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CRISPR/Cas9 genome surgery for retinal diseases

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Retinal diseases that impair vision can impose heavy physical and emotional burdens on patients' lives. Currently, clustered regularly interspaced short palindromic repeats (CRISPR) is a prevalent gene-editing tool that can be harnessed to generate disease model organisms for specific retinal diseases, which are useful for elucidating pathophysiology and revealing important links between genetic mutations and phenotypic defects. These retinal disease models are fundamental for testing various therapies and are indispensible for potential future clinical trials. CRISPR-mediated procedures involving CRISPR-associated protein 9 (Cas9) may also be used to edit genome sequences and correct mutations. Thus, if used for future therapies, CRISPR/Cas9 genome surgery could eliminate the need for patients with retinal diseases to undergo repetitive procedures such as drug injections. In this review, we will provide an overview of CRISPR/Cas9, discuss the different types of Cas9, and compare Cas9 to other endonucleases. Furthermore, we will explore the many ways in which researchers are currently utilizing this versatile tool, as CRISPR/Cas9 may have far-reaching effects in the treatment of retinal diseases.

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Introduction

Inherited retinal diseases affect approximately 1 in 3000 people worldwide and include a number of progressive disorders involving photoreceptor degeneration [1,2]. Many retinal diseases ultimately lead to irreversible blindness. Currently, there are no proven cures for patients suffering from retinal diseases, but developments in medical technologies, pharmacology, and gene and regenerative therapies have yielded promising results [3,4].

The retina contains several layers of cells that convert light stimuli into electrical signals through phototransduction [5]. Photoreceptors capture photons of light and convert them into electrical signals [6], which are then amplified and transmitted to bipolar cells, inner neuron cells (i.e. amacrine, horizontal), and ganglion cells. The ganglion cells then transmit these electrical impulses to the brain through the optic nerve [7]. The flow of signal from the photoreceptors to the brain is disrupted as photoreceptors degenerate, often leading to irreversible blindness.

One method of treating recessive retinal diseases lies in gene augmentation therapy, which involves supplementing the patient with a copy of the wild type (WT) gene to allow for

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diseased cells to create functional protein products [8]. Adeno-associated virus (AAV) vectors are often used to deliver the appropriate gene due to their low toxicity and lack of pathogenicity [1]. Gene augmentation therapy in humans showed promising results in the treatment of Leber congenital amaurosis (LCA) caused by mutations in the retinal pigment epithelium-specific 65-kDa protein (RPE65) gene [9–11]. Researchers demonstrated the long-term survival of the AAV vectors and consistent expression of therapeutic genes in animal disease models [9–11]. On December 19, 2017, the U.S. Food and Drug Administration (FDA) approved the AAV therapy (named ''Luxturna,'' or voretigene neparvovec-rzyl) to treat LCA in humans after the phase 3 trial demonstrated safety and efficacy [10,12].

Despite its successes as a treatment for LCA, AAV gene augmentation therapy currently lacks the ability to treat dominant diseases, as the addition of an exogenous functional gene via gene therapy is insufficient to override the dysfunctional proteins produced by the dominant mutant allele. This is problematic because there are numerous dominant retinal diseases, including but not limited to autosomal dominant retinitis pigmentosa (adRP), pattern macular dystrophies, and Best vitelliform macular dystrophy [13–15]. For this reason, scientists have turned to alternative methods such as CRISPR/Cas9 genome surgery to overcome this hurdle.

CRISPR/Cas9 can serve as a fundamental tool for translational research on retinal diseases affecting widespread populations, such as retinitis pigmentosa (RP), LCA, and age-related macular degeneration (AMD) [16–18]. CRISPR/Cas9 technology has been used to create knock-in and knock-out animal disease models geared towards developing treatments for inherited retinal diseases. In this review, we will provide an overview of CRISPR/Cas9, discuss the different types of Cas9 being used in research today, and compare CRISPR/Cas9 to transcription activator-like nucleases (TALENs) and Zinc Finger Nucleases (ZFNs). Next, we will explore how CRISPR/Cas9 has been used to study disease mechanisms of retinal diseases as well as its potential for application in future clinical treatments.

