· Editorial ·

Gene therapy for retinal diseases: global progress and challenges

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[**Abstract**] In the past three decades, we have witnessed the great promise of gene therapy in treating a variety of inherited and acquired retinal diseases. The large number of preclinical studies in retinal gene therapy has led to over sixty clinical trials at different phases and the approval of Luxturna, a gene therapy product that treats type 2 Leber congenital amaurosis (LCA2) caused by mutations in the *RPE65* gene. While current gene delivery systems have led to some success in clinical studies, safer and more efficient gene delivery calls for improved vectors and alternative intraocular administration routes. Progress has been made in large gene delivery and less invasive vector administration with the advancement of novel adeno-associated viral (AAV) vector technologies. Clustered regularly interspaced short palindromic repeats (CRISPR)-based technology has enlarged the toolbox of gene therapy and will inevitably find wide applications in the treatment of retinal diseases. Although gene therapy faces a number of challenges, in the coming years, it will become a viable treatment option for a number of currently incurable retinal disorders.

[**Key words**] Gene therapy; Adeno-associated viral vector; Retinal diseases; Clustered regularly interspaced short palindromic repeats; Genome editing
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视网膜疾病基因疗法：全球研究进展和挑战

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【**摘要**】 过去 30 年来，基因疗法给多种遗传性和获得性视网膜疾病患者带来了很大希望。在大量临床前研究的基础上，全球已进行了 60 多项视网膜疾病基因疗法的各期临床试验，且 Luxturna，一种用于由 *RPE65* 突变导致的 2 型 Leber 先天性黑朦（LCA2）的基因治疗产品已批准用于临床。目前的基因传递系统虽然在临床研究中取得了一些成功，但为保证安全性和有效性，仍然需要在载体和眼内给药方式上加以改进和变通。新的腺相关病毒载体技术的发展使大基因传递和小创伤载体注射方式的研究都有所进展。规律间隔成簇短回文重复序列（CRISPR）技术也丰富了基因疗法的手段，必将在视网膜疾病的治疗中得到广泛应用。虽然基因疗法仍面临许多挑战，但预计在未来若干年中，它将成为目前许多不可治性视网膜疾病的临床治疗选择。

【**关键词**】 基因疗法；腺相关病毒载体；视网膜疾病；规律间隔成簇短回文重复序列；基因组编辑
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Millions of people worldwide suffer from retinal diseases that cause severe visual loss or blindness. Over the past three decades, gene therapy has been developed as a powerful treatment modality for these diseases, with over 60 clinical protocols having been or currently being conducted in patients (www.clinicaltrials.gov) and many more in the preclinical stage. A majority of these protocols are developed against inherited retinal dystrophies (IRDs) in which the corresponding disease-causing genes have been identified. Notably, Luxturna, an adeno-associated viral (AAV) vector carrying the expression cassette of the *RPE65* gene for the treatment of type 2 Leber congenital amaurosis (LCA2), has been approved by the Food and Drug Administration (FDA) of the U.S.

as the first gene therapy product for retinal diseases[1]. In addition, gene therapy protocols against multifactorial retinal diseases, such as age-related macular degeneration (AMD) and diabetic retinopathy (DR) have also been tested clinically or in disease models. In this editorial, I will briefly summarize the recent progress in this area and introduce a few new technologies that can be applied in the treatment development for retinal diseases.

