High-Throughput Imaging Prioritizes Modulators of Cardiomyocyte and Fibroblast Function Identified by Human Genetics

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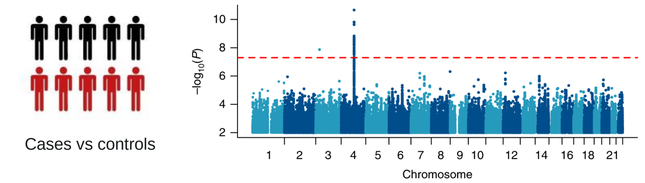
Introduction

Human genetics is a powerful tool to accelerate targets selection and increase the likelihood of success in drug development. An increased understanding of the genetic underpinnings of disease offers the hope of identifying targets that may address the underlying molecular pathologies and developing disease modifying therapeutics. Here we developed a workflow to test genes of interest (GOI) derived from human genetics in high throughput screens (HTS). Using publicly available human genetic data on cardiac structure, function, and disease risk, we prioritized more than 100 targets for cardiomyopathic disease. siRNA and AAV-ORF libraries were created to facilitate the knockdown or overexpression of these genes in both human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and cardiac fibroblasts (CF). Two custom MATLAB HTS image processing scripts named 'Tamarack' and 'Porcupine' were developed for quantifying hiPSC-CM sarcomere morphology and CF activation, respectively using wavelet transforms to detect subcellular structures. Tamarack enables quantification of sarcomere count, length, and orientation. Porcupine enables quantification of fibroblast activation metrics, including α-smooth muscle actin, stress fiber count, length, orientation, nuclei α-SMA overlap, and F-actin α-SMA overlap. We identified top hits from both the CM and CF screens to perform further analysis.

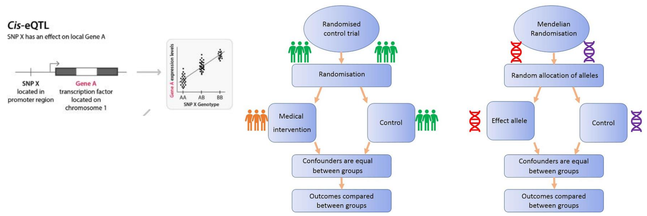
Generation of Gene Libraries

GOI were selected based on genome-wide association studies (GWAS) and Mendelian randomization (MR). siRNA and AAV-ORF libraries were created in order to facilitate HTS.

1. GWAS



2. MR



Identification and Validation of Top Hits

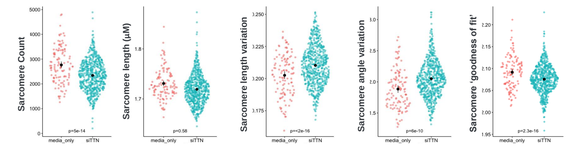


Figure 4: siTTN alters sarcomere parameters in hiPSC-CMS and induces disarray

Sarcomere disarray was evident in titin siRNA (siTTN) treated hiPSC-CMs. GOIs were either knocked down or over-expressed in the siTTN background to look for trends of rescuing or worsening(figure 5). Hits with significant changes, such as FHOD3, were investigated for functional effects in engineered heart tissues(EHTs) (figure 6).

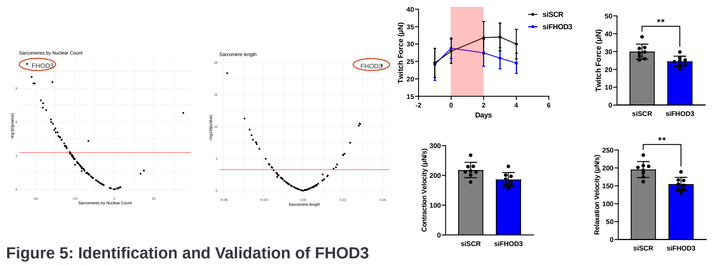


Figure 5: Identification and Validation of FHOD3
Left: siFHOD3 causes a marked increase in measures of sarcomere disarray.
Right: FHOD3 knockdown in EHTs decreases measures of contractility.

