Investigating CRISPR-mediated Knockdown of *ANGPTL3* in Familial Hypercholesterolemia

Meredith Reeves
Advisor: Dr. Renee Cottle
Department of Bioengineering, Clemson University
March 31, 2022, at 3:30 PM | Rhodes Annex 111

Familial hypercholesterolemia (FH) is a genetic condition that affects 1 in 250 people and is characterized by high levels of low-density lipoprotein (LDL) cholesterol.[1] FH is caused by the loss of one or both functional copies of the LDL receptor (LDLR) gene that removes LDL from the bloodstream to regulate plasma LDL levels. Individuals with FH are at an increased risk of developing heart disease or having heart attacks early in life. Current treatment of FH includes diet, weight loss, and exercise along with cholesterol-lowering medications. Gene editing has emerged as a promising approach for the treatment of FH by delivering functional copies of the LDLR gene or knocking down target genes that are associated with lipoprotein metabolism.

Angiopoietin-like 3 (*ANGPTL3*) is an inhibitor of plasma lipid metabolism and has become a promising molecular target for the treatment of FH. Individuals with non-functional copies of *ANGPTL3* have low levels of plasma LDL cholesterol and triglycerides, indicating a protective effect against coronary heart disease.[2] Additionally, clinical trials using inhibitors of *ANGPTL3* have shown reductions in LDL cholesterol and triglyceride levels.

CRISPR-Cas9 is a gene editing tool that uses a guide RNA (gRNA) to direct the Cas9 nuclease to a target site in the genome to induce a double strand break (DSB).[3] This system has been widely used due to its high levels of editing activity and amenability to multiplexing. One of the main challenges associated with gene therapy is selecting for cells that have been edited. Acetaminophen (APAP) selection is a novel method to overcome this barrier that links therapeutic transgenes with the knock down of NADPH-cytochrome P450 oxidoreductase (*CYPOR*) followed by treatment with APAP to provide a selective advantage to edited cells.[4] These principles can be utilized to form a novel multiplex gene editing approach that involves targeting *ANGPTL3* and *CYPOR* using CRISPR-Cas9 for the treatment of FH. The first **aim** of this study is to optimize the design of CRISPR gRNAs targeting *Cypor* and *Angptl3* and quantify the editing efficiency using mouse Hepa 1-6 cells. We will also evaluate the specificity of gRNA designs using in silico tools. In the second **aim**, we will evaluate whether disruption of *Angptl3* using CRISPR-Cas9 plasmid DNA lowers cholesterol levels in an LDLR knockout mouse model when placed on a Western diet. The third **aim** is to evaluate the efficiency of multiplex gene editing in *Cypor* and *Angptl3* genes in Hepa1-6 cells.

References

1. Centers for Disease Control and Prevention. (2022, February 10). *Familial hypercholesterolemia*. Centers for Disease Control and Prevention. Retrieved March 25, 2022, from https://www.cdc.gov/genomics/disease/fh/FH.htm
2. Wang, X., & Musunuru, K. (2019). Angiopoietin-Like 3: From Discovery to Therapeutic Gene Editing. *JACC: Basic to Translational Science*, *4*(6), 755-762. https://doi.org/10.1016/j.jacbts.2019.05.008
3. Hille, Frank and Charpentier, Emmanuelle. (2016). CRISPR-Cas: biology, mechanisms, and relevance. *Phil. Trans. R. Soc. B* **371**20150496http://doi.org/10.1098/rstb.2015.0496
4. Vonada, A., Tiyaboonchai, A., Nygaard, S., Posey, J., Peters, A. M., Winn, S. R., Cantore, A., Naldini, L., Harding, C. O., & Grompe, M. (2021). Therapeutic liver repopulation by transient acetaminophen selection of gene-modified hepatocytes. *Science translational medicine*, *13*(597), eabg3047. https://doi.org/10.1126/scitranslmed.abg3047