1 Major strategies of retinal gene therapy

Gene replacement (or gene augmentation) is the most common gene therapy strategy for the treatment of IRDs with recessive or loss-of-function gene mutations. This is usually achieved by delivering the expression cassette of the corresponding wild-type gene using a viral or non-viral vector to the target cells such as photoreceptors or retinal pigmented epithelium (RPE) cells. For IRDs caused by dominant or gain-of-function mutations, gene suppression (or gene inactivation) is often carried out through the delivery and expression of antisense RNA, ribozyme, RNA interference, or the use of clustered regularly interspaced short palindromic repeats (CRISPR) technology. Both gene replacement and gene suppression are classified as disease gene-dependent strategies, as they are designed to specifically modulate the disease-causing genes. Several other treatment approaches including gene correction, exon skipping, and RNA trans-splicing also fall into this category. In contrast, disease gene-independent strategies are usually applied to tackle multifactorial diseases or IRDs with unidentified disease-causing genes. These often involve the transfer of genes whose products could halt the disease progression, compensate for the function loss, and/or support the survival of the afflicted cells. The antiangiogenic approach for the treatment of AMD, and the optogenetic approach for the treatment of late-stage retinal degeneration fall into this category.

2 Recent progress in clinical and preclinical retinal gene therapy

Over 270 genes have been identified as disease-causing genes for IRDs (https://sph.uth.edu/retnet/) and it is unrealistic to develop a gene therapy treatment for every one of them. Preference has been given to the genes that affect relatively large patient populations (even though each population is still small), are suitable to be delivered by AAV vectors, and have corresponding disease models. Gene therapy for LCA2 due to *RPE65* mutations is no doubt the most successful one so far and has resulted in visual improvement in a few dozen patients undergoing phase 1 to 3 clinical studies[2]. The success of gene therapy in treating the disease can be ascribed to a few reasons. First, the *RPE65* mutations mainly affect RPE cells which can be efficiently transduced by an AAV vector. Second, the relatively slow rate of photoreceptor death in the patients allows for a longer treatment window. Third, because *RPE65* mutations disrupt the normal visual cycle causing vison loss, restoring the visual cycle with AAV-mediated RPE65 expression leads to immediate visual improvement. In contrast, many other IRDs are unlikely to functionally improve with gene therapy treatment; therefore, the treatment goal is to halt disease progression.

Promising results have also been obtained in clinical studies for a few other retinal diseases such as choroideremia (CHM), LCA10, retinitis pigmentosa 3 (RP3), Leber´s hereditary optic neuropathy (LHON), and AMD. CHM is an X-linked disease due to mutations in the *REP1* gene, which causes degeneration of the choroid, photoreceptors, and RPE cells. In a two-year follow-up of a clinical trial in which the patients received subretinal injections of an AAV2 vector encoding the REP1 protein, significant and sustained improvements in visual acuity were achieved in the treated eyes [3]. Recently, Biogen completed the enrollment of 170 adult males with CHM for a phase Ⅲ clinical trial (NCT03496012).

LCA10 is caused by mutations in the *CEP290* gene, and the most common mutation in patients with European ancestry is in intron 26 of the gene, generating a cryptic splice donor site. In a phase Ⅰ/Ⅱ clinical trial, ten patients with the mutation received intravitreal injections of an antisense oligonucleotide (AON) designed to block the aberrant splicing site and restore correct splicing. Sixty percent of the patients demonstrated improvements in visual acuity and the ability to navigate a mobility course[4]. Based on the encouraging results, ProQR has started a 24-month phase Ⅱ/Ⅲ trial.

RP3 is an X-linked retinitis pigmentosa (XLRP)

caused by mutations in the *RPGR* gene. In a recent phase Ⅰ/Ⅱ trial, eighteen patients received subretinal administrations of an AAV8 vector encoding codon-optimized human *RPGR*. In six patients, visual field improvements were observed during the 6 months of follow-up[5]. Significant visual acuity improvements were also observed in a majority of patients involved in a clinical trial of LHON gene therapy led by Dr. Bin Li in a seven-year follow-up[6]. A second clinical study led by the same group has enrolled 149 subjects and the initial results are promising. In a phase Ⅰ/Ⅱ trial for achromatopsia caused by *CNGA3* mutations, minor improvements in cone functions in the treated eyes were observed in a 12-month follow-up[7].

