections can then be transplanted into diseased retina (Fig. 3) [97]. Bassuk et al. [98] induced iPSC formation from skin fibroblasts of patients with X-linked hereditary RP. Because patient-derived iPSCs harbor disease-causing mutations, these mutations can be repaired with CRISPR/Cas9 in vitro to generate healthy patient-derived cells. Cell sequencing showed that 13% of the mutations were corrected or repaired. In theory, these repaired iPSCs can be used as autologous transplanted cells, effectively avoiding allograft immune rejection. Furthermore, Burnight et al. [99] conducted experiments on multiple genes including MAK, CEP290, and RHO; they found that, regardless of whether the mutation site was located in the exon region or the deep intron region, or a dominant mutation, the patient-derived iPSCs could be successfully modified. These findings confirmed the feasibility of using CRISPR/Cas9 to correct or modify patient-derived iPSCs; they also demonstrated the potential for autologous transplantation involving iPSCs. Sanjurjo-Soriano et al. [100] used enhanced specificity Cas9 to correct the two most prevalent USH2A mutations in iPSCs of USH patients with. This promising technology expands the prospects for gene replacement therapy to correct gene defects in situ; the corrected genes can be expressed in the context of their genome and be regulated by the cell's endogenous gene regulatory mechanisms. Moreover, affected cells can be more easily treated, screened, and selected for successful genome editing [101].

RISKS OF GENOME EDITING

There are many potential risks associated with genome editing using the CRISPR/Cas9 system in vivo; these include the harmful

effects of off-target mutations, DSB related oncogenesis, and the immunogenicity associated with genome editing components. Thus, there is a need for risk-benefit analysis of the clinical feasibility of genome editing therapy.

Potential off-target effects

The specificity of a particular CRISPR/Cas system depends on endonuclease fidelity and sgRNA complementarity. When the CRISPR/Cas system is operating, unexpected cleavage may occur at certain sites; these "off-target" sites have been experimentally identified in human cell lines, with up to five or six mismatches with sgRNA [102]. On the basis of accumulated large datasets and machine-learning approaches or whole-genome sequencing, computer algorithms can be used to predict off-target sites in CRISPR/Cas systems and evaluate the potential off-target effects of genome editing therapy [103]. Prime editing exhibits efficiency that is similar to (or better than) than HDR; it is associated with much lower levels of off-target mutations than Cas9 nuclease at known Cas9 off-target sites in human cells [104].

Concerns related to oncogenicity

The second concern is the oncogenic potential of long-term Cas9 expression in vivo. Cas9-induced DSBs can enhance chromosomal integration or chromosomal translocation of the AAV genome [105].

Immune and inflammatory responses

The common bacterial origin of CRISPR-Cas9 components increases the possibility of intraocular immune and inflammatory responses during genome editing of the eyes in vivo; such responses may cause cellular damage and reduce treatment effectiveness. Importantly, 78% and 58% of humans may harbor preexisting antibodies to Cas9 orthologs derived from the bacterial species *Staphylococcus aureus* (SaCas9) and *Streptococcus pyogenes* (SpCas9) [106].

STRATEGIES TO CONTROL CAS9 ACTIVITY AND IMPROVE SAFETY

Because most safety concerns in therapeutic genome editing are related to the sustained activity of Cas9 in vivo, various strategies can be developed to promote transient or controlled expression of Cas9.

Delivery of Cas9/sgRNA RNPs

To achieve transient genome editing activity, non-viral vectors should be considered because they exhibit minimal risk of an immune response and minimal risk of insertional mutations, while maintaining substantial delivery capacity. Indeed, considerable progress has been made in non-viral strategies based on physical methods (e.g., electroporation, gene gun, or nuclear transfection) and chemical methods (e.g., nanoparticle systems).

Drug-inducible Cas9 activity

For viral vector-mediated genome editing, inducible Cas9 constructs can achieve transient and regulated Cas9 expression, thereby preventing long-term accumulation of off-target mutations. Temporal control of Cas9 expression can be achieved by using transient or drug-inducible promoters, such as the steroid-inducible GRE5 promoter and doxycycline (or tetracycline)-inducible Tet-ON system [107, 108]. Another strategy for temporal control of Cas9 activity involves the post-translational induction of small molecules; many such systems have been described [109].

