A CRISPR Knockout Screen Systematically Identifies Critical Epigenetic Barriers in Direct Cardiac Reprogramming

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Heart failure is a leading cause of mortality worldwide, with the limited regenerative ability of the human heart posing a significant obstacle. Ischemic injury such as myocardial infarction causes cardiomyocytes to be replaced by cardiac fibroblasts (CFs), often leading to adverse ventricular remodeling. Considering these challenges, direct cardiac reprogramming aims to restore functionality to the injured heart by converting endogenous CFs into induced cardiomyocytes (iCMs). However, current reprogramming cocktails involving the canonical MEF2C, GATA4, and TBX5 (MGT) factors are insufficient to efficiently reprogram human CFs. With the perspective that removal of epigenetic barriers could enhance reprogramming cocktails, we aim to identify key epigenetic factors that hinder cardiac reprogramming efficiency through CRISPR screening. Here, we establish a reproducible and high-quality CRISPR knock out (CRISPRko) screening platform for direct cardiac reprogramming. We transduced the ACER pooled sgRNA library (8000+ sgRNAs) containing a GFP marker to Cas9-expressing human CFs. These cells were reprogrammed using a MGT + miR-133 retroviral cocktail (MGT133) and cardiac troponin T+/GFP+ double positive cells were isolated via fluorescence-activated cell sorting, selecting only cells expressing sarcomere proteins and ACER sgRNA. Following cell sorting, gDNA was extracted and amplified, and sequencing analysis identified top-hit genes for further investigation. Our study establishes an optimized CRISPRko screening platform for large-scale discovery of critical epigenetic factors in direct cardiac reprogramming. Using this platform, we have identified 25 top-hit genes that are currently undergoing mechanistic study in our lab.

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