CRISPR-Cas9 disruption of VEGF as a strategy to treat Exudative Age-Related Macular Degeneration

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INTRODUCTION

Age-related Macular Degeneration (AMD) is the number one cause of vision loss in people over the age of 50 in developed nations.[1] Estimates suggest that currently over 1.2 million people in the United States have AMD, and this number is expected to only grow to 3 million in 2020.[2] The neovascular "wet" form of AMD leads to the formation of abnormal blood vessels that can leak fluid into the subretinal space leading to vision loss (Figure C). Thus, there has been much work on the development of antiangiogenesis agents targeting vascular endothelial growth factor (VEGF). The two most popular current options include bevacizumab, ranibizumab, and aflibercept, which target secreted isoforms of VEGF, limiting its activity to form new blood vessels.[1] However, these treatments often require monthly intravitreal injections indefinitely, which are invasive and can cost up to $16,114 per patient every year, making up 10% of all Medicare part B drug spending.[3-4]

This study seeks to suppress VEGF secretion with the use of the type II clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing system (Figure 2). A recent study from Dr. Yiu's lab has successfully implemented CRISPR-Cas9 gene editing for VEGF *in vitro* with human retinal pigment epithelium (RPE) cells[5]. This study hopes to further our understanding by demonstrating reproducible results *in vivo* with the use of small animals (mice) as evidence that this strategy may be one day translatable to humans. We expect to see a similar decrease in VEGF and neovascularization in mice. This will provide a more effective and efficient method for addressing many neovascular ocular diseases that would reduce the overall costs of treatment and may even possibly represent a cure for the millions affected.

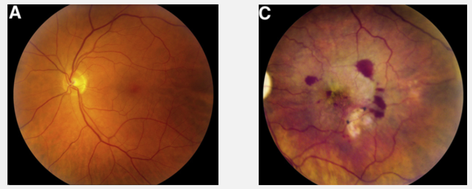

Figure 1. Fundus image of the eye. A) Normal eye. C) Eye with neovascularization (6).

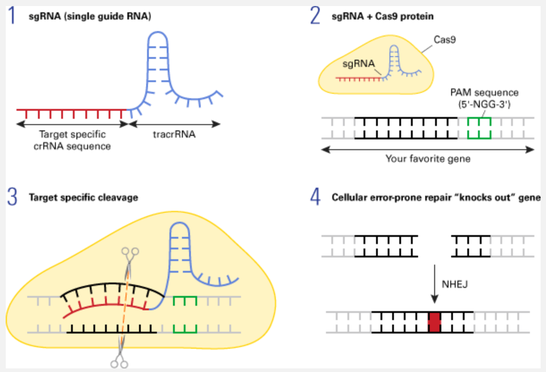

Figure 2. Summary of CRISPR Cas9 gene editing strategy (7)

OBJECTIVES

In this study, we seek to:

1) Use CRISPR-Cas9 to reduce VEGF expression

These aims will help identify whether gene editing of VEGF through the use of CRISPR-Cas9 is a viable ocular antiangiogenesis therapy solution *in vivo* by testing it in a small animal model. These results will help show whether this strategy may ultimately be efficacious in humans.

MATERIALS & METHODS

Design single guide RNA (gRNA) to target mouse VEGF

gRNAs were designed against the protein coding regions of exon 1 of the mouse VEGF-A gene using computer software (Benchling). gRNA with the best specificity as predicted by highest on-target probabilities and lowest off-target probabilities will be used.

Clone gRNAs into adeno-associated virus (AAV) vectors expressing *Staphylococcus aureus* (SaCas9) and *Campylobacter jejuni* (CjCas9)

We will clone gRNAs into a single vector AAV system using SaCas9 or CjCas9 to express the CRISPR-Cas9 components targeting VEGF-A in mice. Viral vectors expressing Cas9 without the VEGF-targeted gRNA sequences will be used as controls.

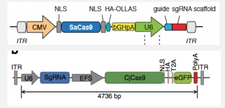

Figure 3. Vector designs for SaCas9 and CjCas9

Inject AAVs subretinally into mouse eyes

Subretinal injection of AAVs expressing Cas9 and gRNA will be injected into one mouse eye, and AAVs expressing Cas9 only without gRNA will be injected into the contralateral eye as a control. Three animals will be used to measure levels of VEGF in vitreous, and another three animals will be used for laser-induced choroidal neovascularization.

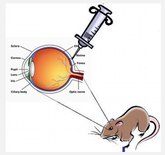

Figure 4. Schematic of subretinal injection in mice. Mice were sacrificed 5 weeks postinjection.

Check vitreous for VEGF levels using ELISA

After the mice are infected with the CRISPR-Cas9 system targeting VEGF-A, we will measure VEGF-A concentrations in the vitreous by ELISA compared to Cas9-only controls. We expect to see VEGF levels reduced in the eyes with gRNA expression compared to Cas9-only controls assessed statistically by ANOVA with Dunnett's post-test.