Self-destructing CRISPR/Cas9

To address the genotoxicity caused by active endonucleases, numerous self-destructing CRISPR systems containing a self-targeting sgRNA have been proposed and validated for use in retinal genome editing without reduced targeting efficacy [110].

mRNA editing

Editing mRNA rather than DNA, avoids the risks associated with genome editing; it also offers an improved safety profile because off-target events do not cause permanent mutations [111].

DISCUSSION

This review has summarized recent research findings concerning the use of CRISPR/Cas9 in the treatment of eye diseases. The development of gene therapy has overcome many obstacles to achieve translation from basic science advances to clinical trials; it has generally been considered safe in clinical trials. As one of the most promising gene therapy technologies for basic research and clinical application, CRISPR/Cas9 has received intense interest in genome editing research; it offers hope for the analysis and treatment of human eye diseases. The incremental knowledge gained in each study may help to simply the procedures needed to advance CRISPR/Cas9 projects. The latest progress in gene therapy has been encouraging; it has generated considerable interest among scientists, clinicians and industry working in the field. This interest and investment will enable stakeholders to address the remaining challenges in this area. Importantly, researchers can use CRISPR/Cas9 technology to target insertion, replacement or knockout of disease-causing genes to construct animal models of diseases that facilitate analyses of eye disease pathogenesis; moreover, CRISPR/Cas9 can be used to target a patient's genome, suggesting potential applications in the treatment of various eye diseases. Although CRISPR/Cas9 technology remains in the early stages of research and clinical applications with many challenges, this new and revolutionary genome editing technology offers additional possibilities for the treatment of genetic eye diseases. Of course, relevant research must be carefully planned and strictly monitored; it should be optimized to ensure effectiveness, safety, and specificity, thus facilitating further basic research and clinical translation. Further improvements of Cas protein stability and target binding specificity, as well as further optimization of genome editing efficiency, are expected to become major research directions in genome editing technology. In the future, we presume that the CRISPR/Cas9 system will be widely used in more medical fields, including ophthalmology; it will have a profound impact on human life. The continuous advancement of this technology is certainly something to look forward to.

SUMMARY

What is known about this topic?

- The development of CRISPR/Cas9.
- The mechanism of CRISPR/Cas9.
- Application of CRISPR/Cas9 in gene therapy of ophthalmological diseases.
- Challenges of CRISPR/Cas9 in gene therapy of ophthalmological diseases.

What this study adds?

- The latest progress in gene therapy of ophthalmological diseases.
- The combination of CRISPR/Cas9 genome editing technology with adeno-associated virus vector and inducible pluripotent stem cells.

