Identification and Validation of Key Proliferative Genes in Cardiomyocytes from CRISPR Inhibition Screen

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Background

Determining genes key in cardiomyocyte (CM) proliferation is important for helping people with cardiovascular disease, especially those who have suffered from myocardial infarction (MI). During MI, heart cells die and are unable to regenerate. This results in reduced function of the heart, impacting blood pumping ability and heart rhythm. Being able to regenerate cardiomyocytes would allow people to regain much of the function lost after a heart attack.

Genetic screening is a powerful and promising tool used to determine the function and biological processes within which genes of interest are involved. Gaining an understanding of the function and mechanism of genes will aid in increasing our understanding of disease and lead to an overall better understanding of human health.

CRISPR introduced the use of RNA to direct gene editing, making genomic engineering much less expensive and time-consuming and more precise and scalable than precursors[1]. CRISPR genomic screens utilize a catalytically dead Cas9 nuclease (dCas9) to either upregulate or downregulate gene expression[2]. For CRISPR interference (CRISPRi) screens, small guide RNA (sgRNA) directs dCas9 to specific binding sites in the genome where transcription of the targeted genes is blocked. A combination of dCas9 with the Krüppel-associated box (KRAB) domain facilitates CRISPRi screens in human cells[2,3]. Using CRISPRi screens with a dCas9 and a guide RNA serves to identify regions of interest within the genome and interferes with transcription, RNA polymerase binding, or transcription factor binding[4] to inhibit gene expression.

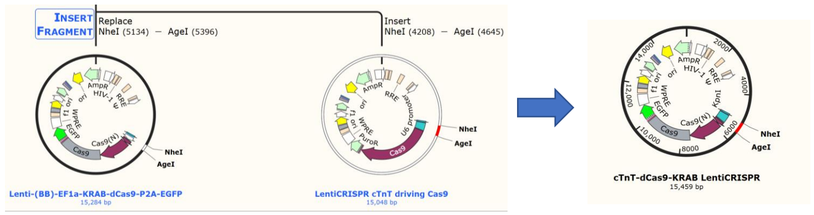

Figure 1: Steps for cloning the cTnT-dCas9-KRAB LentiCRISPR vector to replace the CMV promoter with cTnT. The final product will be ligated with sgRNAs and used in the creation of virus for delivery to hiPSC-CMs.

Objectives

A CRISPRi screen was performed to identify genes that influence cardiomyocyte proliferation. The goal of the following project is to continue the work started by Sophia DeLucal in her CRISPRi screen by validating if inhibition of the top five genes of interest results in cardiomyocyte proliferation.

Results

Genes of interest identified from a previous CRISPRi screen are NT5C, TLK2, EIF2AK2, PCTP, and ATP2C1. Viral transduction efficiency was between 60-80%. Transduction efficiency was measured 2 weeks post-transduction and after 48 hour EdU incorporation (Figure 3). Statistical analysis was performed using a One-Way ANOVA with $p<0.05$ considered statistically significant and with n=3 for each guide and N=1 differentiation batch (Figure 2).

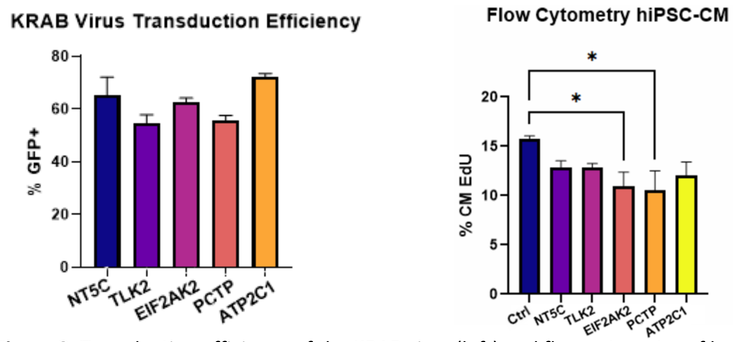

Figure 2: Transduction efficiency of the KRAB virus (left) and flow cytometry of human induced pluripotent stem cell (hiPSC)-CMs.

Flow cytometry data showed no increased proliferation in samples with inhibited genes above the control proliferation level. With a p-value of $p<0.05$, guides for NT5C, TLK2, and ATP2C1 produced samples that were not found to have significantly different proliferation levels from the control. Guides for EIF2AK2 and PCTP produced samples that were found to have significantly reduced proliferation when compared to the control (Figure 2).

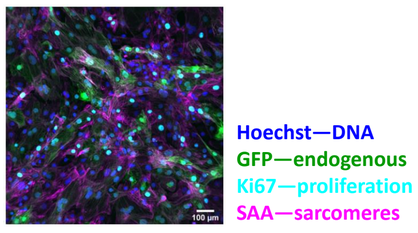

Figure 3: Hoechst, GFP, Ki67, and SAA overlap. Combining all stains allows for quantification of transduced cardiomyocytes that express Ki67, a proliferation marker.

Methods

Various molecular biology techniques were used in validating the selected genes including cloning of the LentiCRISPR vector for transfection (Figure 1), bacterial transformations, PCR, cell culture of Human Embryonic Kidney (HEK) cells, flow cytometry, image processing with Cell Profiler, and statistical analysis. Additionally, echocardiology was learned to aid in future *in vivo* studies in mice.

Conclusions

Our original flow cytometry showed proliferation levels not different from or significantly lower than the control in the samples with genes of interest inhibited. This was unexpected because these genes were selected from overrepresented guides in the CRISPRi screen. When reflecting on transduction efficiency (60-80%), we realized that transduction efficiency is typically higher in hiPSC-CMs. Additionally, titer for our synthesized lentivirus was ten-fold lower than normal. Due to the reduced transduction efficiency and low titer, we suspected that there was a problem with our virus and found that some plasmids used (PAX2 and VSVG) were impure. We planned to remake the lentivirus, repeat transduction of the cardiomyocytes, and repeat flow cytometry analysis. Additionally, we created a non-targeting KRAB guide to use as a future control as it could potentially be the case where the presence of dCas9 or KRAB reduces proliferation. We plan to use this new control in addition to a non-transduced control for comparison in further flow cytometry analysis.

Future Research

The overall goal of this project is to identify genes that are potentially key in CM proliferation, and we are doing this with CRISPR inhibition, activation, and knockout screens. We are currently working on a CRISPR activation screen as well as researching the mechanism behind a gene identified in a knockout screen with *in vitro* and *in vivo* studies.

Acknowledgements

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