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INSIGHTS

Eye on genome editing

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CRISPR/Cas9 genome editing techniques have the potential to treat previously untreatable inherited genetic disorders of vision by correcting mutations that cause these afflictions. Using a prime editor, Qin et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20220776) restored visual functions in a mouse model (*rd10*) of retinitis pigmentosa.

Since its development as a platform for gene editing, a number of animal models of human diseases have demonstrated the utility of the CRISPR/Cas9 gene editing system. Using a genome editor (PE^{SpRY}), Qin et al. (2023) corrected a pathogenic mutation in *Pde6b*, restoring retinal function and morphology. The authors report the first successful retinal photoreceptor prime editing of a clinically relevant gene (Qin et al., 2023). The first human clinical trials have begun to report the results of both ex vivo and in vivo gene editing (Gillmore et al., 2021). However, CRISPR/Cas9 has major drawbacks, including the inability to precisely control the editing outcomes and genotoxicity, either through homology-directed recombination or random insertions and deletions (Wang and Doudna, 2023). Even base editors, though they circumvent many of the issues of nuclease Cas9, including avoidance of double-strand breaks in DNA, still could lead to unwanted bystander editing of accessible bases within the editing window (Wang and Doudna, 2023). In particular, conversion of mutated alleles back to wildtype sequences, as opposed to knocking out dominant or pathogenic alleles, requires extremely precise editing. Indeed, impurity of editing outcomes can limit the effectiveness of such attempted gene correction or even nullify it. Within this context, a new generation of precision genome editors, termed prime editors, offers even more flexibility and applicability to correcting disease mutations. These new editors can install or reverse every kind of point mutation, as well as effect small insertions and deletions; continual improvements will undoubtedly expand the broad utility of prime editors (Anzalone et al., 2019; Chen et al., 2021). Although prime editors are powerful tools, there remain several limitations, such as the overall efficiency of editing, the need for properly placed protospacer adjacent motifs (PAMs) for accurate target recognition and editing, and the need to empirically define and screen prime editing guide RNAs (pegRNAs).

The eye has been a proven testbed for new technologies and therapies, as evidenced by the FDA approval of Luxturna (voretigene neparvovec-rzyl) for biallelic *RPE65* Leber congenital amaurosis (Darrow, 2019), the very first FDA-approved gene therapy for any indication. Building on this success, the use of Cas9 editing in the eye has led to numerous reports of successful mutation corrections. Importantly, in vivo base and prime editors have been demonstrated in the retinal pigment epithelium (RPE), with high levels of editing reported along with physiological rescue of retinal function (Choi et al., 2022; Jang et al., 2022; Jo et al., 2022; Suh et al., 2021). Undeniably, these are important proof-of-concept studies, but further improvements in editing efficiency and purity are required, and application to other retinal cell types besides the RPE is necessary. For instance, many more rod and cone photoreceptor genes are involved in retinal dysfunction than those RPE genes that have been tested (Suh et al., 2022; Travis et al., 2007).

Insights from Samuel W. Du and Krzysztof Palczewski.

To address these issues, Qin et al. (2023) combined the prime editor effector domain (Moloney murine leukemia virus reverse transcriptase; Qin et al., 2023) with a recently described engineered Cas9 variant from *Streptococcus pyogenes* (SpRY; see Fig. 1; Walton et al., 2020). As SpRY is almost unconstrained by PAM placement and sequence, the combined prime editing construct is highly flexible and theoretically can repair nearly every kind of mutation that leads to disease (Miller et al., 2020; Walton et al., 2020). In a masterful investigation, the authors systematically screened and optimized pegRNAs for correction of the murine $Pde6b^{rd10}$ (*rd10*) mutation in the critical enzyme phosphodiesterase 6 β. This naturally occurring mutation in humans is responsible for photoreceptor cell death and blindness, recapitulated in the *rd10* mouse model. They then packaged prime editor SpRY (PE^{SpRY}), with the optimal pegRNA and a nicking guide RNA to improve editing, into dual adeno-associated viruses (AAVs)

