High-Throughput Imaging of Cardiomyocytes Using Biomimetic Tools Help Identify Therapeutic Targets

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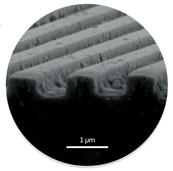
Introduction

Mutations resulting in sarcomere disarray and loss of contractile function are causal in many forms of genetic cardiomyopathies. In order to improve disease models in vitro, we have developed a highly specialized work flow starting with in house cell differentiation and maturation of human iPSC-Cardiomyocytes lines. Here, we modeled sarcomere disarray *in vitro* by culturing human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) on tissue culture plates with biomimetic patterning designed to simulate the topographical features of native cardiac extracellular matrix (ECM). These patterned plates promoted human iPSC-CM alignment and allowed for the quantification of sarcomere organization. We incorporated this platform into a high throughput screening (HTS) process to examine the effects of overexpression of genes of interest (GOIs) on sarcomere morphology in a dilated cardiomyopathy (DCM) context. Images of RBM20 S635F$^{+/-}$ patient-derived human iPSC-CMs treated with overexpression GOI libraries were run through a custom MATLAB HTS image processing script named 'Tamarack' that enables the quantification and analysis of sarcomere count, length, and orientation.

Methods: Biomimetic Patterned Plates

Cardiomyocyte and sarcomere alignment in hearts is driven by ECM cues; however, human iPSC-CMs in standard cell culture plates have no such cues for anisotropic organization. The design of patterned substrates that mimic cardiac ECM topography can therefore induce proper cardiomyocyte development.



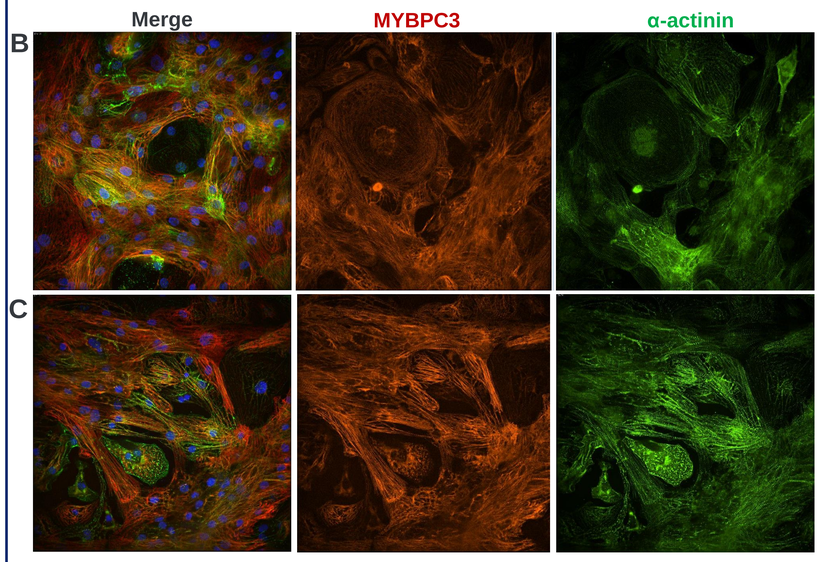


Figure 1. The presence of biomimetic nanopatterning promotes increased organization of cardiomyocytes. (A) Scanning electron microscope image of the 1 µm-wide grooves and ridges of the CuriBio NanoSurface patterned cell culture plate. (B) Representative confocal images of RBM20 S635F$^{+/-}$ human iPSC-CMs in a standard 384-well cell culture plate. Note disordered organization of cells. (C) RBM20 S635F$^{+/-}$ human iPSC-CMs cultured on 384-well NanoSurface plate. Cells are anisotropically aligned along nanopatterned grooves. All confocal images were taken with a 40X water objective.

Methods: Cells and Imaging

Screening Workflow for Overexpressing Genes of Interest

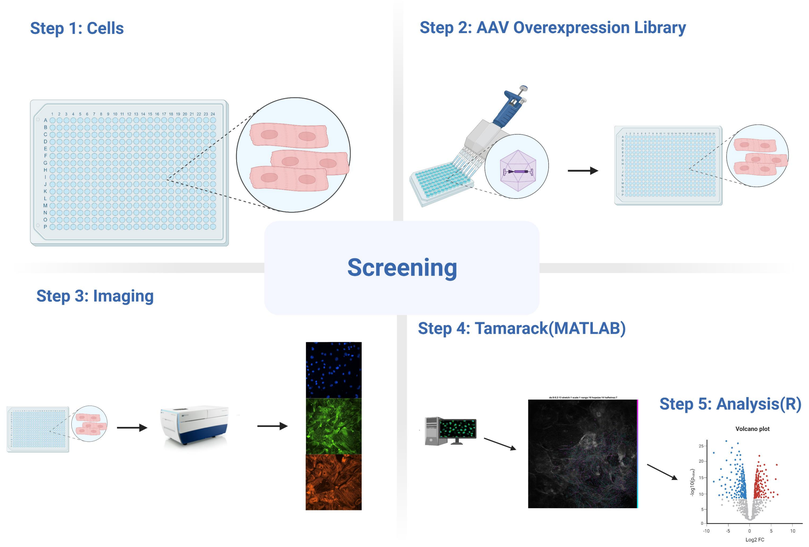


Figure 2. High throughput screening of GOIs. Tenaya has developed a precision medicine-based approach to drug discovery for genetic cardiomyopathies. Potential druggable GOIs are identified through human genetics and are initially screened in a high-throughput manner in 2D. Screens are conducted against a variety of cardiac disease model backgrounds that have been optimized and validated in-house.

