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Saving eyesight, one gene at a time

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Abstract

Kai Yao's group used prime editing to repair a blindness-causing mutation in the *Pde6b* gene in the mouse retina. This breakthrough was made possible by a Cas9 nickase that is not constrained by a PAM sequence requirement. This innovation brings prime-editing technology one step closer to correcting disease-causing mutations at will.

Keywords

CRISPR-Cas; base editing; prime editing; genome editing; retinal degeneration

CRISPR-Cas technology has come a long way since 1987, when Yoshizumi Ishino and coworkers discovered peculiar repeated sequences in the bacterial genome [1]. Through 25 years of research, scientists unraveled the purpose of these sequences and decoded the RNA-guided restriction mechanism of prokaryotes [2]. A major breakthrough occurred in 2013, when CRISPR-Cas was first successfully implemented for genome editing in mammalian cells [3]. Important developments followed, especially when Cas9 was mated to the enzymatic activities of DNA-specific cytosine and adenine deaminases to create base editors [4, 5], and to reverse transcriptase to create prime editors [6]. These new classes of genome editors enable designed alterations in DNA independently from the double-strand nuclease activity of Cas9, significantly enhancing precision and versatility. Ultimately, base editors and prime editors constitute a toolbox with the potential to precisely repair disease-causing mutations by introducing targeted transitions (base editors); or substitutions, small insertions, and deletions (prime editors); or larger scale modifications (twin prime editing with DNA integration). In the canonical approach, both base and prime editors are limited

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by a short sequence known as a protospacer adjacent motif (PAM). The PAM is located immediately downstream from a target DNA sequence determined by the guide RNA and is involved in the initial recognition of the target site by Cas9. For example, the PAM for *Streptococcus pyogenes* Cas9 (SpCas9), the most often used ortholog, is NGG. Flexibility in the PAM sequence can be achieved by utilizing modified SpCas9 or different Cas9 orthologs, such as *Staphylococcus aureus* Cas9 which has NNGRRT as its PAM.

CRISPR-Cas technology and the eye

Over the years, many models of inherited blindness have been identified in mice, providing excellent preclinical models for studying approaches to treat blinding diseases. Precision genome editing using CRISPR-Cas technology, more specifically base and prime editors, has the potential to correct blinding mutations in humans. Major roadblocks to the clinical application of the technology include low efficiency of editing in the target tissue, especially in the case of prime editing, and inadequate delivery to the cell population affected by the disease-causing gene. In the eye, therapeutic efforts are focused on the retinal pigment epithelium (RPE) and the photoreceptors, of which the latter are especially difficult targets for the genome-editing tools due to low permeability of the retinal extracellular matrix. An example of an RPE-centered model is the *rd12* mouse, where the gene encoding the central enzyme of the visual cycle, retinoid isomerase RPE65, is not expressed due to a transition mutation that introduces a premature stop codon. Consequently, functional visual chromophore is not produced, leading to severely impaired visual function from birth, accompanied by a rapid loss of cone photoreceptors, resembling Leber congenital amaurosis (LCA). The *rd10* mouse instead serves as a great model for a disease affecting photoreceptors, retinitis pigmentosa. In this case, the gene *Pde6b* encoding an essential component of the phototransduction pathway, cGMP phosphodiesterase, is mutated and its activity decreased. In these mice, insufficient activity of phosphodiesterase leads to accumulation of cGMP in the photoreceptor cells, causing rod and cone photoreceptor cell death.

Viral vector for the delivery of nickase

Robust restoration of functional vision was achieved in *rd12* mice by using lentiviral vectors carrying an adenine base editor (ABE) and a specific guide RNA leading to the recovery of expression of RPE65 [7]. In the case of the photoreceptors, use of an adeno-associated virus (AAV) is a currently applicable paradigm for viral delivery. Indeed, Kai Yao's group demonstrated that genome-editing machinery can be delivered to the photoreceptors of *rd10* mice using AAV, and result in highly efficient genome editing, restoration of PDE6 activity, and functional vision [8]. Remarkably, they have demonstrated a potential solution for improving editing efficiency; namely, to avoid the PAM requirement altogether and rely exclusively on the specificity provided by the guide RNA. A preliminary assessment of this strategy in cell culture showed that a prime editor with a conventional Cas9 nickase recognizing the NGG-PAM was not effective in correcting the *rd10* mutation. In contrast, the use of Cas9 SpRY nickase, also known as "PAMless", led to highly efficient editing *in vitro*, in some cases exceeding 60% conversion of the mutant allele to wild type. This motivated the researchers to develop dual AAV constructs for the delivery of prime editor in

two parts for sustained expression in mouse photoreceptors. In this way, editing efficiency reached 40% in the whole retina and 80% in the cells that received the vector, with minimal editing of off-target sites. Over 15 weeks, prime-editor activity in the photoreceptors led to a gradual increase of PDE expression, increased activity of the enzyme, normalized cGMP levels, and maintenance of functional vision, as evidenced by electroretinography (ERG) and an extensive battery of behavioral tests.

In another recent study, the *rd10* mutation was targeted using ABE [9], which was delivered to the photoreceptors, also using AAV. Collectively, these studies provide an interesting insight into the relative efficacy of ABE and PE with the same mouse model [8, 9]. In both reports, precision genome editing resulted in the restoration of vision in the treated *rd10* mice, as evidenced by ERG and behavioral tests. Remarkably, retinal morphology was also preserved, especially for the rod photoreceptors. Thus, both the ABE and unconstrained prime-editing approaches demonstrated efficiency in maintaining vision in the model mice, with a slight advantage to the prime editing. However, prime editing had a clear advantage over ABE in terms of precision. Deep sequencing showed that besides unedited and precisely edited outcomes, imprecise edits constituted only a small fraction of the outcomes in the prime-edited animals. By contrast, with the base-edited animals, a large share of the outcomes had a neighboring adenine edited alongside the target. This comparison is an early indication that prime editing in general has greater potential to become the precise genome-editing modality of choice. We believe that with further development of prime editors, patients with debilitating inherited diseases will soon have their vision protected from progressive degeneration.

