Modeling Hypertrophic Cardiomyopathy Due to MYBPC3 Haploinsufficiency with Engineered Heart Tissues

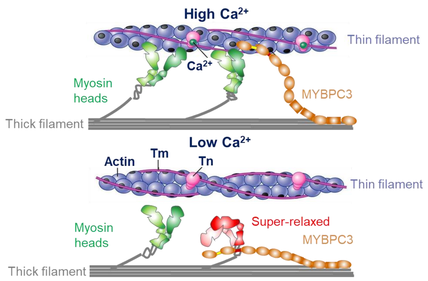
Jonathan H. Tsui[1], Cassady E. Rupert[2], Benjamin Archer[2], Shannon Cirilli[2], Viet Dau[2], Laura M. Lombardi[1], James R. Priest[1], Stuart G. Campbell[2,3], and Timothy Hoey[1]

[1]Tenaya Therapeutics, Inc., South San Francisco, CA, USA; [2]Propria, LLC, Branford, CT, USA; [3]Department of Biomedical Engineering, Yale University, New Haven, CT, USA

Introduction

Human induced pluripotent stem cells (hiPSCs) harboring cardiomyopathy-associated mutations are increasingly used to generate models of heart disease for study and therapeutic discovery. However, cardiomyocytes differentiated from these hiPSCs often fail to reliably reproduce clinical phenotypes when cultured in 2D, thereby reducing the translatability of readouts from these assays. Developments in engineered heart tissue (EHT) technologies have improved the potential of hiPSC models of cardiomyopathy, leveraging significantly improved maturity in cardiomyocytes through the presentation of microenvironmental cues akin to those seen *in vivo*. Success of these approaches would enable the ability to validate and prototype therapeutic approaches in humanized models, thereby improving the probability of novel treatments reaching the clinic. Additionally, these models can greatly assist with the study of disease pathologies and mechanisms of action, leading to new discoveries of potential drug targets.

MYBPC3 Mutations: Leading Cause of Genetic HCM and Tenaya's Gene Therapy Approach



MYBPC3 acts as a "brake" by stabilizing the super-relaxed state of myosin

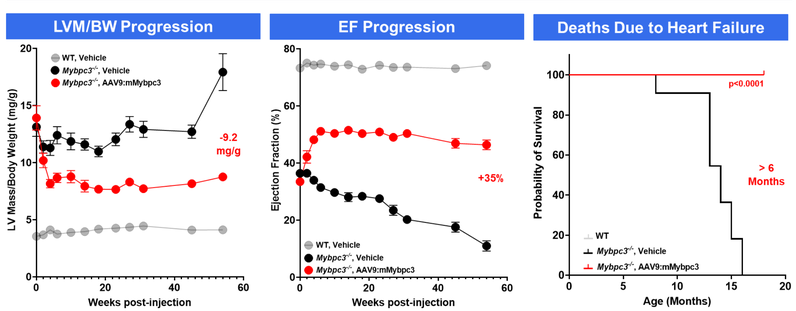
Disease Symptoms and Severity

- Loss of MYBPC3 protein disrupts contraction, leading to remodeling of ventricles
- Cardiomyocyte hypertrophy, disarray and fibrosis contribute to diastolic dysfunction and abnormal heart rhythms
- Sudden cardiac death is possible in adults and children

Epidemiology

- *MYBPC3* mutations accounts for ~19% of all HCM
- Estimated >115K patients in U.S. alone

TN-201 AAV9:MYBPC3 gene therapy modality improves hypertrophy, cardiac dysfunction and premature lethality of *Mybpc3*$^{-/-}$ mice



Symptomatic mice injected intravenously at 2 weeks of age.

Figure 1. Tenaya's TN-201 gene replacement therapy significantly improves outcomes in *Mybpc3*$^{-/-}$ mice. Administration of a AAV9-MYBPC3 in symptomatic mice induced a durable reduction in hypertrophy over time while untreated mice continued to worsen. Ejection fraction was also significantly improved with treatment, and survival rates were also dramatically improved over a 6-month monitoring period.