RESULTS

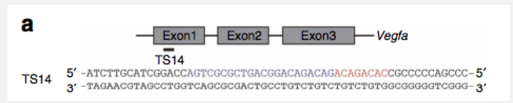

***Figure 5. Vegfa* target sequence in CjCas9 with PAM sequence (red) and sgRNA (blue) (8).**

	Position	Strand	Sequence	PAM	On-Target Score	Off-Target Score
☐	712	+	GCGCCGCAGGAGACAAACCGAT	CGGAG	91.4	48.4
☐	696	+	GTAGTAGCCGTGGTCTGCGCCG	CAGGA	76.9	49.6
☐	576	+	GTGGACGCGGCGGCGAGCCGCG	AGGAA	73.1	47.2
☐	1042	–	AGCCAGGGTCCAGTGCACCCAA	GAGAG	63.3	45.7
☐	970	+	GCGGGCTGCCTCGCAGTCCGAG	CCGGA	62.5	47.5

Figure 6. Highest On-Target Score gRNA sequences for SaCas9 with PAM site NNGRR(N)

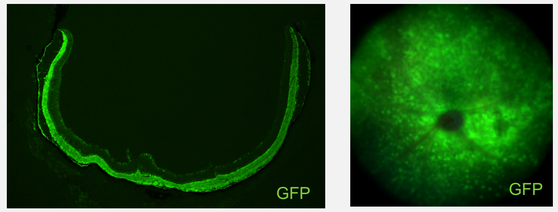

Figure 7. Micron IV retinal images showing transduction efficiency in choroid following subretinal injection after 3 – 5 weeks

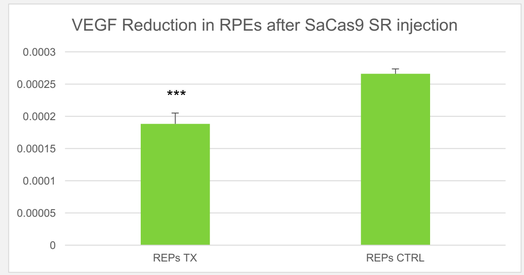

Figure 8. Enzyme-linked Immunosorbent assay (ELISA) results showing decreased levels of VEGF following SaCas9 subretinal injection. n = 5-7 *** p-value < 0.05

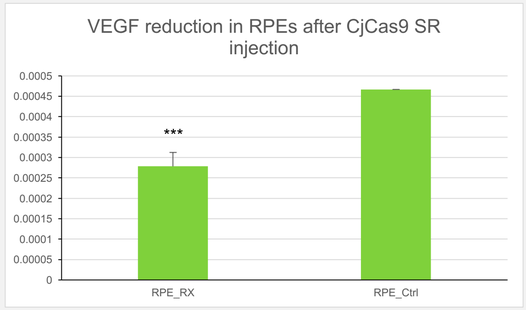

Figure 9. Enzyme-linked Immunosorbent assay (ELISA) results showing decreased levels of VEGF following CjCas9 subretinal injection. n = 4-5 *** p-value < 0.05

CONCLUSIONS/FUTURE DIRECTIONS

- **SaCas9 and CjCas9 constructs were both able to reduce VEGF levels in subretinally injected mice**
- **Laser-induced choroidal neovascularization will be assessed to show functional decrease**
- **Compare efficiency of designs *in vitro***

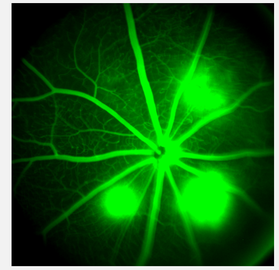

Figure 10. Fluorescein angiography showing laser-induced neovascularization

REFERENCES

1) Do D, Fallano K, Adyanthaya R, et al. Neovascular Age-Related Macular Degeneration. *Focal Points*. 2010;28:1-9.

2) Friedman DS, O' Colmain BJ, Munoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol.* 2004;122: 564-72.

3) Shienbaum G, Gupta OP, Fecarotta C, et al. Bevacizumab for neovascular age-related macular degeneration using a treat-and-extend regimen: clinical and economic impact. *Am J Ophthalmol.* 2012;153:468-73.

4) Martin DF, Maguire MG, Fine SL, et al. Ranibizumab and bevacizumab for treatment of neovascular age-related macular degeneration: two-year results. *Ophthalmology*. 2012;119:1388-98.

5) Yiu G, Tieu E, Nguyen AT, Wong B, Smit-McBride Z. Genomic disruption of VEGF-A expression in human retinal pigment epithelial cells using CRISPR-Cas9 endonuclease. *Invest Ophthalmol Vis Sci.* 2016;57:5490-5497.

6) Ambati J and Fowler Benjamin. Mechanisms of Age-Related Macular Degeneration. *Neuron*. 2012; 75: 26-39.

7) Takara Bio USA, Inc. http://www.clontech.com/US/About/Takara_Bio_USA, 2017.

8) Kim E, et al. *In vivo* genome editing with a small Cas9 orthologue derived from *Campylobacter jejuni*. *Nature Communications.* 2017; 8: 1-8.

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