Fibroblast screening identified that siRNA knockdown of a specific tyrosine kinase(TK) was found to modulate fibrotic response to TGF- β, a response validated by using a commercially available tool compound from GSK.

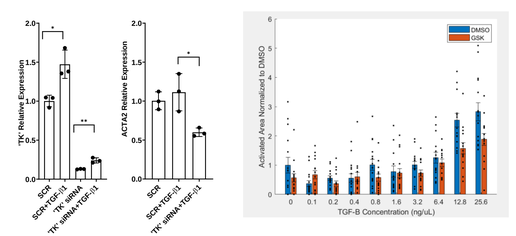


Figure 6: Validation of a tyrosine kinase hit in fibroblast screens.
Left: Knockdown of the TK blunts the response to TGF- β
Right: A specific TK-inhibitor was used to confirm modulation of fibrosis.

Generation of iPSC-Derived Cardiomyocytes

Different hiPSC-CM models were used in the screening including siRNA knockdown models, isogenic engineered models, and patient derived models in order to mimic various types of sarcomere disarray and cardiomyopathy-causing mutations.

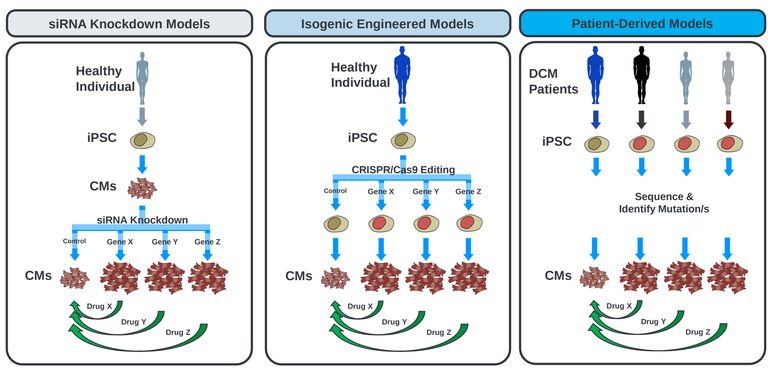


Figure 1: Generation of hiPSC-CM models. From left to right: Cardiomyocytes are differentiated from healthy-donor cells and modified using siRNA to mimic specific phenotypes; Cardiomyocytes are differentiated from healthy-donor cells and edited using CRISPR; Cardiomyocytes are differentiated from patient donor cells.

High-Throughput Imaging and Analysis

Cardiomyocytes

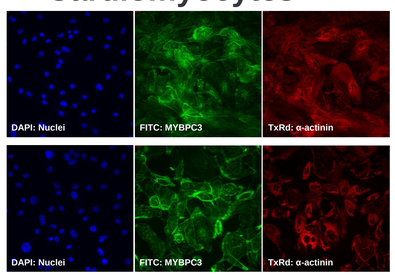


Figure 2: Cardiomyocytes
Top: Nuclei(DAPI), cMYBPC(FITC), and α-actitin(TxRed) stains
Left: Sarcomeres identified using 'Tamarack'

Fibroblasts

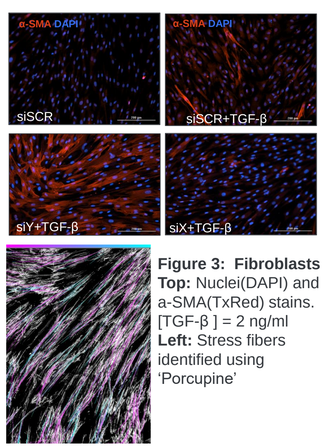


Figure 3: Fibroblasts
Top: Nuclei(DAPI) and a-SMA(TxRed) stains. [TGF-β] = 2 ng/ml
Left: Stress fibers identified using 'Porcupine'

Two custom scripts, 'Tamarack' and 'Porcupine' were used to identify sarcomere parameters (cardiomyocytes) and stress fibers (fibroblasts) respectively. Alterations to these parameters were used to rank GOI for further investigation.

Future Directions

We have advanced multiple targets from our initial screens into further validation. We will continue to validate top hits both *in vitro* and *in vivo* as well as perform additional silencing and over-expression screens with new GOIs.

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