REFERENCES

1. Bainbridge JW, Mehat MS, Sundaram V, Robbie SJ, Barker SE, Ripamonti C, et al. Long-term effect of gene therapy on Leber's congenital amaurosis. N Engl J Med. 2015;372:1887–97.
2. Zhou R, Caspi RR. Ocular immune privilege. F1000 Biol Rep. 2010;2:3.
3. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J Bacteriol. 1987;169:5429–33.
4. Jansen R, Embden JD, Gaastra W, Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. Mol Microbiol. 2002;43:1565–75.
5. Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol Evol. 2005;60:174–82.
6. Pourcel C, Salvignol G, Vergnaud G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology. 2005;151:653–63.
7. Bolotin A, Quinquis B, Sorokin A, Ehrlich SD. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology. 2005;151:2551–61.
8. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315:1709–12.
9. Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. Science. 2008;321:960–4.
10. Marraffini LA, Sontheimer EJ. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science. 2008;322:1843–5.
11. Garneau JE, Dupuis MÈ, Villion M, Romero DA, Barrangou R, Boyaval P, et al. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature. 2010;468:67–71.
12. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pirzada ZA, et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature. 2011;471:602–7.
13. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337:816–21.
14. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. Science. 2013;339:819–23.
15. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science. 2013;339:823–6.
16. Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell. 2013;153:910–8.
17. Liu B, Xu H, Miao J, Zhang A, Kou X, Li W, et al. CRISPR/Cas: a faster and more efficient gene editing system. J Nanosci Nanotechno. 2015;15:1946–59.
18. Bassett AR, Tibbit C, Ponting CP, Liu JL. Highly efficient targeted mutagenesis of Drosophila with the CRISPR/Cas9 system. Cell Rep. 2013;4:220–8.
19. Kong J, Kim SR, Binley K, Pata I, Doi K, Mannik J, et al. Correction of the disease phenotype in the mouse model of Stargardt disease by lentiviral gene therapy. Gene Ther. 2008;15:1311–20.
20. Komáromy AM, Alexander JJ, Rowlan JS, Garcia MM, Chiodo VA, Kaya A, et al. Gene therapy rescues cone function in congenital achromatopsia. Hum Mol Genet. 2010;19:2581–93.
21. MacLaren RE, Groppe M, Barnard AR, Cottriall CL, Tolmachova T, Seymour L, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. Lancet. 2014;383:1129–37.
22. Hassan H, Thaung C, Ebenezer ND, Larkin G, Hardcastle AJ, Tuft SJ. Severe Meesmann's epithelial corneal dystrophy phenotype due to a missense mutation in the helix-initiation motif of keratin 12. Eye. 2013;27:367–73.
23. Irvine AD, Corden LD, Swensson O, Swensson B, Moore JE, Frazer DG, et al. Mutations in cornea-specific keratin K3 or K12 genes cause Meesmann's corneal dystrophy. Nat Genet. 1997;16:184–7.
24. McLean WH, Moore CB. Keratin disorders: from gene to therapy. Hum Mol Genet. 2011;20:R189–R197.
25. Courtney DG, Moore JE, Atkinson SD, Maurizi E, Allen EH, Pedrioli DM, et al. CRISPR/Cas9 DNA cleavage at SNP-derived PAM enables both in vitro and in vivo KRT12 mutation-specific targeting. Gene Ther. 2016;23:108–12.
26. Nishino T, Kobayashi A, Mori N, Masaki T, Yokogawa H, Fujiki K, et al. In vivo histology and p.L132V mutation in KRT12 gene in Japanese patients with Meesmann corneal dystrophy. JPN J Ophthalmol. 2019;63:46–55.
27. Han KE, Choi SI, Kim TI, Maeng YS, Stulting RD, Ji YW, et al. Pathogenesis and treatments of TGFBI corneal dystrophies. Prog Retin Eye Res. 2016;50:67–88.
28. Taketani Y, Kitamoto K, Sakisaka T, Kimakura M, Toyono T, Yamagami S, et al. Repair of the TGFBI gene in human corneal keratocytes derived from a granular corneal dystrophy patient via CRISPR/Cas9-induced homology-directed repair. Sci Rep. 2017;7:16713.
29. Kitamoto K, Taketani Y, Fujii W, Inamochi A, Toyono T, Miyai T, et al. Generation of mouse model of TGFBI-R124C corneal dystrophy using CRISPR/Cas9-mediated homology-directed repair. Sci Rep. 2020;10:2000.