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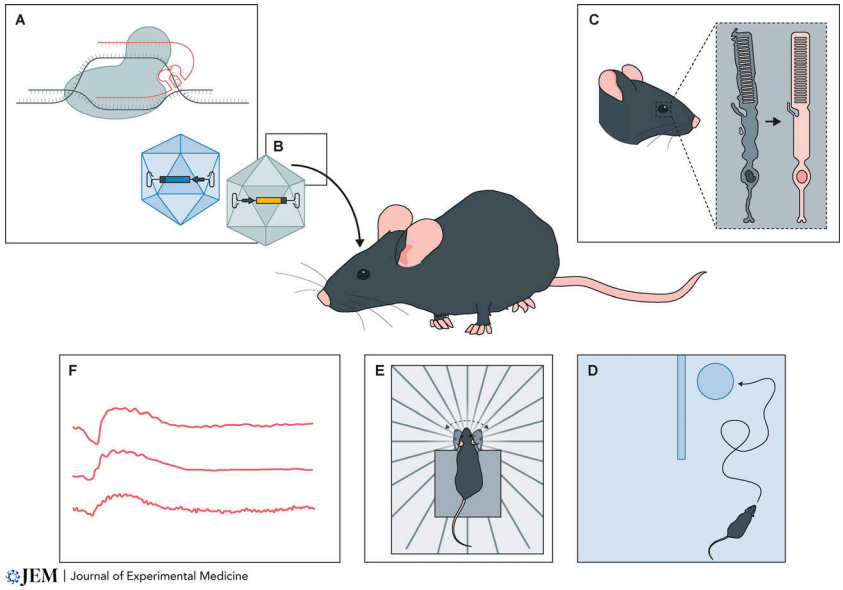

Figure 1. PE^{SpRY} prime editing strategy rescues the *rd10* mouse. (A) After in vitro screening of pegRNAs, the PE^{SpRY} construct, pegRNA, and nicking guide RNA were packaged into two AAVs. (B) The two AAVs were then injected into the subretinal space of the *rd10* mouse. (C) Once photoreceptors were prime edited to restore wildtype *Pde6b*, the photoreceptors were rescued and became healthy again. (D) The treated mice successfully completed a water maze test, where the mouse is dropped into a tank of water and has to pick a side to swim to: one has a submerged platform and the other does not, and the mouse is guided by a visual cue. (E) Another behavioral task successfully completed by the treated mouse is the optomotor test, where a mouse is placed on a platform and a computer tracks head movements in response to varying visual stimuli. (F) The mice demonstrated restored electroretinography curves, where electrophysiological responses to flashes of light are recorded to measure retinal function.

that reconstitute in vivo to effect gene editing. The editing constructs were co-injected subretinally into *rd10* mice and achieved an average editing rate of ∼41% in a total retinal cell population, and ∼76% in a virally transduced and enriched cell population. The successfully edited *rd10* mice also displayed preserved retinal morphology and phosphodiesterase biochemical activity. Importantly, not only were the authors able to restore the electrophysiological responses of the retina, they also thoroughly documented recovery of vision-driven behaviors through a comprehensive battery of tests, including a light-dark transition test, optomotor responses, and a visually guided water maze task. Overall, the genetic, anatomical, biochemical, and behavioral evidence presented in this paper are a convincing demonstration of the efficiency and therapeutic potential of prime editing in this mouse model, providing a compelling proof of principle of the utility of prime editing for inherited retinal diseases that affect photoreceptors. Along with the previous body of work examining precision genome editing in the eye, we believe that this study adds to the evidence that base and prime editing have huge therapeutic potential for the treatment of previously untreatable genetic diseases leading to blindness. Indeed, the applicability of the PE^{SpRY} construct extends well beyond the eye and conceivably could be applied to nearly every human disease with known genetics, with the possible exceptions of copy number variations and large structural rearrangements.

As nuclease CRISPR/Cas9 gene editing, base editing, and prime editing are still relatively new, there are outstanding concerns remaining about their safety. While it is known that nuclease Cas9 and base editing can result in unintended genome- and transcriptome-wide off-target editing, little is known about prime editor off-targets. As further methodologies are developed, it will be critical to examine genetic off-target effects of any gene editor before physicians can confidently use them to treat patients. Numerous protocols exist to explore off-target effects (Doman et al., 2020), but it is still unclear how exhaustively and thoroughly one must assess these off-targets, i.e., whether one approach or a combination of approaches would be sufficient. Furthermore, in vivo gene editing comes with additional challenges in assessing tissue- and body-wide side effects, especially with human clinical trials.

The delivery of the PE^{SpRY} gene editing components via AAV raises another consideration. Although AAVs have a mostly favorable safety profile, there are still risks to viral delivery of genome editors, such as immune reactivity and toxicity from strong promoters and viral sequences. Additionally, recent work has highlighted that prolonged expression of genome editors can lead to extensive off-target mutations and unwanted effects that can be mitigated, in part, by the transient delivery of gene editors (Banskota et al., 2022). There is concern within the field that dosage with AAV or another viral vector could preclude a patient from receiving a repeat dose of a gene therapy for the same disease or another disease (e.g., inherited retinal degeneration therapy in childhood, and age-related macular degeneration or diabetic retinopathy therapy later in life). These are important considerations for genome editing that should be addressed now, before gene therapy and gene editing become commonplace. We think that transient delivery of genome editors as proteins or mRNA could circumvent some of these issues, but further work needs to be done before these alternative delivery vectors could replace AAV.