In addition to clinical studies for IRDs, positive results have also been obtained in a phase Ⅰ/Ⅱ trial for wet AMD conducted by Regenxbio. Patients receiving administrations of an AAV8 vector encoding an antibody fragment designed to inhibit VEGF exhibited improved visual acuity and demonstrated reductions in anti-VEGF treatment burden over the two years of follow-up.

However, no significant improvement was observed or reported in a few other clinical studies of X-linked retinoschisis (XLRS)[8], RP caused by *MERTK* mutations, USH1B, and Stargardt disease. In the XLRS trial, the intravitreally injected AAV8 vector may not be able to pass the inner limiting membrane and reach the target retinal cells, leading to insufficient transgene expression. In the trials for USH1B and Stargardt disease due to mutations in *MYO7A* and *ABCA4* respectively, lentiviral vectors were used for gene delivery, as both disease-causing genes are too large to be packaged into an AAV vector. It has been reported that lentiviral vectors do not transduce adult retina efficiently. More preclinical studies are needed for these diseases to address these potential problems. In preclinical studies, retinal expression of MYO7A and ABCA4 were achieved using a "dual AAV vector" approach, which will be discussed in a later part of this article.

For LCA10 caused by *CEP290* mutations, in addition to the AON treatment, other approaches have also been pursued. Due to its size, the 7.4 kb *CEP290* coding sequence cannot be delivered by a single AAV vector. However, a dual AAV vector approach was able to restore the full length *CEP290* expression in photoreceptors and rescued the retinal function in a mouse model with a *Cep290* mutation[9]. A mini-gene approach in which functional domains of the *Cep290* gene were delivered by AAV vectors also demonstrated treatment benefits in the same mouse model in two independent studies, including our own[10-11]. In another study, which employed a CRISPR-based genome editing method, an AAV5 vector carrying expression cassettes of Cas9 and a pair of sgRNAs was used to remove the aberrant splice donor and restore normal CEP290 expression[12]. Successful preclinical results in this study led to a phase Ⅰ/Ⅱ trial by Editas, which was the first *in vivo* application of the CRISPR technology in humans.

The disease gene-independent approaches, as exemplified by *in vivo* photoreceptor reprogramming and optogenetic treatment, have also been actively pursued in preclinical studies. Results from our group showed that the CRISPR-mediated disruption of neural retina leucine zipper (*Nrl*) was able to reprogram rod photoreceptors into cone-like cells that are less susceptible to RP-specific gene mutations, prolonging their survival and thereby delaying secondary cone degeneration[13]. This approach could be used for the treatment of mid-stage RP when rods have not died out. For late stage RP or LCA when all photoreceptors have degenerated, the optogenetic approach could be a better option, as it could convert bipolar cells or ganglion cells into photoreceptor-like cells by introducing genes encoding light-activated photosensitive proteins[14]. Several clinical trails using this approach are ongoing and the efficacy results have not been reported.

3 Novel vector technologies and safer administration routes

The appropriate use of vector and administration routeis the key to the success for retinal gene therapy. Among the large variety of viral and non-viral gene delivery vectors, AAV vectors are the most commonly used for *in vivo* gene therapy due to their excellent safety profile, low immunogenicity, wide tissue tropism, and the ability to mediate long-term transgene expression in postmitotic cells. After infection, the vector genomes exist as episomes in the nuclei and rarely integrate to the host genome,

which reduces the concern of insertional mutagenesis. AAV vectors are by far the most efficient gene delivery tool to the retina, especially to photoreceptors and RPE cells, the two cell types most affected in retinal diseases. In addition to AAV serotype 2 (AAV2) that has been used in the FDA-approved Luxturna, hundreds of AAV variants, either naturally occurring or genetically engineered are available in the AAV toolbox. While AAV2 is usually used to transduce RPE cells, AAV5 and AAV8 are the most commonly used AAV serotypes to transduce photoreceptors in rodents and non-human primates (NHPs). Although other gene delivery systems such as lentiviral vector, adenoviral vector and non-viral nanoparticles have also been tested in preclinical and even clinical studies, none of them have exhibited the ability to mediate stable and persistent transgene expression in photoreceptors *in vivo*.

