CRISPR-Cas9 Genome Editing of Target Gene B to Mediate Brain Tumor Angiogenesis *In Vivo*

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INTRODUCTION

Angiogenesis is a hallmark of tumor growth and plays a critical role in the control of cancer progression. Newly formed tumor blood vessels deliver oxygen and nutrients to the tumor microenvironment, enabling tumor progression and metastasis. Targeting tumor-associated endothelial cells (ECs) has emerged as a fundamental strategy for cancer treatment. However, CAR T cell-based immunotherapy for glioblastoma (GBM) is challenged due to limited T cell infiltration and T cell exhaustion caused by the immunosuppressive tumor microenvironment.

Our recent studies reveal that endothelial plasticity in GBM induces vessel abnormality, resistance to chemotherapy, anti-angiogenic therapy, and immunotherapy. Our goal for this study is to reprogram tumor vasculature and improve CAR T immunotherapy. Whole-genome CRISPR-Cas9 library screening in tumor-associated ECs has identified gene B as a key regulator for endothelial-mesenchymal transformation (Endo-MT). Inhibition of EC-specific gene B *in vivo* by lipid nanoparticles (LNP) delivered siRNA reversed Endo-MT, improved EGFRviii CAR T cell infiltration, and decreased CAR T cell exhaustion. In this study, we will generate inducible EC-specific gene B KO mice with AAV-delivered CRISPR technology. The accomplishment of this study will provide a novel target and new therapeutic strategy to improve CAR-T therapy in solid tumors.

METHODS & HYPOTHESIS

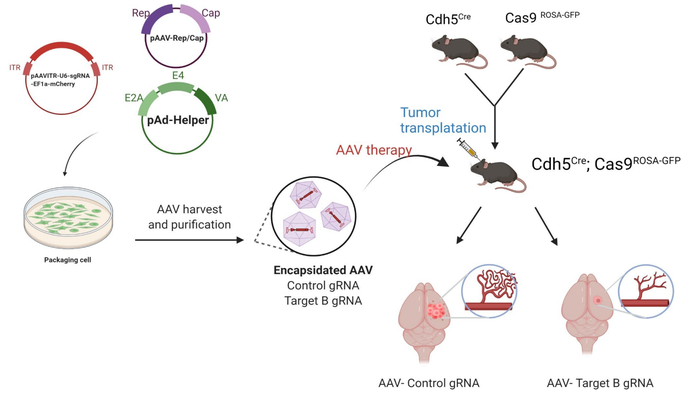

Figure 1. Reprogramming tumor vasculature by *in vivo* CRISPR technology. AAV delivered sgRNA will be intracranially injected into $Cdh5^{Cre}$; $Cas9^{ROSA\text{-}GFP}$ mice to generate EC-specific gene B KO mice. GBM will be induced in AAV-CRISPR engineered mice.

RESULTS

Transfected $Cas9^{GFP}$ Cells express RFP along with GFP

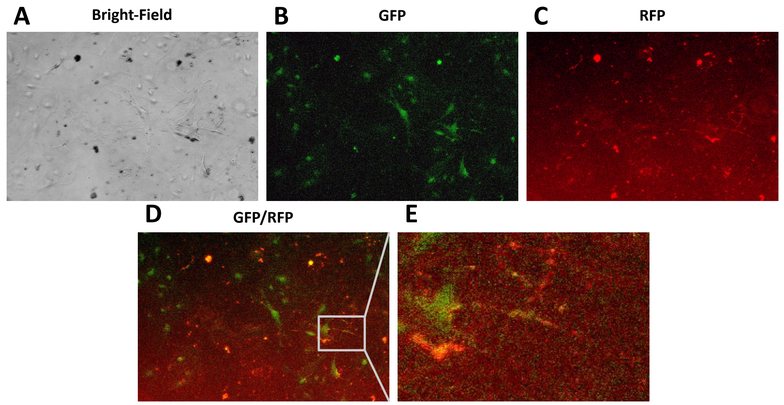

Figure 2. Representative immunofluorescence images of primary ECs sorted from $Cas9^{GFP}$ mice after transient transfection with AAV-sgRNA-mCherry plasmid. (A) Bright-field image (B) ECs from $Cas9^{GFP}$ mice express GFP (C) ECs from $Cas9^{GFP}$ mice express mCherry (D) Colocalization of GFP and mCherry (E) Magnification of the indicated area in (D).

Gene B Expression qPCR Analysis for Each Target B sgRNA

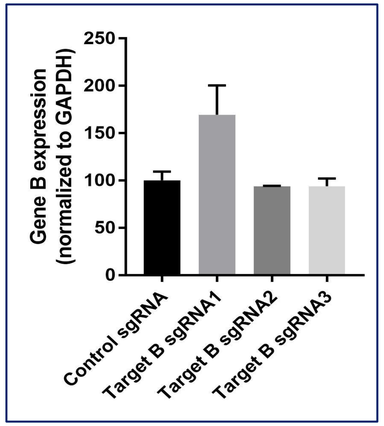

Figure 3. Expression of gene B with sgRNA knockdown in ECs. $Cas9^{GFP}$ ECs were transfected with either a control sgRNA or one of the three different Target B sgRNAs. Quantitative polymerase chain reaction (qPCR) of Gene B expression in ECs were analyzed and normalized with GAPDH.

DISCUSSION & FUTURE DIRECTIONS

As shown in **Figure 1.**, we observed the Cas9 endothelial cells expressing RFP along with GFP, indicating that the transfection processes with the plasmids containing the sgRNA and mCherry reporter were successful. However, we observed similar Gene B knockdown across all the endothelial cells, as shown in **Figure 2**. One possible explanation is that the transfection performed was a transient transfection, meaning the constructed plasmids were introduced into the ECs, but not integrated into the genome of the cells. As a result, the genes carried by these plasmids could be lost with the growth and division of cells, leading to a low transfection efficiency and therefore insignificant Gene B knockdown. To overcome this problem, we plan on packaging the plasmids in 293T cells to construct AAV virus, then transfecting ECs with the constructed virus. This will allow for a more stable and efficient transfection.

Future steps include:

- AAV production through 293T cell packaging
- AAV purification & infectious viral titer assay
- Gene B knockout efficiency test in Cas9 EC through qPCR & western blotting
- Large-scale AAV production
- Knockdown of Target B *in vivo* in $Cdh5^{Cre}$; $Cas9^{ROSA\text{-}GFP}$ mice
- Construct GBM model using 3D Lightsheet imaging

ACKNOWLEDGEMENTS

I would like to thank everyone at the Fan Lab for hosting me this summer. Special thanks to **Dr. Yi Fan** for giving me this amazing opportunity and **Dr. Menggui Huang** for her close guidance and mentorship on my research. Finally, I would like to thank **Penn CURF** and the **Penn Undergraduate Research Mentoring Program (PURM)** program for funding my research.