Soluble CX3CL1-Expressing Cells Delay Rod Photoreceptor Degeneration in a Mouse Model of Retinitis Pigmentosa

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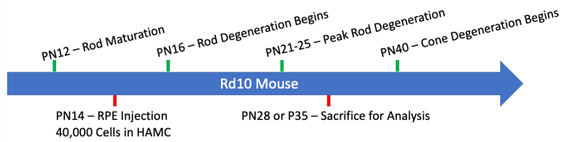
Introduction

- Retinitis pigmentosa (RP) causes blindness due to inherited mutations that result in photoreceptor degeneration[1].
- Therapeutics delay disease progression, but sustained release over long-term is required[2].
- Gene replacement therapy shows promise but is infeasible as any single mutation in over 70 genes contribute to RP; and this strategy poses safety concerns as viral vectors may give rise to insertional mutagenesis or non-specific transduction[3].
- Increase in the CX3CL1-CX3CR1 signaling axis has been shown to delay photoreceptor degeneration in mouse models of RP, possibly by inhibiting microglia activation[4,5].
- Here, we propose a combined cell and gene therapy, using human embryonic stem cell (hESC)-derived retinal pigment epithelium (RPE) cells to provide a sustained release of sCX3CL1 in the degenerating retina.
- Cells have been modified with our FailSafe™ (FS) technology to enhance cell therapy safety[6].

Hypothesis

Soluble CX3CL1-expressing retinal pigment epithelium cells delay the progression of retinitis pigmentosa by inhibiting microglia activation.

In Vivo Experiment Timeline



Rd10 mouse model of RP was used to evaluate *in vivo* efficacy of transgenic cells (Figs. 4-6). Mice were treated with cyclosporine A. Timeline of retinal degeneration in the Rd10 mouse (green) and the corresponding experiment timepoints (red). PN, post-natal.

Conclusion and Future Directions

- sCX3CL1-expressing retinal pigment epithelium cells delay rod degeneration in the Rd10 mouse model of RP.
- The therapeutic mechanism of action is currently being investigated via minocycline co-treatment, a drug inhibitor of microglia activation.
- Despite its presumptive local-acting effect, this method of delivery for biologics could be employed for the treatment of specific tissue regions, such as the human macula.

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Results

1. hESC Genetically Engineered using *piggyBac* Transposons

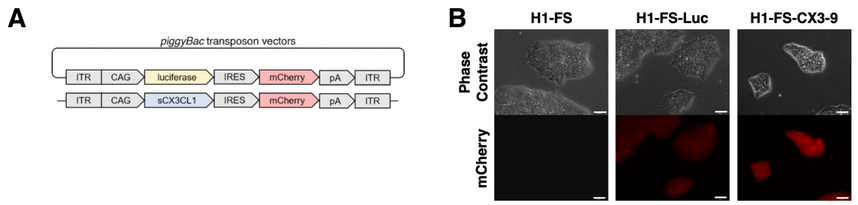

Figure 1. *piggyBac*-mediated genome engineering of hESC. (**A**) H1 hESC equipped with FailSafe™ (H1-FS) were genetically modified with *piggyBac* transposons to overexpress either luciferase (H1-FS-Luc) or sCX3CL1 (H1-FS-CX3-9). C-terminal FLAG and His tags added to sCX3CL1 construct via Gly-Ser linkers. IRES, internal ribosome entry site; ITR, inverted terminal repeat; pA, rabbit globin poly-A signal. (**B**) Selected subclones that express luciferase (H1-FS-Luc) or sCX3CL1 (H1-FS-CX3-9) co-express the mCherry reporter. Scale is 65μm.

2. Transgenic hESC Differentiate into RPE Cells

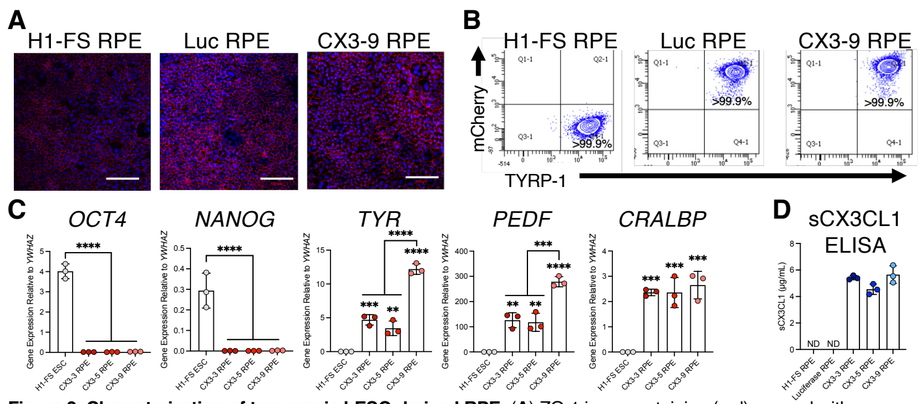

Figure 2. Characterization of transgenic hESC-derived RPE. (**A**) ZO-1 immunostaining (red) merged with DAPI counterstain (n=3, scale is 100μm). (**B**) Flow cytometry analysis of TYRP-1 and mCherry expression (n=2-3). (**C**) RT-qPCR analysis for markers of pluripotency and RPE cells (n=3). **p<0.0021, ***p<0.0002, ****p<0.0001. (**D**) sCX3CL1 ELISA of RPE culture supernatant. ND, not detected.