As mentioned above, a major drawback of the AAV vector is its limited packaging capacity. As only a maximum of 5 kilobases (kb) DNA fragment can be packaged into an AAV virion [15], it cannot deliver larger genes such as *ABCA4*, *CEP290* and *MYO7A*. To over come this limitation, a "dual AAV" approach has been pursued, in which a large coding sequence is split into two parts and delivered by two separate AAV vectors. Expression of the full-length protein can be achieved in cells co-infected by the two vectors either through homologous recombination or hetero-dimerization and the subsequent intron-mediated trans-splicing. Varying degrees of protein expression and phenotype correction were reported in preclinical studies using this approach, which may not be ready for clinical applications due to the overall low efficiency of homologous recombination or hetero-dimerization between the two vectors. In a recent study, a split intein-mediated protein trans-splicing was incorporated into the dual AAV approach, which efficiently facilitated full-length protein reconstitution in the retina of mice and pigs, and in human retinal organoids [9].

Subretinal injection has been employed in most retinal gene therapy trials, as it provides an efficient way for the vectors to be taken up by photoreceptors or RPE cells. However, it is an invasive procedure, associated with a few complications and only allows the injected vector to spread to a limited retinal area. Intravitreal injection is a preferred route for vector administration, as it is much less invasive, can be done in an outpatient setting, and allows the vector to spread to larger retinal area. However, most of the existing AAV variants are not capable of penetrating the multiple retinal layers to reach the outer layers where the photoreceptors and RPE cells are located. Therefore, developing AAV vectors suited for intravitreal injection has been a focus in the retinal gene therapy field. Although rational design based on existing knowledge of AAV may help generate such vectors, directed evolution appears to be a more viable approach. This has been pursued by a few groups and has shown some success in rodents and NHPs[16-17]. Another less invasive administration route is suprachoroidal injection, which has been used to deliver an AAV8 vector encoding anti-VEGF Fab to treat angiogenesis in the preclinical treatment of wet AMD. Following suprachoroidal injection, similar transduction efficiency in rat retina was observed when compared to subretinal injection. Widespread transgene expression was also achieved in the eyes of large animals such as pigs and NHPs [18].

4 CRISPR-based technologies: new powerful weapons of retinal gene therapy

CRISPR has brought revolutionary changes to the life science field and provides powerful weapons to retinal gene therapy. Mechanism and principles of CRISPR-based technology can be found in recent reviews [19] and will not be described in detail here. In general, the Cas nuclease is guided by the guide RNA (gRNA) and to create a double-strand DNA break (DSB) at the genome locus where the DNA sequence is complementary to the gRNA. The two major DNA repair pathways are used for different genome editing purposes. The error-prone non-homologous end joining (NHEJ) pathway is usually used for gene inactivation, in which the insertion and deletion (indel) generated during DNA repair process disrupts the reading frame of the coding sequence. This approach has been widely applied in preclinical treatment of dominant and gain-of-function diseases, and has been tested to knock down the mutant *RHO* alleles in rodent models by several research groups[20-21]. The homologous recombination (HR) pathway is used to achieve precise gene

correction, which in theory could be applied to the treatment of IRDs caused by both dominant and recessive mutations. However, the HR efficiency is usually too low to achieve meaningful therapeutic effects, especially in postmitotic photoreceptors. Efforts have been made to improve its efficiency for the treatment of retinal degeneration, such as incorporating an Escherichia coli recombinase A (RecA) protein into the CRISPR system, which catalyzes DNA strand exchange reactions [22].

In addition to the previously mentioned approaches for gene inactivation and gene correction, a few novel CRISPR-based techniques have recently emerged. One of them is called homology-independent targeted integration (HITI), which was developed for targeted DNA insertion at the specific locus through the NHEJ repair pathway. Integration efficiency of the HITI approach is much higher than that of the HR-mediated integration, and has been used to correct the deletion of the *Mertk* gene that causes retinal degeneration in Royal College of Surgeons (RCS) rats[23].