Modeling Dilated Cardiomyopathy with Patient-Derived Cells

Patient-derived human iPSC-CMs with a RBM20 S635F mutation were used to model RBM20 DCM *in vitro* as described in Briganti, et al. (Briganti, et al. 2020 Cell Reports).

Results: Increased Sarcomere Angle Alignment in Patterned Plates

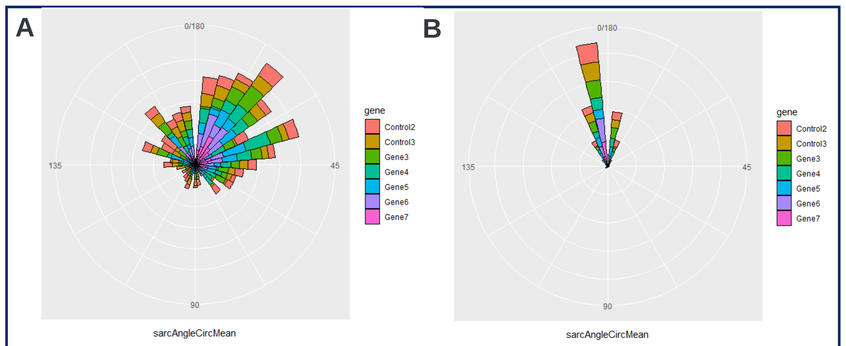


Figure 3. Sarcomere angle can be quantified from confocal imagery and plotted. Highly aligned sarcomeres are defined as those that fall within a small range of angles. (A) In the case of WT human iPSC-CMs cultured in standard 384-well plates, sarcomere angles are highly dispersed regardless of genetic background. (B) When human iPSC-CMs were cultured on patterned plates, cells were significantly more aligned along the direction of the patterning which is designated here as 0° and 180°.

Results: GOI Effect on Sarcomere Angle

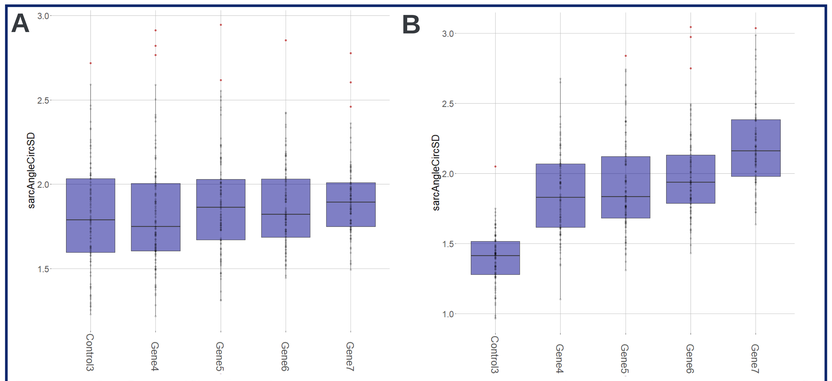


Figure 4. Quantifiable changes in sarcomere organization due to gene of interest overexpression in an *in vitro* model of RBM20 DCM. (A) Standard non-patterned cell culture plates induce no change in the standard deviation of sarcomere angle when genes of interest are overexpressed in RBM20 S635F$^{+/-}$ human iPSC-CMs. (B) However, once these cells are cultured on patterned plates and the same genes are overexpressed, a significant change in the standard deviation of sarcomere angle is observed due to GOI overexpression when compared to controls.

Summary

Sarcomere disarray is a marker for cardiomyopathic phenotypes previously not utilized in HTS protocols. Biomimetic nanopatterned cell culture plates are effective in promoting enhanced alignment and organization of cultured cardiomyocytes, thereby producing images and data from which sarcomere counts and sarcomere lengths can be quantified. This allows for the assessment of perturbations of specific GOIs on disease genotypes that may not have been possible on standard cell culture plates. The introduction of biomimetic patterned plates to established screening workflows is seamless, and the use of such tools allows for deeper investigations into potential therapeutic targets and for the validation of *in vitro* models of genetic cardiomyopathy. This protocol can be adapted to several different screens beyond genetic overexpression, including small molecule and gene knockdown.

Future Directions

- We aim to continue to use human genetic evidence to direct our screening efforts.
- We plan on continuing to investigate the mechanisms of action regarding sarcomere disarray for GOIs.
- As new technologies and techniques emerge, we may integrate them into our validation pipeline.
- With these efforts, we hope to significantly increase the success rates of new therapies for heart failure.

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