30. Matthaei M, Hribek A, Clahsen T, Bachmann B, Cursiefen C, Jun AS. Fuchs endothelial corneal dystrophy: clinical, genetic, pathophysiologic, and therapeutic aspects. Annu Rev Vis Sci. 2019;5:151–75.
31. Fautsch MP, Wieben ED, Baratz KH, Bhattacharyya N, Sadan AN, Hafford-Tear NJ, et al. TCF4-mediated Fuchs endothelial corneal dystrophy: Insights into a common trinucleotide repeat-associated disease. Prog Retin Eye Res. 2021;81:100883.
32. Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sunden SL, et al. Identification of a gene that causes primary open angle glaucoma. Science. 1997;275:668–70.
33. Sheffield VC, Stone EM, Alward WL, Drack AV, Johnson AT, Streb LM, et al. Genetic linkage of familial open angle glaucoma to chromosome 1q21-q31. Nat Genet. 1993;4:47–50.
34. Jain A, Zode G, Kasetti RB, Ran FA, Yan W, Sharma TP, et al. CRISPR-Cas9-based treatment of myocilin-associated glaucoma. Proc Natl Acad Sci USA. 2017;114:11199–204.
35. Wu J, Bell OH, Copland DA, Young A, Pooley JR, Maswood R, et al. Gene therapy for glaucoma by ciliary body aquaporin 1 disruption using CRISPR-Cas9. Mol Ther J Am Soc Gene Ther. 2020;28:820–9.
36. Deng H, Yuan L. Molecular genetics of congenital nuclear cataract. Eur J Med Genet. 2014;57:113–22.
37. Shiels A, Hejtmancik JF. Genetics of human cataract. Clin Genet. 2013;84:120–7.
38. Wu Y, Liang D, Wang Y, Bai M, Tang W, Bao S, et al. Correction of a genetic disease in mouse via use of CRISPR-Cas9. Cell Stem Cell. 2013;13:659–62.
39. Yuan L, Sui T, Chen M, Deng J, Huang Y, Zeng J, et al. CRISPR/Cas9-mediated GJA8 knockout in rabbits recapitulates human congenital cataracts. Sci Rep. 2016;6:22024.
40. Yuan L, Yao H, Xu Y, Chen M, Deng J, Song Y, et al. CRISPR/Cas9-mediated mutation of αA-crystallin gene induces congenital cataracts in rabbits. Invest Ophth Vis Sci. 2017;58:O34–O41.
41. Zhao D, Jones JL, Gasperini RJ, Charlesworth JC, Liu GS, Burdon KP. Rapid and efficient cataract gene evaluation in F0 zebrafish using CRISPR-Cas9 ribonucleoprotein complexes. Methods. 2021;194:37–47.
42. den Hollander AI, Roepman R, Koenekoop RK, Cremers FP. Leber congenital amaurosis: genes, proteins and disease mechanisms. Prog Retin Eye Res. 2008;27:391–419.
43. Yzer S, Hollander AI, Lopez I, Pott JW, de Faber JT, Cremers FP, et al. Ocular and extra-ocular features of patients with Leber congenital amaurosis and mutations in CEP290. Mol Vis. 2012;18:412–25.
44. Zhong H, Chen Y, Li Y, Chen R, Mardon G. CRISPR-engineered mosaicism rapidly reveals that loss of Kcnj13 function in mice mimics human disease phenotypes. Sci Rep. 2015;5:8366.
45. Jo DH, Song DW, Cho CS, Kim UG, Lee KJ, Lee K, et al. Rpe65CRISPR-Cas9-mediated therapeutic editing of ameliorates the disease phenotypes in a mouse model of Leber congenital amaurosis. Sci Adv. 2019;5:x1210.
46. Maguire AM, Simonelli F, Pierce EA, Pugh EN, Mingozzi F, Bennicelli J, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008;358:2240–8.
47. Bainbridge JW, Ali RR. Success in sight: the eyes have it! Ocular gene therapy trials for LCA look promising. Gene Ther. 2008;15:1191–2.
48. Bennett J. Taking stock of retinal gene therapy: looking back and moving forward. Mol Ther J Am Soc Gene Ther. 2017;25:1076–94.
49. 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.
50. 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;79:556–61.
51. Ruan GX, Barry E, Yu D, Lukason M, Cheng SH, Scaria A. CRISPR/Cas9-mediated genome editing as a therapeutic approach for leber congenital amaurosis 10. Mol Ther J Am Soc Gene Ther. 2017;25:331–41.
52. Maeder ML, Stefanidakis M, Wilson CJ, Baral R, Barrera LA, Bounoutas GS, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nat Med. 2019;25:229–33.
53. Verbakel SK, van Huet R, Boon C, den Hollander AI, Collin R, Klaver C, et al. Non-syndromic retinitis pigmentosa. Prog Retin Eye Res. 2018;66:157–86.