Several other CRISPR-based techniques use the DNA search function of the deficient Cas9 (dCas9) and gRNA complex without generating DSBs, which reduces safety concerns. One such technique is called CRISPR interference (CRISPRi), in which a gene repressor protein is fused with the dCas9 and down-regulates transcription when it binds to the promoter region of a gene with the guidance of gRNA. CRISPRi is therefore a viable option to treat dominant diseases. The opposite approach to CRISPRi is CRISPR activation (CRISPRa), which fuses a transcriptional activator to dCas9.

Base editing and prime editing are two recently developed and most attractive CRISPR-based techniques for precise gene modification. Base editing facilitates the conversion of C to T or A to G without creating DSBs, which could be an ideal tool for treating genetic diseases caused by point mutations. High efficiency of precise gene correction has been achieved in the mouse retina using dual AAV vector-delivered base editors [24]. Prime editing seems to be even more attractive than base editing, as it can mediate targeted insertions, deletions, and all base-to-base conversions [25]. Although these two techniques have not been tested in a preclinical setting for a retinal disease, their wide applications in the field are foreseeable as long as *in vivo* delivery of their components is efficient.

5　Challenges and future perspectives

Because IRDs are considered rare diseases – with each affecting a relatively small population – they have generally been considered less attractive to industry as it is difficult to develop a profitable gene therapy treatment for these diseases. While this may have been true in the past, the approval of Luxturna and several other gene therapy products has now attracted a number of large pharmaceutical companies and numerous biotech startups to the field. Although the current cost of gene therapy products is very high and unaffordable for a majority of patients, this may change in the coming years due to competition in the field and the synergistic efforts of patient advocates, policy makers, insurance companies, and drug developers.

It is generally believed that optimal treatment effects to IRDs can only be achieved if gene therapy is given to patients at an early phase of the disease. However, it is not uncommon for an IRD patient to miss the treatment window, with most photoreceptors or RPE cells already dead at the time of diagnosis. Early genetic diagnosis is therefore critical for IRD patients. Alternatively, developing treatment options against late stage diseases, such as the optogenetic approaches, is equally important.

From the technical perspective, there are a few problems to be overcome in retinal gene therapy. Most preclinical efficacy studies are conducted in rodent models which may not recapitulate the disease pathology and progression in humans. In addition, extrapolating preclinical results to clinical trials may not always be reliable due to the differences between rodent and human retinal anatomy. To account for these issues, vector biodistribution studies in NHPs and efficacy studies using patients´ iPSC derived retinal organoids would be helpful. As previously mentioned, a single conventional AAV vector cannot deliver a DNA fragment larger than 5 kb, which has hindered gene replacement for a number of IRDs with large disease-causing genes. Although the dual AAV approach is an option, the transduction efficiency is usually too low to achieve meaningful therapeutic effects; therefore, novel methods for more efficient delivery of large genes are needed. Another reason these methods are needed is for the purposes of CRISPR genome editing

technology. Although shorter Cas variants and orthologues have been identified, *in vivo* applications of a few newly emerged CRISPR-based techniques, including base editing and prime editing, are unlikely to succeed without a viable large gene delivery system. Indeed, better vectors are constantly in demand. No vector used in any retinal gene therapy clinical trial was designed to precisely control the expression level of the transgene. In addition, inflammation or immune response usually occurs following vector administration, especially after intravitreal injection. Thus, the goals of novel vector development should be toward designing a vector with more potent gene transfer, less immunogenicity in the eye, and controllable gene expression to ensure safety, efficacy, and specificity.