Overview of the CRISPR/Cas9 system

Background of CRISPR/Cas9

The CRISPR/Cas system was first discovered in prokaryotes in the 1990s, and its role in adaptive immunity was further characterized in the 2000s [19–22]. By incorporating a copy of the pathogenic viral DNA into their own DNA sequences, bacteria retain a genetic memory of former viral invaders and utilize their CRISPR/Cas machinery to cleave and destroy DNA of future viral invaders. Cas9 binds to the DNA target site by recognizing a CRISPR RNA (crRNA) bound to a trans-activating crRNA (tracrRNA) strand. The crRNA binds to the complementary DNA sequence, or spacer sequence, which lies next to a G-rich protospacer adjacent motif (PAM) [23,24]. Then Cas9 makes a double-stranded break (DSB) to the target DNA using its HNH and RuvC nuclease domains [23,25,26]. In 2012, Jinek et al. demonstrated that a single guide RNA (sgRNA)—created by joining crRNA and tracrRNA with a hairpin-shaped linker—effectively mimics the tracrRNA:crRNA complex and enables a more efficient and versatile design [27].

Different types of Cas9

A number of Cas9 endonucleases have been discovered thus far. *Streptococcus pyogenes* Cas9 (SpCas9) is a frequently utilized variant of Cas9 spanning 4.2 kb [20,27,28]. Due to its large size, however, researchers have explored alternative Cas9 strains that fit the carrying capacity of AAV (~4.7 kb) [24,28–30]. Ran et al. reported *Staphylococcus aureus* Cas9 (SaCas9) to have a gene editing efficiency comparable to that of SpCas9, with the added benefit of being over 1 kb shorter in length [28]. In 2017, Kim et al. reported *Campylobacter jejuni* (CjCas9) to be an even shorter Cas9 orthologue (2.95 kb) that demonstrated highly specific *in vivo* gene editing [30]. In 2015, Zetsche et al. reported a unique single RNA-guided endonuclease, Cpf1, which differs in structure from Cas9 but is capable of facilitating gene-editing in a mammalian cell line [24]. Unlike Cas9, Cpf1 has: (1) a crRNA but no tracrRNA domain (2) a T-rich PAM instead of a G-rich PAM, and (3) a RuvC-like endonuclease that creates a staggered double stranded break and DNA overhang. After analyzing 16 Cpf1-family proteins, authors claimed that the endonucleases from *Acidominococcus sp. Bv3L6* and *Lachnospiracae bacterium ND2006* display the best efficacy for gene editing in human cells [24].

Catalytically dead Cas9 (dCas9) possesses inactive nuclease domains due to mutations in RuvC and HNH [31]. As such, it employs the DNA targeting and binding function of Cas9 while lacking the ability to create DSBs. Fusion to an effector domain, however, allows dCas9 to affect levels of gene expression [32]. When fused to Krüppel associated box (KRAB), a transcription repressor domain, the dCas9-KRAB complex blocks the transcription of target sequences in a modular and precise fashion [32]. This gene knockdown technique is called CRISPR interference (CRISPRi) because dCas9 blocks RNA Polymerase (RNAP) activity during the initiation of translation and elongation in place of cleaving a targeted site like WT Cas9 [31]. A highly efficacious endonuclease for gene knock down, dCas9 is capable of repressing gene expression in human HEK293 cells [31,33] and exhibits high specificity, reversibility, and an impressive on-target rate [31]. Although dCas9 expands the application of CRISPR/Cas9, it is challenging to use *in vivo*, requiring two AAVs to account for the size of both dCas9 and effector domain (e.g. KRAB) [34]. The effector is usually fused to an adaptor protein (e.g. Pumilio/FBF) and packaged into an AAV separately from dCas9 [35]. The dCas9

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