54. Ferrari S, Di Iorio E, Barbaro V, Ponzin D, Sorrentino FS, Parmeggiani F. Retinitis pigmentosa: genes and disease mechanisms. Curr Genomics. 2011;12:238–49.
55. Bakondi B, Lv W, Lu B, Jones MK, Tsai Y, Kim KJ, et al. In vivo CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa. Mol Ther J Am Soc Gene Ther. 2016;24:556–63.
56. Latella MC, Di Salvo MT, Cocchiarella F, Benati D, Grisendi G, Comitato A, et al. In vivo editing of the human mutant rhodopsin gene by electroporation of plasmid-based CRISPR/Cas9 in the mouse retina. Mol Ther Nucleic Acids. 2016;5:e389.
57. Tsai YT, Wu WH, Lee TT, Wu WP, Xu CL, Park KS, et al. Clustered regularly interspaced short palindromic repeats-based genome surgery for the treatment of autosomal dominant retinitis pigmentosa. Ophthalmology. 2018;125:1421–30.
58. Lv JN, Zhou GH, Chen X, Chen H, Wu KC, Xiang L, et al. Targeted RP9 ablation and mutagenesis in mouse photoreceptor cells by CRISPR-Cas9. Sci Rep. 2017;7:43062.
59. Giannelli SG, Luoni M, Castoldi V, Massimino L, Cabassi T, Angeloni D, et al. Cas9/sgRNA selective targeting of the P23H Rhodopsin mutant allele for treating retinitis pigmentosa by intravitreal AAV9.PHP.B-based delivery. Hum Mol Genet. 2018;27:761–79.
60. 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.
61. Moreno AM, Fu X, Zhu J, Katrekar D, Shih YV, Marlett J, et al. In situ gene therapy via AAV-CRISPR-Cas9-mediated targeted gene regulation. Mol Ther J Am Soc Gene Ther. 2018;26:1818–27.
62. Wu WH, Tsai YT, Justus S, Lee TT, Zhang L, Lin CS, et al. CRISPR repair reveals causative mutation in a preclinical model of retinitis pigmentosa. Mol Ther J Am Soc Gene Ther. 2016;24:1388–94.
63. Suzuki K, Tsunekawa Y, Hernandez-Benitez R, Wu J, Zhu J, Kim EJ, et al. In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. Nature. 2016;540:144–9.
64. Mathur P, Yang J. Usher syndrome: Hearing loss, retinal degeneration and associated abnormalities. Biochim Biophys Acta. 2015;1852:406–20.
65. Fuster-García C, García-García G, González-Romero E, Jaijo T, Sequedo MD, Ayuso C, et al. USH2A gene editing using the CRISPR system. Mol Ther Nucleic Acids. 2017;8:529–41.
66. Liu X, Lillywhite J, Zhu W, Huang Z, Clark AM, Gosstola N, et al. Generation and genetic correction of USH2A c.2299delG mutation in patient-derived induced pluripotent stem cells. Genes. 2021;12:805.
67. Bressler SB. Introduction: Understanding the role of angiogenesis and anti-angiogenic agents in age-related macular degeneration. Ophthalmology. 2009;116:S1–S7.
68. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003;9:669–76.
69. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989;246:1306–9.
70. Kim K, Park SW, Kim JH, Lee SH, Kim D, Koo T, et al. Genome surgery using Cas9 ribonucleoproteins for the treatment of age-related macular degeneration. Genome Res. 2017;27:419–26.
71. Ling S, Yang S, Hu X, Yin D, Dai Y, Qian X, et al. Lentiviral delivery of co-packaged Cas9 mRNA and a Vegfa-targeting guide RNA prevents wet age-related macular degeneration in mice. Nat Biomed Eng. 2021;5:144–56.
72. Huang X, Zhou G, Wu W, Duan Y, Ma G, Song J, et al. Genome editing abrogates angiogenesis in vivo. NAT Commun. 2017;8:112.
73. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J Off Publ Federation Am Societies Exp Biol. 2000;14:835–46.
74. Edwards AO, Ritter R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005;308:421–4.
75. Whitmore SS, Sohn EH, Chirco KR, Drack AV, Stone EM, Tucker BA, et al. Complement activation and choriocapillaris loss in early AMD: implications for pathophysiology and therapy. Prog Retin Eye Res. 2015;45:1–29.
76. Armento A, Ueffing M, Clark SJ. The complement system in age-related macular degeneration. Cell Mol life Sci CMLS. 2021;78:4487–505.
77. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005;308:419–21.
78. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA. 2005;102:7227–32.
79. Li M, Atmaca-Sonmez P, Othman M, Branham KE, Khanna R, Wade MS, et al. CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration. Nat Genet. 2006;38:1049–54.