3. Donor RPE Survive and are Functional in the Subretinal Space

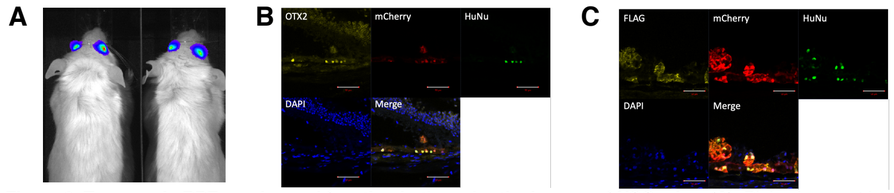

Figure 3. Transgenic RPE survive and function in the subretinal space of NSG mice. (**A**) Bioluminescent imaging of NSG mice 7 weeks after subretinal injection of Luc RPE. (**B-C**) NSG retinas 2 weeks after subretinal injections of CX3-9 RPE were co-stained for (**B**) OTX2 and human nuclear antigen (HuNu) or (**C**) FLAG (to detect exogenous sCX3CL1) and HuNu. Scale bar is 50μm.

4. Donor RPE Survive Short-Term in the Degenerating Retina

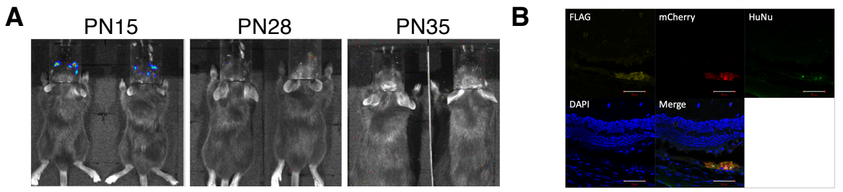

Figure 4. Donor RPE survive short-term in the subretinal space of the Rd10 mouse model of RP. (**A**) Bioluminescent imaging of Rd10 mice treated with subretinal injections of Luc RPE on PN14. (**B**) FLAG and HuNu immunostaining of a PN28 Rd10 retina treated with CX3-9 RPE on PN14. Scale bar is 50μm.

5. sCX3CL1-RPE Delay Rod Photoreceptor Degeneration

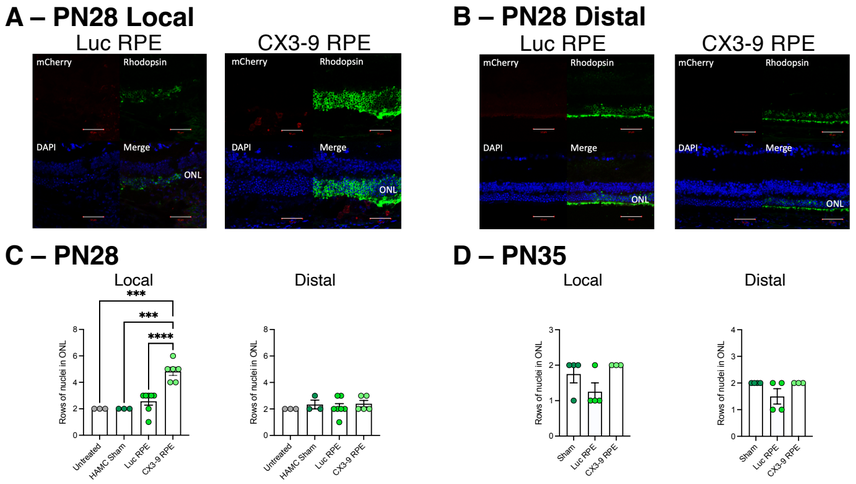

Figure 5. sCX3CL1-expressing RPE delay photoreceptor degeneration in the Rd10 mouse. Rhodopsin immunostaining of PN28 retinas treated with subretinal injections of Luc RPE or CX3-9 RPE on PN14 (**A, local**) at injection site where graft is present or (**B, distal**) absent (in the opposite region of the retina equidistant from the optic nerve). (**C-D**) ONL (outer nuclear layer) quantification of Rd10 retinas on (**C**) PN28 and (**D**) PN35 in local and distal regions according to panels **A-B**. Scale bar is 50μm. **p<0.0021, ***p<0.0002, ****p<0.0001.

6. Rescue is Potentially Independent of Donor Cell Survival

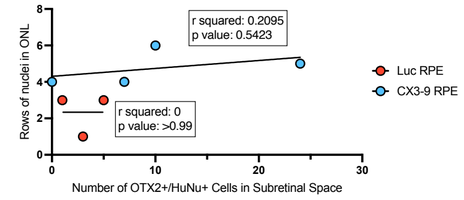

Figure 6. Magnitude of photoreceptor rescue might not be affected by number of donor cells in subretinal space. Number of OTX2+/HuNu+ donor cells in the subretinal space was plotted against rows of nuclei in ONL of Rd10 mice on PN28 that were treated on PN14.

References

(**1**) Hartong, D. T. *et al. Lancet.* **368**, 1795-1809 (2006); (**2**) Kolomeyer A. M. and Zarbin M. A. *Surv. Ophthalmol.* **59**(2):1795-1809 (2014); (**3**) Thomas C. E., Ehrhardt A., Kay M. A. *Nat Rev Genet.* **4**(5):346-358 (2003); (**4**) Zabel, M. K. et al. Glia. 64, 1479-1491 (2016); (**5**) Wang, S. K. *et al. PNAS.* **116**(20):10140-49 (2019); (**6**) Liang, Q. *et al. Nature.* **563**, 701-704 (2018).