

Development of an *in vitro* model to evaluate novel genes regulating inflammation and fibrosis

Peyton Kuhlers, Michael Greene

Non-alcoholic steatohepatitis (NASH) is a condition in which fat accumulates in the liver and is accompanied by inflammation. This condition can eventually lead to liver fibrosis, cirrhosis, and hepatocellular carcinoma. The goal of this project was to use the CRISPR-Cas9 system to develop an *in vitro* model capable of identifying novel genes that regulate the development of inflammation and fibrosis in the context of NASH. The mechanism of the disease is not entirely understood, and the CRISPR-Cas9 system has not been used in this context.

To validate our *in vitro* NASH model, AML-12 mouse liver cells were treated with either 0.4 mM palmitic acid (PA), 25 ng/ml TNF- α , or hypoxia for 24 hours to induce Collagen I expression, which was assessed by western blot. Collagen I is a marker for fibrosis and NASH. Next, the AML-12 cells were infected with a plasmid containing the gene for the Cas9 enzyme. This plasmid also included resistance to the antibiotic blasticidin, which allowed for selection of stable AML-12 cells expressing Cas9. Multiple single cell colonies were cultured and Cas9 expression was determined by western blot (Figure 1A). The Cas9 expressing AML-12 cells were then treated with the same experimental NASH conditions and Collagen I expression was analyzed by western blot.

As shown in Figure 1, both regular AML-12 and Cas9 expressing AML-12 cells exhibited significant elevations in collagen I expression, normalized to the expression of GAPDH, when treated with palmitic acid (2.4 ± 0.2 -fold and 3.3 ± 1.5 -fold, respectively), but not when treated with TNF α (0.6 ± 0.2 -fold and 0.9 ± 0.2 -fold, respectively). We observed that there was not a significant difference in Collagen I expression between the two cell types (Figure 1). These results verify that AML-12 cells and our Cas9 expressing AML-12 cells can be used as a model for NASH and fibrosis.

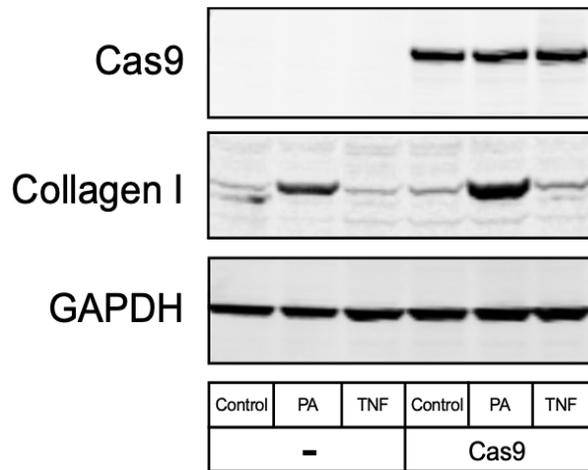
The next step for our project is to utilize the CRISPR-Cas9 system to mutate the gene *TGFBR2*, which

has a known role in the development of NASH. We hypothesize that by mutating this gene we will see a reduction in Collagen I expression. The final step will be to use the CRISPR-Cas9 system to mutate genes that were found to be differentially expressed in the livers of mice with NASH. The reduction in Collagen I expression found from knocking down *TGFBR2* will be used as a benchmark to assess the role that these novel genes may have in the development of NASH.

Statement of Research Advisor:

Peyton has performed the key experiments necessary to validate our new *in vitro* model of NASH. Our Cas9 expressing cells will be used to screen for novel regulators of NASH. Our novel model may transform the manner in which new targets for NASH are screened —*Michael Greene, Nutrition, Dietetics, & Hospitality Management*

A.



B.

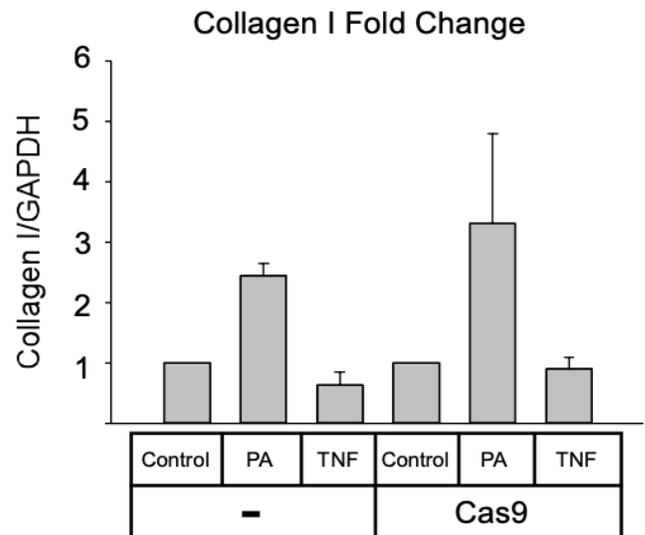


Figure 1. Induction of collagen I expression in AML-12 cells. **A.** Representative images of Cas9, Collagen I and GAPDH Western blots. **B.** Quantitation (mean \pm standard error) of Collagen I expression from 3 independent treatments.