Modeling Genetic Dilated Cardiomyopathies with Engineered Heart Tissues From Patient-Derived and Isogenic Mutant Induced Pluripotent Stem Cells

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Introduction

The availability of human induced pluripotent stem cells (hiPSCs) has offered the possibility to study human-derived models of different genetic strata of cardiomyopathy for mechanistic discovery and therapeutic development. However, the phenotypes derived from cardiomyocytes differentiated from hiPSCs in conventional 2-dimensional culture systems often fail to reproducibly model clinical presentations, thereby reducing the translatability of readouts from these assays. Using a variety of cell lines harboring patient-derived mutations (TTN, RBM20, BAG3) known to cause dilated cardiomyopathy (DCM), we developed 3-dimensional engineered heart tissues (EHTs) which recapitulated contractile deficits associated with DCM. EHTs also recapitulate contractile defects with small interfering RNA (siRNA) knockdown models of haploinsufficiency across multiple disease states. Our observed findings with EHTs suggests improved maturity in cardiomyocytes through the presentation of microenvironmental cues akin to those seen *in vivo*. With continued advances in EHT technologies and capabilities, this platform may serve as a novel method for validating and prototyping therapeutic approaches in addition to providing new insights into pathologies and mechanisms of action that were previously difficult to obtain.

Drug Discovery at Tenaya

High-Throughput 2D Screening

Functional Validation in 3D EHTs

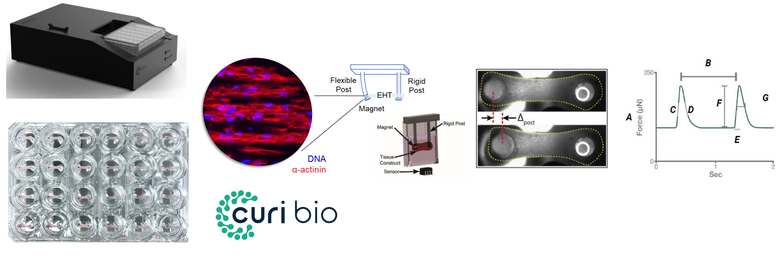

Figure 1. Tenaya's drug discovery program. Tenaya has developed a precision medicine-based approach to drug discovery for genetic cardiomyopathies. Potential druggable genes of interest are identified through human genetics and are initially screened in a high-throughput manner in 2D. Screens are conducted against a variety of DCM model backgrounds that have been optimized and validated in-house. Hits from screens are then validated with additional assays including measuring functional changes in 3D EHTs. The CuriBio Mantarray platform enables medium-throughput assessment of cardiac tissue contractile function.

Isogenic DCM Mutant hiPSC Lines

Tissues generated from hiPSC-cardiomyocytes with DCM-associated mutations exhibited significantly reduced contractile function compared to wild-type controls.

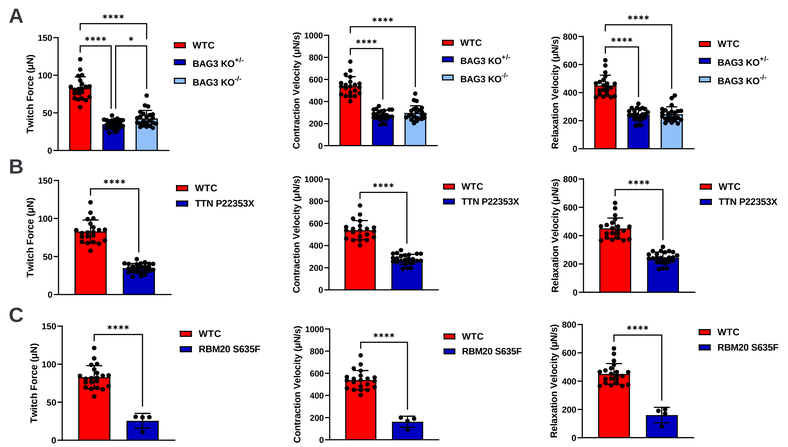

Figure 2. Contractile dysfunction of several DCM mutant lines produced at Tenaya from a WT background. (A) Knockout of BAG3, a known DCM-causing gene in humans, induced reductions in twitch forces and kinetics. (B) Similarly, a line harboring a TTN truncation mutation displayed functional deficits in line with TTN-related DCM. (C) A hiPSC line replicating a known patient mutation in RBM20 also produced EHTs with impaired contractile function compared to WT. ****p<0.0001; *p<0.05.