Most importantly, genome editing of animal models of inherited retinal diseases and other human diseases can only teach us so much. While the authors (and our group) have proven that precision genome editing is a viable way to assess physiological rescue of these animal models, the variable genetics among rodents, dogs, non-human primates, and humans means that these genome editing strategies will not be directly translatable. We hope that the reports of successful genetic rescues will spur others to develop and commercialize analogous therapies for humans, but a robust genetic screening and modeling pipeline must be developed in humans. Because they have been shown to be efficacious and safe in animal models, the next step for base and prime editors is application in humans. For this advance, we propose two complementary approaches: patient-derived induced pluripotent stem cells (iPSCs) and organoids, and human donor tissues (see Fig. 2). If patients are identified early enough in their disease course, we can generate iPSCs,

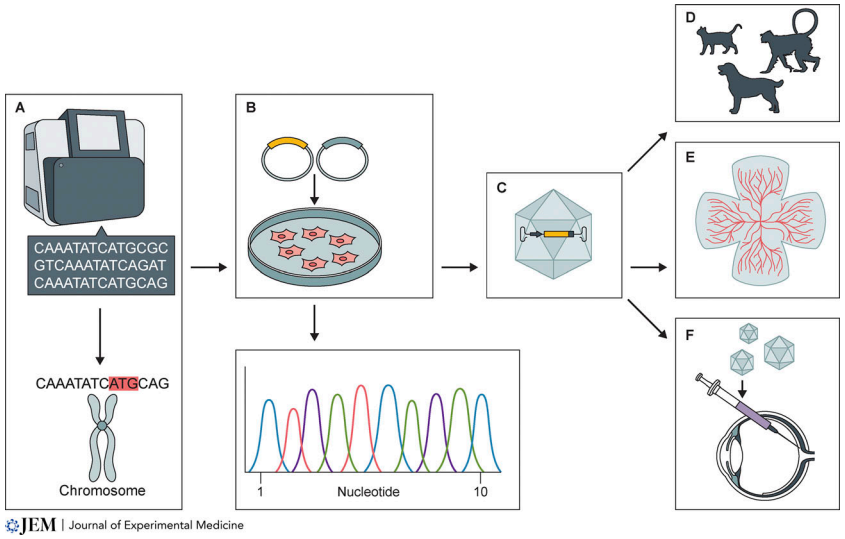

Figure 2. Conceptual gene editing pipeline for the treatment of inherited retinal diseases. (A) Patients with suspected inherited retinal diseases will have whole genome sequencing to identify causal variants. In some cases, this may include sequencing of close relatives as well. (B) Once mutations are identified, an appropriate gene editor can be selected, and appropriate guide RNAs can be designed. These will be then delivered onto patient-derived cells in vitro, and editing outcomes will be analyzed by sequencing. (C) The optimal gene editor and guide RNA combination can then be packaged into AAV for delivery. (D) For inherited retinal diseases for which we have appropriate animal models, these can be assessed for long-term physiological rescue and outcomes. (E) We also propose treating human donor eyes and retinas kept in culture to assess genome-wide effects, expression, and detect any potential off-target mutations. (F) Lastly, the validated AAVs can then be delivered to patients via subretinal injection for curative therapy.

differentiate them to the target tissue, and then assay the efficacy of the editors and any potential off-target effects. This concern is especially relevant in light of reports that off-target effects that are only assayed in cell culture or against a target genome could miss off-targets that are specific to single nucleotide polymorphisms or variations found in different human populations, which becomes an important issue considering that reference genomes and biomedical investigation is predominantly focused on people of European descent (Cancellieri et al., 2023). Thus, a cell line or tissue organoid of the patient's exact genetic makeup will be invaluable for precisely assessing any potential unwanted genetic alterations. More interestingly, to provide a proof of concept of human genome editing, we envision genome editing in human donor tissues. Recent reports of human donor eyes being kept in culture long enough for assessment of gene editing outcomes, as well as physiological responses close to in vivo processes (Abbas et al., 2022), could provide a stepping-stone model for the analysis of genome delivery method tropism, efficacy, and unwanted side effects, and excitingly, could teach us if in vivo gene editing in the retina could lead to changes in retinal physiology. We envision that this approach could be applicable to other tissue and cell types, and are excited to see the adoption of gene editing as a future standard of care. The future of genome engineering is bright, and the potential is unconstrained, so keep an eye on genome editing.

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