Development of a Genome Engineering Approach for the Modulation of Vascular Phenotypes

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Introduction

Vascular smooth muscle cells (VSMCs), a major cell type in arteries, contribute to the progression of atherosclerotic lesions via the phenotypic switch from contractile to synthetic phase [1]. By reducing the proliferation of VMSCs, the progression of atherosclerotic lesions can be attenuated. Upregulation of KLF4 in VSMCs has been shown to promote disease progression, and previous reports, including ours, indicated that KLF4 modulates the phenotypic switch of VSMCs [2][3]. The development of CRISPRi with nuclease-dead Cas9 (dCas9) enables fine tuning of gene expression at the transcriptional level, thus allowing for the manipulation of genes contributing to lesion progression [4].

We **hypothesize** that the downregulation of KLF4 via a CRISPRi mediated mechanism will decrease the proliferative phenotypes of synthetic VSMCs and attenuate atherosclerotic lesions, serving as a potential therapeutic.

Research Objective

The **goal** of this project is to construct a recombinant adeno associated virus to repress KLF4 in VSMCs via a CRISPRi mediated mechanism, to reduce the proliferative phenotype and attenuate lesion progression.

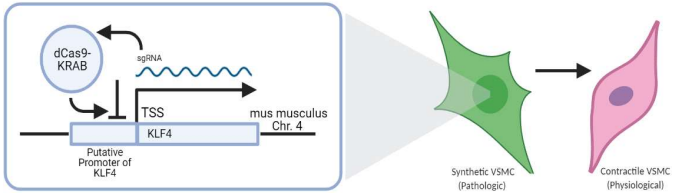

Figure 1. CRISPRi mediated mechanism occurring in the nucleus of VSMCs using the repressor domain KRAB. The mechanism will reduce the phenotypic switch of VMSCs to the proliferative phenotype.

Materials and Methods

- For DNA cloning the sgRNA sequences were inserted into the plasmid using restriction enzyme SapI and DNA ligase.
- The transfer plasmid carrying the sgRNA sequences were co-transfected with the packaging plasmids into HEK293 cells to produce a recombinant adeno associated virus.
- HEK293 cells are maintained in DMEM complete medium at 37°C and 5% CO2 and supplemented with 10% FBS.

Results

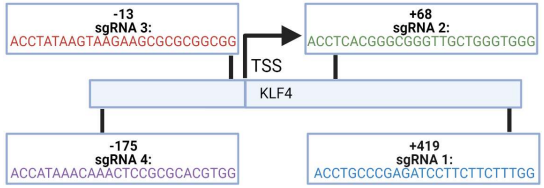

Figure 2. Design of the sgRNA sequences targeting the transcriptional start site (TSS) on KLF4. The sequences were designed using PNA Bio and Chop Chop.

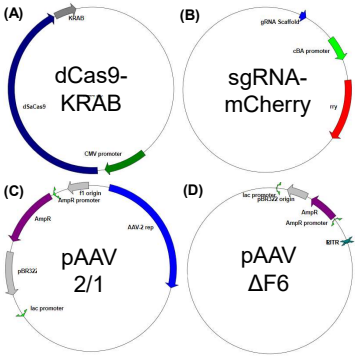

Figure 3a. Transfer (A&B) and packaging (C&D) plasmids for rAAV production. All plasmids were purchased from Addgene.

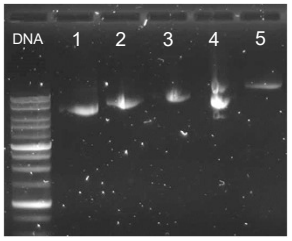

Figure 3b. Validation of the following plasmids: mCherry (1), KRAB (2), VPR (3), pAAV 2/1 (4), and pAAV ΔF6 (5).

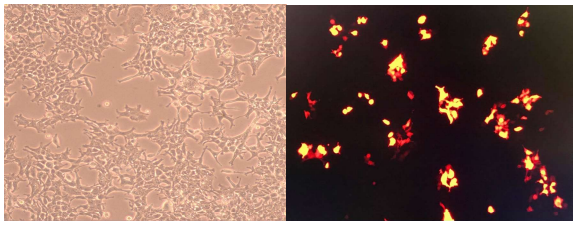

Figure 4. Production of rAAV via transfection of the sgRNA mCherry plasmid into HEK293 cells observed in bright field (left) and mCherry (right) channels.

Ongoing Work

Ongoing work for this project includes the synthesis of the gRNA sequences used for gene targeting for both the repression and activation domains, KRAB and VPR respectively, continued production the recombinant adeno associated virus, and continued testing of the virus via cell transfection of HEK293 cells for early proof of efficacy in vitro.

Future Work

- Isolation of VSMCs from mice
- Culturing of isolated VSMCs
- Continued testing of generated virus
  - Viral efficacy in vitro
- Continued optimization of DNA cloning conditions for both activation and repression domains
  - Continued research of CRISPRi/a methods
  - Modification of gRNA sequences

Acknowledgements

I would like to acknowledge Dr. Kuei-Chun (Mark) Wang for his immense support and continued mentorship throughout this project, Dr. Huang, and fellow undergraduate researcher Chamonix Michaud. I would also like to thank the FURI program for all the support, as well as W.L. Gore & Associates for the additional financial support and recognition.

References

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