DCM Patient-Derived hiPSC Lines

Engineered heart tissues formed with cells from patients with RBM20 mutations show robust functional deficits that are not captured in 2D.

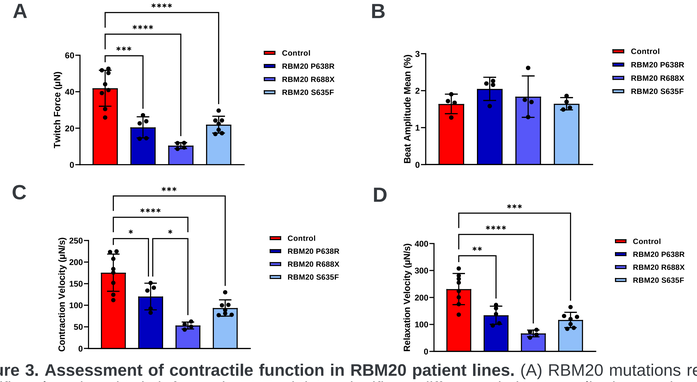

Figure 3. Assessment of contractile function in RBM20 patient lines. (A) RBM20 mutations result in significantly reduced twitch forces in EHTs. (B) No significant difference in beat amplitude was detected in the same cardiomyocytes cultured in 2D (Axion MaestroPro). (C) RBM20 mutation tissues also exhibited reduced contraction and (D) relaxation velocities. ****p<0.0001; ***p<0.001; **p<0.005; *p<0.05.

siRNA Knockdown Models

Contractile abnormalities replicated those observed in patients harboring haploinsufficiency or loss of function (LoF) mutations of these genes of interest.

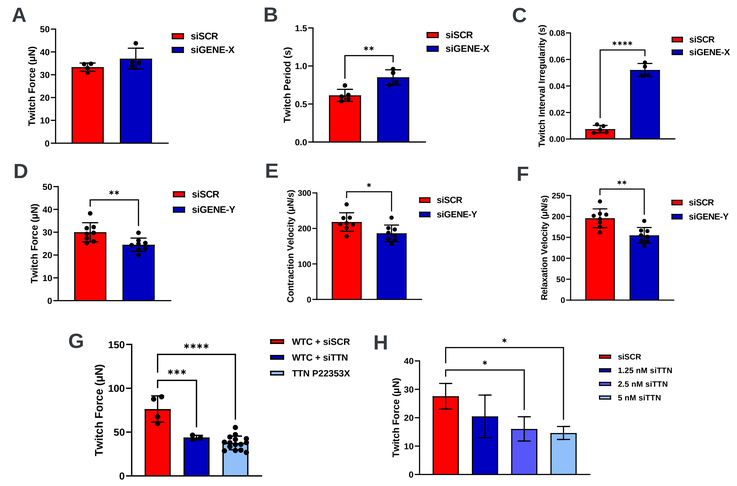

Figure 4. siRNA knockdown models in EHTs also recapitulate disease phenotypes *in vitro*. (A) Knockdown of GENE-X resulted in no significant change in twitch force. (B) However, twitch periods were increased, suggesting an underlying prolongation of the action potential duration (APD). (C) Furthermore, twitch interval irregularity, a proxy for arrhythmia, was significantly increased in siGENE-X tissues. This correlates with the predicted impact of a loss of function (LoF) of this particular gene. (D) Knockdown of a gene known to be associated with DCM, GENE-Y, resulted in a decrease in twitch force. (E) Similarly, decreases were observed in contraction velocity and (F) relaxation velocity in siGENE-Y EHTs. (G) Haploinsufficiency can also be modeled in EHTs. For example, siRNA knockdown of TTN produces twitch force reduction similar to that observed in the P22353X truncation mutant line. (H) Knockdown effects are also dose-dependent. ****p<0.0001; ***p<0.001; **p<0.005, *p<0.05.

Summary and Future Directions

Through the application of mutant hiPSC cell lines and siRNA knockdown of genes of interest in EHTs, we have demonstrated the utility of this 3D culture platform for modeling DCM phenotypes and for validating the results of our drug target screening and discovery efforts. Work is underway to optimize and achieve the therapeutic rescue of disease phenotypes in EHTs so that prototyping and efficacy assays can be conducted with these tissues in conjunction with *in vivo* studies.

Acknowledgements

The authors would like to thank Dr. Ray Hershberger (The Ohio State University) and the California Institute for Regenerative Medicine (CIRM) for their generous contribution of the patient-derived cells. We would also like to thank CuriBio for their continued collaboration and assistance with the Mantarray platform.

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