Development of a CRISPR screen to identify molecular regulators of photoreceptor intercellular material exchange

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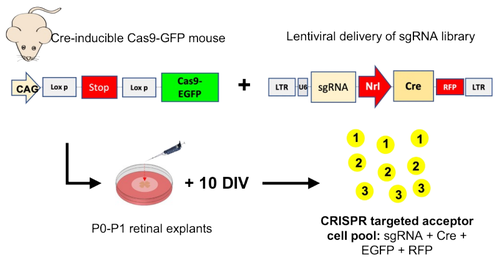
Introduction

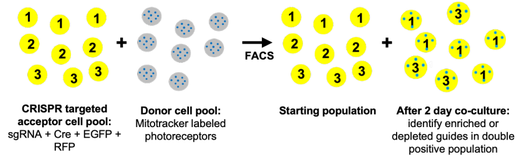
- The degeneration of photoreceptors (PRs) leads to irreversible and untreatable vision loss that is associated with diseases such as Retinitis Pigmentosa[1].
- This unmet clinical need has prompted several research groups to investigate cell replacement to restore photoreceptor function.
- While early studies of PR precursor transplantation reported the presence of host cells expressing the donor cell tracker (e.g. cytosolic GFP)[2], recent studies show that donor cells rarely, if ever, integrate into the host retina; instead, positive results of transplantation are due to material exchange (ME) of proteins from donor to host photoreceptors[3,4,5].
- There remains a gap in understanding of the molecular players involved in photoreceptor ME.
- We will use CRISPR-mediated gene inactivation in PRs to identify novel modulators of photoreceptor ME.

Methods

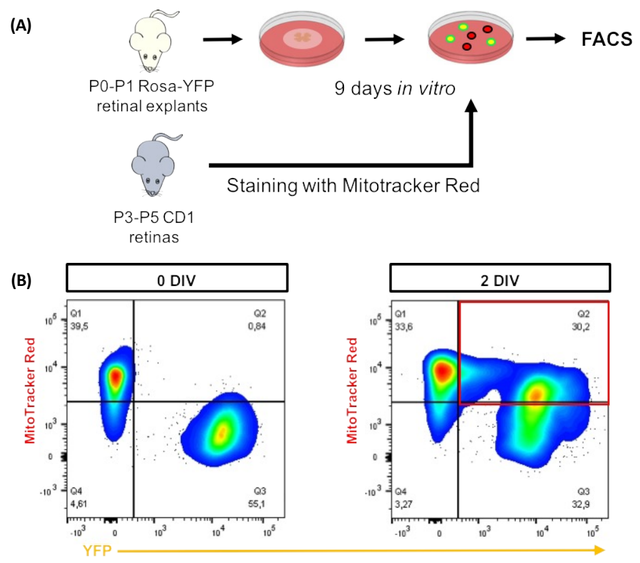
Retinal explants of cre-inducible Cas9 mice are infected with lentivirus carrying a library of over 3000 sgRNAs against 772 druggable targets, cre-recombinase, and RFP reporter regulated by the rod-specific Nrl promotor. After 10 days, to allow gene inactivation, infected explants are dissociated to generate a pool of gene-targeted acceptor photoreceptors.

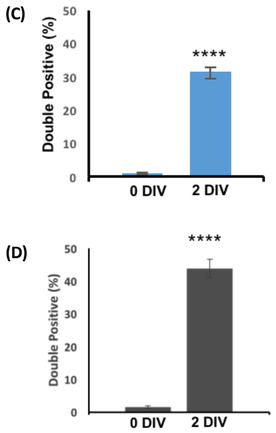


These guide-targeted acceptor cells are then either co-cultured with a donor pool of PRs labeled with MitoTracker Red (MTR), to track transfer of mitochondria, or cultured alone. After 2 DIV, the double positive (RFP+MTR+) acceptor cells are enriched by flow cytometry, gDNA is isolated and subjected to NGS and analyzed by changes in the frequency of sgRNAs to the pool of single RFP+ cells cultured alone. In the example below, sgRNA 1 targets inhibitors of ME, sgRNA 2 targets enhancers of ME, and sgRNA 3 targets a gene unrelated to ME.



Results





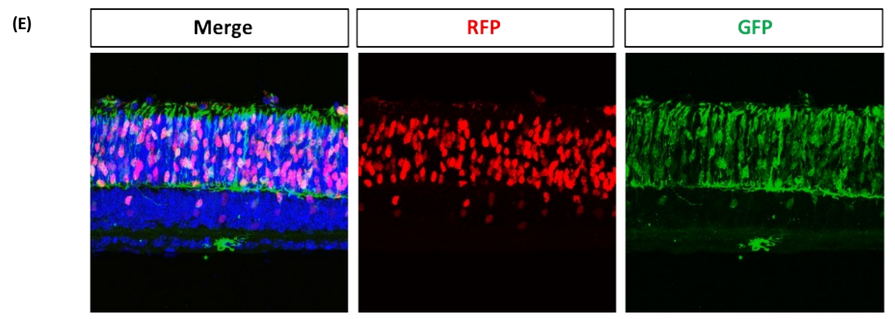


Figure 1. ***Generating CRISPR targeted pool of acceptor photoreceptors*** **(A)** To test the feasibility of using *in vitro*-cultured PRs as acceptors of mitochondria, P1 Rosa-YFP retinal explants (n=4) were maintained in explant culture for 9 DIV, then dissociated, using a papain-based kit (Worthington Biochemical, UK), and co-cultured with P3 MTR-labeled CD1 retinal dissociates (MTR^+CD1) (n=3). Flow cytometry analysis was performed after 2-day co-culture using BD LSR Fortessa and analyzed using FlowJo software. **(B)** Flow cytometry analysis of Rosa-YFP explant-derived and MTR^+CD1 photoreceptor co-culture showing transfer of MTR after 2 DIV compared to 0 DIV. Events collected were gated for $eFluor^-$ (live) and $CD73^+$ photoreceptors. Q1: MTR^+; Q2: MTR^+ and YFP^+; Q3: YFP^+; MTR^- and YFP^-. **(C)** Proportion of double positive (MTR^+YFP^+) cells out of entire $CD73^+$ population. **(D)** Proportion of Rosa-YFP explant-derived photoreceptors accepting MTR out of entire YFP^+ photoreceptor population. **E)** Confocal images of cryo-sectioned cre-inducible Cas9-GFP retinal explants, infected at P0 with lentivirus carrying sgRNA library, cre-recombinase, and RFP reporter, under rod-specific Nrl promotor, and analyzed after 10 DIV, showing photoreceptor specific infection and efficient GFP induction. One-way ANOVA; n.s. no statistical significance, ****p≤0.0001,***p≤0.001,**p≤0.01, *p≤0.05

Conclusions

- As dissociated retinal cells have poor viability *in vitro*, we developed an explant-based assay that enhances the feasibility of this screen.
- We demonstrated that explant-derived PRs can be recipients of mitochondria *in vitro*.
- In future experiments, we will FACS purify double positive CRISPR targeted recipients, PCR amplify sgRNA sequences, and perform NGS.
- We will validate hits using individual sgRNA *in vitro* and *in vivo*.
- Understanding the mechanism of ME can present new therapeutic applications of delivering functional proteins to a diseased host photoreceptor population.

References

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