Retina Australia Research Grant 2022: Final Report

Project Title:

RNA base editing strategies as potential therapeutic of inherited retinal dystrophies

Investigators:

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Project synopsis:

Inherited retinal diseases (IRDs) are collectively one of the most common causes of blindness in Australia and affect 1 in 2000 individuals globally. Patients with IRD suffer progressive degeneration of the retina leading to irreversible blindness. Nearly 300 genes have been implicated in IRDs, making the disease incredibly heterogenous and challenging to treat. Only one form of IRD, known as Leber's Congenital Amaurosis (LCA), can currently be treated. Traditional gene therapy is also unable to address many IRDs due to delivery limitations, causing an urgent clinical need for alternative therapeutic options.

Advanced genetic technology offers solutions in this front. Precise gene editing methods, specifically the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-CRISPR associated protein (Cas) technology, allows targeted manipulation of genes to achieve therapeutic benefit. Recent advances in CRISPR-Cas technology, particularly that of base editing, has proven immense clinical potential in cases of sickle cell anaemia or familial hypercholesterolemia. Preclinical studies have also shown the potential of base editing against retinal degenerations. While these advances are ground-breaking developments for gene therapy, they are not widely applicable due to targeting limitations of DNA base editing systems. A more flexible approach, through RNA base editing, is now feasible and can be achieved using clinically proven vectors for safe and long-term therapeutic benefit. Overall, RNA base editing can offer a widely applicable treatment option for IRDs, one that can adequately address the heterogeneous nature of the disease.

In this project, we developed an adeno-associated virus (AAV)-compatible RNA base editor (dCas13e-ADAR) to target a nonsense mutation found in *Rpe65*, causative for LCA. To establish the potential of our RNA base editor, we investigated its capacity to correct the *Rpe65* mutation efficiently and specifically against the only other established AAV-compatible RNA base editor, known as CRISPR-Cas-inspired RNA targeting system (CIRTS)-ADAR. Firstly, we screened various guide (g)RNAs to identify the optimal gRNA for each system using a dual-luciferase assay. Both dCas13e-ADAR and CIRTS-ADAR were then delivered with their optimal gRNAs and a mutant *Rpe65* gene into human retinal pigment epithelium cells. We have thereby shown that the dCas13e-ADAR proves far superior to CIRTS-ADAR in correcting the *Rpe65* mutation. It can also achieve up to 36% editing in human cell lines, as well as recovery of Rpe65 protein in human retinal pigment epithelium cells. Through this study, we have been able to show proof-of-concept for a novel gene editing treatment strategy that is safer and more flexible than current treatment options for IRDs.

The team is truly grateful for the support from Retina Australia towards this project. Our findings have led us to advance our technology to address more significant IRD mutations, as well as preclinical studies which would have direct implications on how these diseases are treated in the future.

Scientific Outcomes resulting from this funding:

1) We have first demonstrated the feasibility of CRISPR-dCas13e-ADAR RNA base editing by targeting a mutant mCherry gene *in vitro*. The introduction of a stop codon within the mCherry sequence with a G>A mutation abolished mCherry fluorescence when expressed as a plasmid within HEK239FT cells. When the CRISPR-Cas13e-ADAR base editor was specified to target the mutated adenosine, we observed ~50% recovery of fluorescence. We also targeted the same mutation using the CIRTS base editor (a protein engineering strategy for constructing programmable RNA control elements), however, this demonstrated a lower recovery of fluorescence (**Figure 1**). Our data suggest that gene delivery of an AAV-compatible RNA base editor (dCas13e-ADAR) can effectively edit the single-base mutation and restored functional protein expression *in vitro*.