Sustained expression of base editors and prime editors

Concerns remain over sustained expression of base editors and prime editors. The studies described above all used viral vectors and prolonged expression of Cas9 along with other components. It is plausible that such prolonged expression would lead to the accumulation of detrimental byproducts of base-editor or prime-editor activity outside of the target site. To avoid unwanted mutagenic activity, it is imperative to develop transient, non-viral delivery methods for converting mutants to functional alleles with clinically measurable benefits, while limiting the duration of exposure to genome-editing machinery. Moreover, the safety data gathered in the preclinical stage of a recently paused trial for CEP290 restoration in LCA10 showed that there was an immune response to the AAV vector and a lower, yet detectable immune response to the Cas9 [10]. When developing the next generation of non-viral vectors, this issue must be included among the considerations for improvement.

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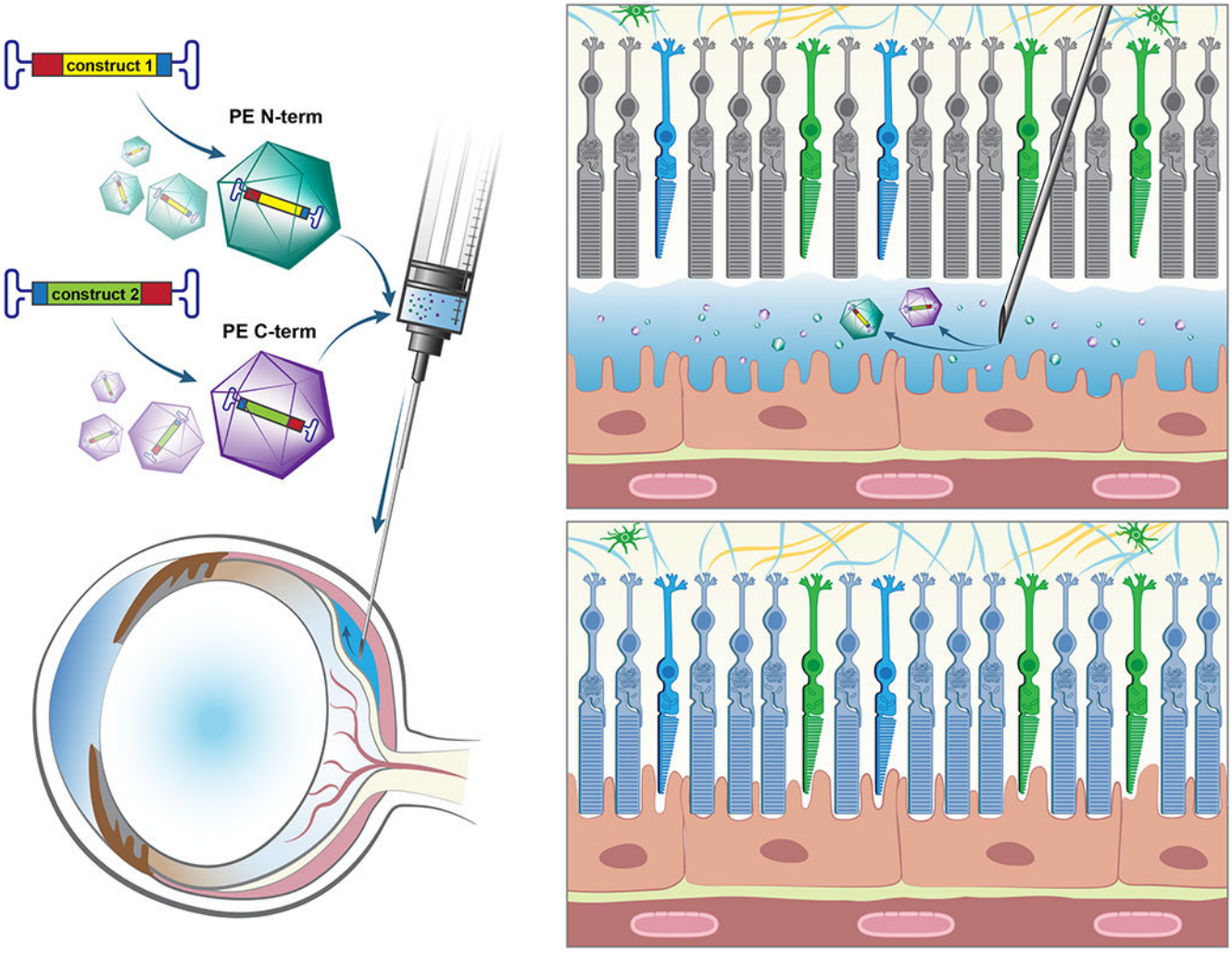

Figure 1. Rescue of vision loss associated with retinitis pigmentosa by correcting a mutation on the β-subunit of phosphodiesterase 6.

Dual adeno-associated viruses (AAVs) were delivered subretinally. AAV vector 1 contained a gene encoding a fragment of Cas9 nickase with fused reverse transcriptase. Independently, AAV2 contained genes encoding the remaining part of the Cas9 nickase, prime-editing guide RNA, nicking guide RNA, and a GFP reporter. After the subretinal injection, fluid containing the virions is absorbed by the RPE cells and retina, and the RPE becomes reattached. Healthy phenotype of the photoreceptor cells is restored after successful prime editing. For details see Ref. [8].