80. Saksens NT, Geerlings MJ, Bakker B, Schick T, Daha MR, Fauser S, et al. Rare genetic variants associated with development of age-related macular degeneration. JAMA Ophthalmol. 2016;134:287–93.
81. Hong N, Shen Y, Yu CY, Wang SQ, Tong JP. Association of the polymorphism Y402H in the CFH gene with response to anti-VEGF treatment in age-related macular degeneration: a systematic review and meta-analysis. Acta Ophthalmol. 2016;94:334–45.
82. Schnabolk G, Parsons N, Obert E, Annamalai B, Nasarre C, Tomlinson S, et al. Delivery of CR2-fH using AAV vector therapy as treatment strategy in the mouse model of choroidal neovascularization. Mol Ther Methods Clin Dev. 2018;9:1–11.
83. Tran M, Khalid M, Pébay A, Cook AL, Liang HH, Wong R, et al. Screening of CRISPR/Cas base editors to target the AMD high-risk Y402H complement factor H variant. Mol Vis. 2019;25:174–82.
84. Devi TS, Hosoya K, Terasaki T, Singh LP. Critical role of TXNIP in oxidative stress, DNA damage and retinal pericyte apoptosis under high glucose: implications for diabetic retinopathy. Exp Cell Res. 2013;319:1001–12.
85. Devi TS, Somayajulu M, Kowluru RA, Singh LP. TXNIP regulates mitophagy in retinal Müller cells under high-glucose conditions: implications for diabetic retinopathy. Cell Death Dis. 2017;8:e2777.
86. Wiggenhauser LM, Qi H, Stoll SJ, Metzger L, Bennewitz K, Poschet G, et al. pdx1activation of retinal angiogenesis in hyperglycemic zebrafish mutants. Diabetes. 2020;69:1020–31.
87. Machemer R. Proliferative vitreoretinopathy (PVR): a personal account of its pathogenesis and treatment. Proctor lecture. Invest Ophth Vis Sci. 1988;29:1771–83.
88. Priglinger CS, Obermann J, Szober CM, Merl-Pham J, Ohmayer U, Behler J, et al. Epithelial-to-mesenchymal transition of RPE cells in vitro confers increased β1,6-N-glycosylation and increased susceptibility to galectin-3 binding. Plos One. 2016;11:e146887.
89. Liu B, Song J, Han H, Hu Z, Chen N, Cui J, et al. Blockade of MDM2 with inactive Cas9 prevents epithelial to mesenchymal transition in retinal pigment epithelial cells. Lab Investig J Tech methods Pathol. 2019;99:1874–86.
90. Horsthemke B. Genetics and cytogenetics of retinoblastoma. Cancer Genet Cytogenetics. 1992;63:1–7.
91. Naert T, Colpaert R, Van Nieuwenhuysen T, Dimitrakopoulou D, Leoen J, Haustraete J, et al. CRISPR/Cas9 mediated knockout of rb1 and rbl1 leads to rapid and penetrant retinoblastoma development in Xenopus tropicalis. Sci Rep. 2016;6:35264.
92. Zheng C, Schneider JW, Hsieh J. Role of RB1 in human embryonic stem cell-derived retinal organoids. Dev Biol. 2020;462:197–207.
93. Dalkara D, Kolstad KD, Caporale N, Visel M, Klimczak RR, Schaffer DV, et al. Inner limiting membrane barriers to AAV-mediated retinal transduction from the vitreous. Mol Ther J Am Soc Gene Ther. 2009;17:2096–102.
94. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, et al. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron. 2000;28:41–51.
95. Hung SS, Chrysostomou V, Li F, Lim JK, Wang JH, Powell JE, et al. AAV-mediated CRISPR/Cas gene editing of retinal cells in vivo. Invest Ophth Vis Sci. 2016;57:3470–6.
96. Jüttner J, Szabo A, Gross-Scherf B, Morikawa RK, Rompani SB, Hantz P, et al. Targeting neuronal and glial cell types with synthetic promoter AAVs in mice, non-human primates and humans. Nat Neurosci. 2019;22:1345–56.
97. Wiley LA, Burnight ER, Songstad AE, Drack AV, Mullins RF, Stone EM, et al. Patient-specific induced pluripotent stem cells (iPSCs) for the study and treatment of retinal degenerative diseases. Prog Retin Eye Res. 2015;44:15–35.
98. 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.
99. Burnight ER, Gupta M, Wiley LA, Anfinson KR, Tran A, Triboulet R, et al. Using CRISPR-Cas9 to generate gene-corrected autologous iPSCs for the treatment of inherited retinal degeneration. Mol Ther: J Am Soc Gene Ther. 2017;25:1999–2013.
100. Sanjurjo-Soriano C, Erkilic N, Baux D, Mamaeva D, Hamel CP, Meunier I, et al. USH2AGenome editing in patient iPSCs corrects the most prevalent mutations and reveals intriguing mutant mRNA expression profiles. Mol Ther Methods Clin Dev. 2020;17:156–73.