After three decades of ups and downs, gene therapy has now entered an exciting era in which retinal gene therapy is at the forefront of the field. With more and more disease-causing genes identified and disease mechanisms elucidated, combined with rapid advancements in gene transfer and genome editing technology, gene therapy may become a leading choice for the treatment of a large variety of inherited and acquired retinal diseases. However, this is not an easy task and requires the combined efforts of patients, scientists, clinicians, industry and regulatory agencies.

Conflicts of interest: Zhijian Wu is employed by PTC Therapeutics and holds stocks and options in the company

References

[1] Apte RS. Gene therapy for retinal degeneration[J/OL]. Cell, 2018, 173(1): 5[2020-07-10]. https://pubmed.ncbi.nlm.nih.gov/29570997/. DOI:10.1016/j.cell.2018.03.021.

[2] Russell S, Bennett J, Wellman JA, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial[J]. Lancet, 2017, 390(10097): 849-860. DOI: 10.1016/S0140-6736(17)31868-8.

[3] Xue K, Jolly JK, Barnard AR, et al. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia[J]. Nat Med, 2018, 24(10): 1507-1512. DOI:10.1038/s41591-018-0185-5.

[4] Cideciyan AV, Jacobson SG, Drack AV, et al. Effect of an intravitreal antisense oligonucleotide on vision in Leber congenital amaurosis due to a photoreceptor cilium defect[J]. Nat Med, 2019, 25(2): 225-228. DOI:10.1038/s41591-018-0295-0.

[5] Cehajic-Kapetanovic J, Xue K, de la Camara CM, et al. Initial results from a first-in-human gene therapy trial on X-linked retinitis pigmentosa caused by mutations in RPGR[J]. Nat Med, 2020, 26(3): 354-359. DOI:10.1038/s41591-020-0763-1.

[6] Yuan J, Zhang Y, Liu H, et al. Seven-year follow-up of gene therapy for Leber's Hereditary optic neuropathy [J/OL]. Ophthalmology, 2020, S0161-6420(20)30188-3[2020-07-10]. https://pubmed.ncbi.nlm.nih.gov/32284191/. DOI:10.1016/j.ophtha.2020.02.023.

[7] Fischer MD, Michalakis S, Wilhelm B, et al. Safety andvision outcomes of subretinal gene therapy targeting cone photoreceptors in achromatopsia: a nonrandomized controlled trial [J]. JAMA Ophthalmol, 2020, 138(6): 1-9. DOI: 10.1001/jamaophthalmol.2020.1032.

[8] Cukras C, Wiley HE, Jeffrey BG, et al. Retinal AAV8-RS1 gene therapy for X-linked retinoschisis: initial findings from a phase Ⅰ/Ⅱ A trial by intravitreal delivery[J]. Mol Ther, 2018, 26(9): 2282-2294. DOI:10.1016/j.ymthe.2018.05.025.

[9] Tornabene P, Trapani I, Minopoli R, et al. Intein-mediated protein trans-splicing expands adeno-associated virus transfer capacity in the retina [J/OL]. Sci Transl Med, 2019, 11(492): eaav4523[2020-07-11]. https://pubmed.ncbi.nlm.nih.gov/31092694/. DOI:10.1126/scitranslmed.aav4523.

[10] Zhang W, Li L, Su Q, et al. Gene therapy using a miniCEP290 fragment delays photoreceptor degeneration in a mouse model of Leber congenital amaurosis[J]. Hum Gene Ther, 2018, 29(1): 42-50. DOI:10.1089/hum.2017.049.

[11] Mookherjee S, Chen HY, Isgrig K, et al. A CEP290 C-terminal domain complements the mutant CEP290 of Rd16 mice in trans and rescues retinal degeneration [J]. Cell Rep, 2018, 25(3): 611-623. DOI:10.1016/j.celrep.2018.09.043.

[12] Maeder ML, Stefanidakis M, Wilson CJ, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10[J]. Nat Med, 2019, 25(2): 229-233. DOI:10.1038/s41591-018-0327-9.

