Nonviral nanoparticles to deliver CRISPR for in vivo genome editing

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Enabling CRISPR gene therapy requires the development of new biomaterial systems to target specific cells of the body, especially for cells outside of the liver. In complex tissues like the retina and brain, efficient delivery without adverse events to postmitotic, functional cells is still challenging with conventional synthetic vectors. Here we apply a new nanoplatform, the silica nanocapsule (SNC), to edit the retina.[1] We demonstrate our work on a rare inherited vision disorder, Leber Congenital Amaurosis (LCA16). This disorder is caused by point mutations, such as W53X (c.158G>A), in the *KCNJ13* gene, which encodes an inwardly rectifying potassium channel, Kir7.1, expressed in the retinal pigmented epithelium (RPE). We used SNC-mediated delivery of adenine base editor (ABE8e) mRNA and single-guide RNA to precisely and efficiently correct the $KCNJ13^{W53X/W53X}$ mutation. We observed editing in patient fibroblasts (47%) and human-induced pluripotent stem cell-derived RPE (LCA16-iPSC-RPE) (17%) with negligible off-target editing. Editing resulted in fully functional channels in the LCA16-iPSC-RPE. In heterozygous knock-in mice ($Kcnj13^{W53X/+}$), we obtained base correction in 16% of the RPE cells in vivo via targeted subretinal delivery of ABE8e by SNCs (Figure 1A). RPE cells were functional following base editing in our newly established LCA16 mouse model ($Kcnj13^{W53X/+\Delta R}$) and restoration of responses to a light stimulus in the treated eyes (Figure 1B). This comprehensive study of ion channel functional rescue, a challenge for pharmacological and genomic interventions, strongly supports the effectiveness of non-viral CRISPR base editing as an effective gene therapy for rare inherited visual disorders.

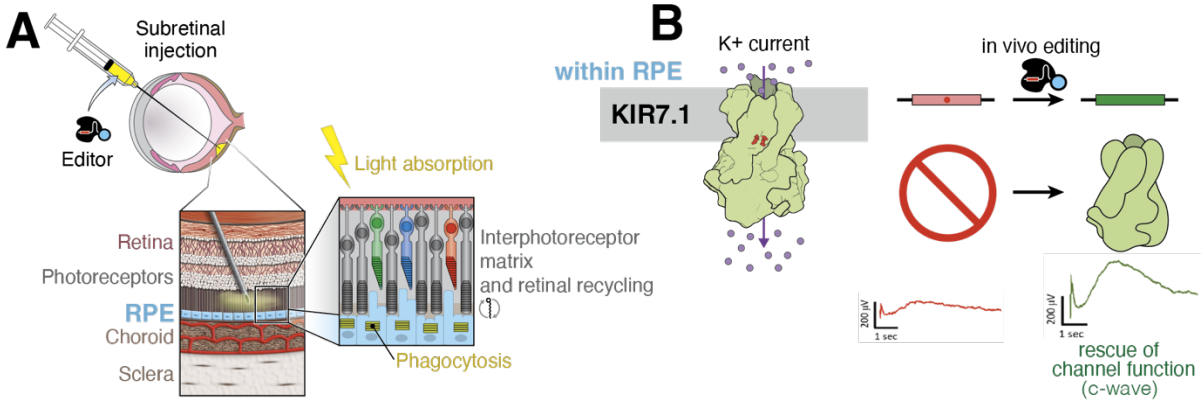

Figure 1: Functional rescue of visual acuity in mice after injection of nanoparticles containing genome editors. A) Overview of subretinal injection to edit the eye. **B)** Silica nanoparticle encapsulated delivery of CRISPR base editor ABE8e mRNA to correct a W53X disease mutation of *KCNJ13* gene. We report high editing efficiency and negligible on-target indels and substitutions in base-edited patient-derived fibroblasts, iPSC RPE, and in vivo mouse RPE. Patch-clamp electrophysiology results showed restored Kir7.1 channel function in base-edited iPSC RPE. In base-edited mice, RPE function was detected by the presence of light-induced electroretinogram (ERG) c-wave.

References:

[1] Kabra, M., et al. *bioRxiv* **2022** doi.org/10.1101/2022.07.12.499808.