101. Yanik M, Müller B, Song F, Gall J, Wagner F, Wende W, et al. In vivo genome editing as a potential treatment strategy for inherited retinal dystrophies. Prog Retin Eye Res. 2017;56:1–18.
102. Tsai SQ, Zheng Z, Nguyen NT, Liebers M, Topkar VV, Thapar V, et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. Nat Biotechnol. 2015;33:187–97.
103. Liu Q, Cheng X, Liu G, Li B, Liu X. Deep learning improves the ability of sgRNA off-target propensity prediction. BMC Bioinformatics. 2020;21:51.
104. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576:149–57.
105. Wang D, Zhang F, Gao G. CRISPR-based therapeutic genome editing: strategies and in vivo delivery by AAV vectors. Cell. 2020;181:136–50.
106. Charlesworth CT, Deshpande PS, Dever DP, Camarena J, Lemgart VT, Cromer MK, et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat Med. 2019;25:249–54.
107. Mader S, White JH. A steroid-inducible promoter for the controlled over-expression of cloned genes in eukaryotic cells. Proc Natl Acad Sci USA. 1993;90:5603–7.
108. Puppo A, Cesi G, Marrocco E, Piccolo P, Jacca S, Shayakhmetov DM, et al. Retinal transduction profiles by high-capacity viral vectors. Gene Ther. 2014;21:855–65.
109. Gangopadhyay SA, Cox KJ, Manna D, Lim D, Maji B, Zhou Q, et al. Precision control of CRISPR-Cas9 using small molecules and light. Biochemistry. 2019;58:234–44.
110. Li F, Hung S, Mohd Khalid M, Wang JH, Chrysostomou V, Wong V, et al. Utility of self-destructing CRISPR/Cas constructs for targeted gene editing in the retina. Hum Gene Ther. 2019;30:1349–60.
111. Fry LE, Peddle CF, Barnard AR, McClements ME, MacLaren RE. RNA editing as a therapeutic approach for retinal gene therapy requiring long coding sequences. Int J Mol Sci. 2020;21:777.

AUTHOR CONTRIBUTIONS

XMH, ZMS, and BBZ conceived and drafted the review outline. XMH wrote the paper. ZMS, BBZ, XLL, ML, YGW, HDD, JMZ, YMW, KKG, and PL provided critical review of the paper.

FUNDING

This work was supported by grants from the Natural Science Foundation of China (82101162), the Joint Construction Program of Henan Medical Science and Technology Research Plan (LHGJ20200067) and the Basic Research Project of Henan Eye Hospital (22JCQN011 and 20JCZD001).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Zongming Song.