[13] Yu W, Mookherjee S, Chaitankar V, et al. Nrl knockdown by AAV-delivered CRISPR/Cas9 prevents retinal degeneration in mice[J/OL]. Nat Commun, 2017, 8: 14716[2020-07-11]. https://pubmed.ncbi.nlm.nih.gov/28291770/. DOI:10.1038/ncomms14716.

[14] Simunovic MP, Shen W, Lin JY, et al. Optogenetic approaches to vision restoration[J]. Exp Eye Res, 2019, 178: 15-26. DOI: 10.1016/j.exer.2018.09.003.

[15] Wu Z, Yang H, Colosi P. Effect of genome size on AAV vector packaging[J]. Mol Ther, 2010, 18(1): 80-86. DOI: 10.1038/mt.2009.255.

[16] Dalkara D, Byrne LC, Klimczak RR, et al. *In vivo*-directed evolution of a new adeno-associated virus for therapeutic outer retinal gene delivery from the vitreous[J/OL]. Sci Transl Med, 2013, 5(189): 189ra76[2020-07-11]. https://pubmed.ncbi.nlm.nih.gov/23761039/. DOI:10.1126/scitranslmed.3005708.

[17] Byrne LC, Day TP, Visel M, et al. *In vivo*-directed evolution of adeno-associated virus in the primate retina [J/OL]. JCI Insight, 2020, 5(10): e135112[2020-07-11]. https://pubmed.ncbi.nlm.nih.gov/32271719/. DOI:10.1172/jci.insight.135112.

[18] Ding K, Shen J, Hafiz Z, et al. AAV8-vectored suprachoroidal gene transfer produces widespread ocular transgene expression[J]. J Clin Invest, 2019, 129(11): 4901-4911. DOI:10.1172/JCI129085.

[19] Yu W, Wu Z. Ocular delivery of CRISPR/Cas genome editing components for treatment of eye diseases [J/OL]. Adv Drug Deliv Rev, 2020, S0169-409X(20)30057-0[2020-07-12]. https://pubmed.ncbi.nlm.nih.gov/32603815/. DOI:10.1016/j.addr.2020.06.011.

[20] Giannelli SG, Luoni M, Castoldi V, et al. Cas9/sgRNA selective targeting of the P23H Rhodopsin mutant allele for treating retinitis pigmentosa by intravitreal AAV9. PHP. B-based delivery[J]. Hum Mol Genet, 2018, 27(5): 761-779. DOI:10.1093/hmg/ddx438.

[21] Li P, Kleinstiver BP, Leon MY, et al. Allele-specific CRISPR-Cas9 genome editing of the single-base P23H mutation for rhodopsin-associated dominant retinitis pigmentosa[J]. CRISPR J, 2018, 1: 55-64. DOI:10.1089/crispr.2017.0009.

[22] Cai Y, Cheng T, Yao Y, et al. *In vivo* genome editing rescues photoreceptor degeneration via a Cas9/RecA-mediated homology-directed repair pathway[J]. Sci Adv, 2019, 5(4): eaav3335[2020-07-12]. https://pubmed.ncbi.nlm.nih.gov/31001583/. DOI:10.1126/sciadv.Aav3335.

[23] Suzuki K, Tsunekawa Y, Hernandez-Benitez R, et al. *In vivo* genome editing via CRISPR/Cas9 mediated homology-independent targeted integration[J]. Nature, 2016, 540(7631): 144-149. DOI:10.1038/nature20565.

[24] Levy JM, Yeh WH, Pendse N, et al. Cytosine and adenine base editing of the brain, liver, retina, heart and skeletal muscle of mice via adeno-associated viruses[J]. Nat Biomed Eng, 2020, 4(1): 97-110. DOI:10.1038/s41551-019-0501-5.

[25] Anzalone AV, Randolph PB, Davis JR, et al. Search-and-replace genome editing without double-strand breaks or donor DNA [J]. Nature, 2019, 576(7785): 149-157. DOI: 10.1038/s41586-019-1711-4.

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