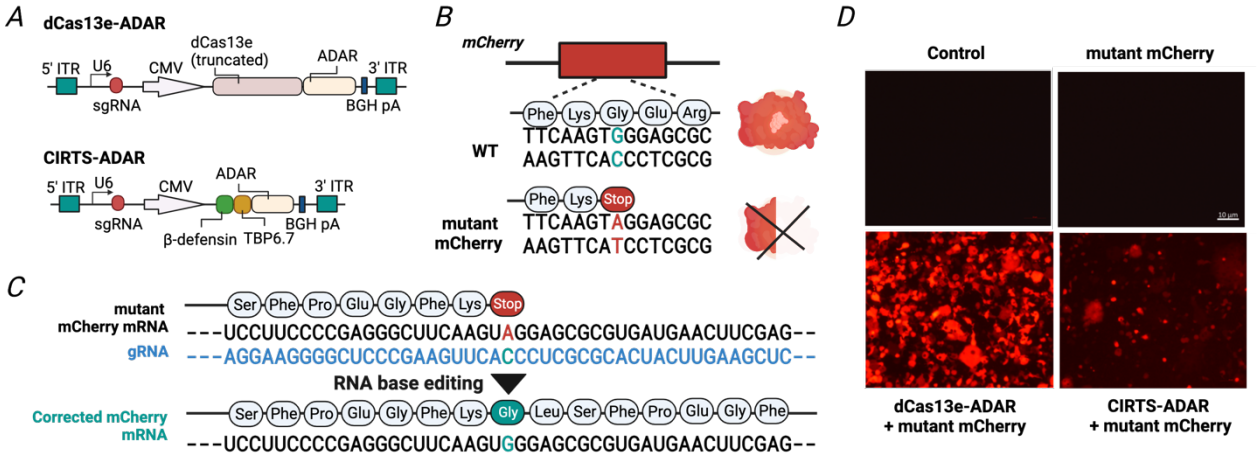

Figure 1. Remove premature stop codon by gene delivery of an all-in-one AAV RNA base editor. (A) CRISPR-dCas13e and CIRTS was attached to ADAR to develop compact RNA base editors. **(B)** The mutant mCherry has a G>A cDNA nonsense mutation, changing glycine (green) to a stop codon (red). **(C)** A 50nt guide RNA targeting the mutant mCherry RNA was used to remove the stop codon. **(D)** Fluorescence images of HEK293FT cells transfected (48 hrs) with mutant mCherry only, dCas13e-ADAR/mutant mCherry and CIRTS-ADAR/mutant mCherry, Scale bar: 10µm. mCherry expression is indicated by red fluorescence signal.

2) We then further employed dCas13e-ADAR and CIRTS-ADAR RNA base editors against a nonsense mutation found in *Rpe65* (**Figure 2A**). Using a dual-luciferase assay, we screened a range of gRNAs with mismatches denoting target base and identified that placing the mismatch at the 24th or 26th position of a 50nt gRNA resulted in high on-target editing with the dCas13-ADAR system. With the CIRTS-ADAR system, a 26th position mismatch proved efficient (**Figure 2B**). We subsequently validated these base editors in a mutant *Rpe65* gene engineered cells, carrying the same nonsense mutation, and found up to 36% editing with the dCas13e-ADAR system, while no editing was observed with CIRTS-ADAR (**Figure 2C and 2D**). Notably, the dCas13e-ADAR system was also able to recover Rpe65 expression in both HEK293FT and ARPE19 (human RPE) cells (**Figure 2E and 2F**).

Overall, these experiments demonstrate clinical viability of single vector gene delivery of RNA base editor against IRD mutant. We are now expanding this study to investigate the feasibility of using this RNA base editor to correct the mutations in other inherited retinal diseases, such as Usher syndrome type 2A, Usher syndrome type IF, and X-linked Retinoschisis.

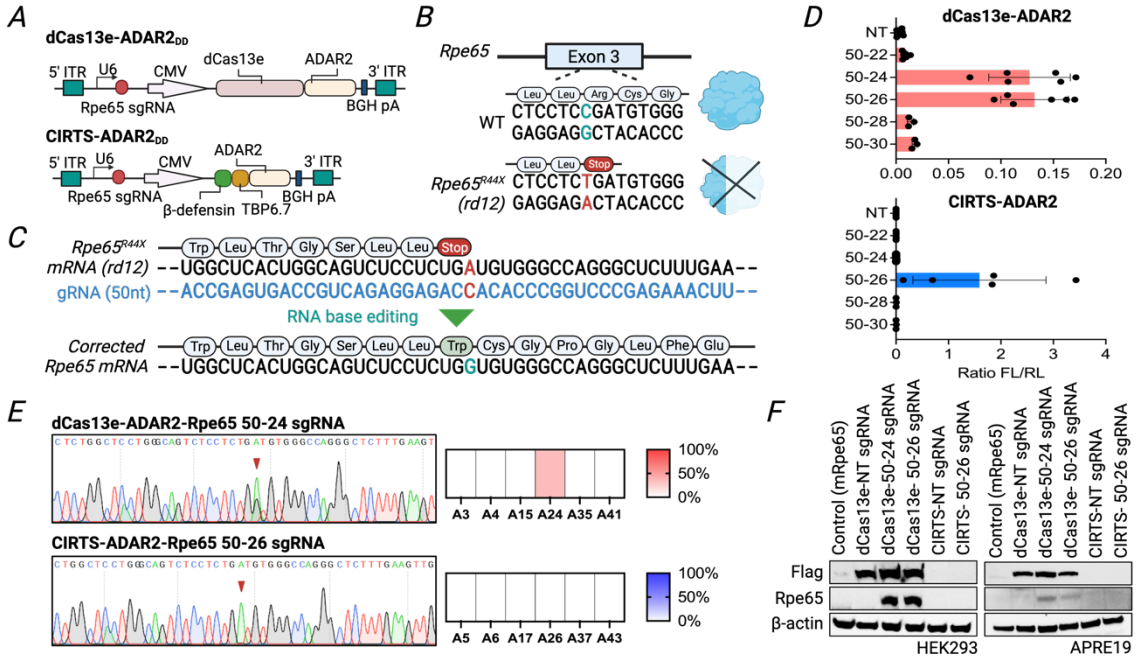

Figure 2. Remove premature stop codon of *Rpe65*^{R44X} mRNA by gene delivery of an all-in-one AAV RNA base editors. (A) dCas13e and CIRTS was attached to ADAR to develop compact RNA base editors. **(B)** The mutant *Rpe65*^{R44X} has a C>T cDNA nonsense mutation. **(C)** A 50nt guide RNA targeting the *Rpe65*^{R44X} mRNA was used to remove the stop codon. **(D)** Guide RNA screening using dual-luciferase assay with dCas13e-ADAR and CIRTS-ADAR RNA base editors **(E)** Sanger sequencing chromatogram and heat map showing correction of *Rpe65*^{R44X} mutation with dCas13e-ADAR and CIRTS-ADAR RNA base editors. **(F)** Western blot showing recovery of Rpe65 protein in HEK293FT and ARPE19 cells. Target base is denoted by red arrow.