Generating *MYBPC3*$^{-/-}$ Human EHTs

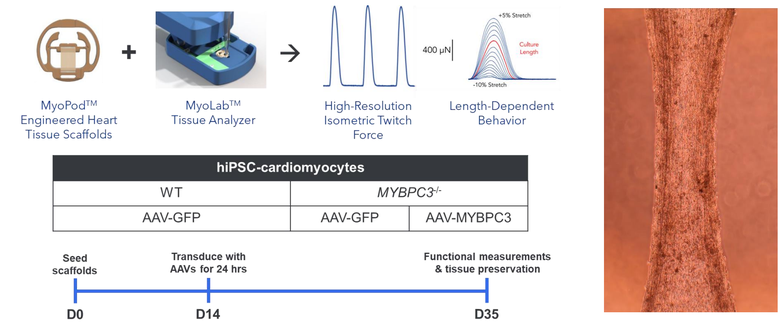


Figure 2. Capabilities of the Propria EHT platform and study design. *MYBPC3*$^{-/-}$ hiPSCs were generated by excising exons 1 & 2 in both alleles. WT and *MYBPC3*$^{-/-}$ hiPSC-CMs were seeded onto MyoPod scaffolds and cultured for 14 days, after which they were transduced for 24 h with either AAV-GFP or AAV-MYBPC3. EHTs were cultured for an additional 21 days before contractile function measurements were collected using the MyoLab. Baseline contractile behavior, force-frequency response (FFR), and length-dependent activation (LDA) of EHTs were measured. For FFR, tissues were held at 0% stretch and paced from 1 Hz to the maximum capture rate at 0.2 Hz intervals. EHTs were held at each pacing frequency for 1 minute while recordings of twitch contractions were taken. To determine LDA, tissues were stretched from -5% to 5% of culture length at 1% steps. EHTs were held at each length for approximately 2 minutes while recordings of twitch contractions were taken.

MYBPC3$^{-/-}$ EHTs Exhibited HCM-Associated Contractile Dysfunction

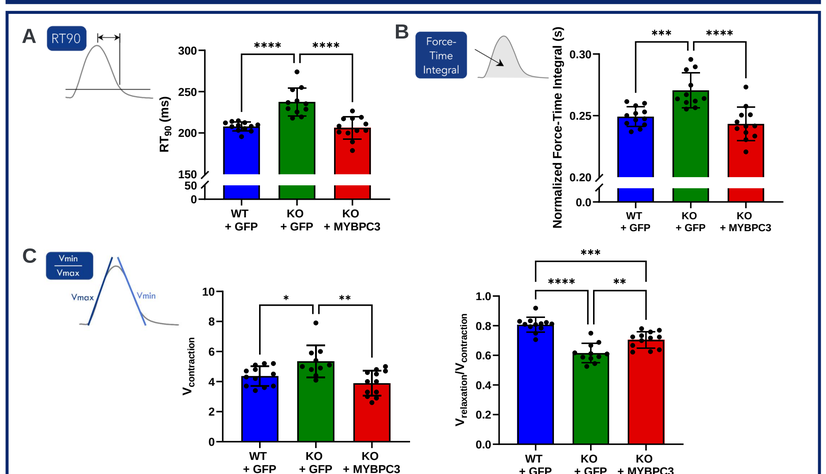


Figure 3. The impaired relaxation and hypercontractility of *MYBPC3*$^{-/-}$ EHTs were reversed with the administration of AAV-MYBPC3. (A) RT_{90} was significantly increased in *MYBPC3*$^{-/-}$ EHTs, and this increase was reduced to WT levels with AAV-MYBPC3 transduction. (B) The increase in relaxation time was also reflected in increased force-time integrals, or work, produced by the untreated KO tissues, and this was also returned to values comparable to WT with treatment. (C) *MYBPC3*$^{-/-}$ EHTs also exhibited hypercontractile behavior with increased contractile velocities compared to WT. Coupled with the impaired relaxation, this resulted in a significant decrease in $V_{relaxation}/V_{contraction}$ in these tissues. ****p<0.0001; ***p<0.001; **p<0.005; *p<0.05.

Force-Frequency Perturbations in KO EHTs

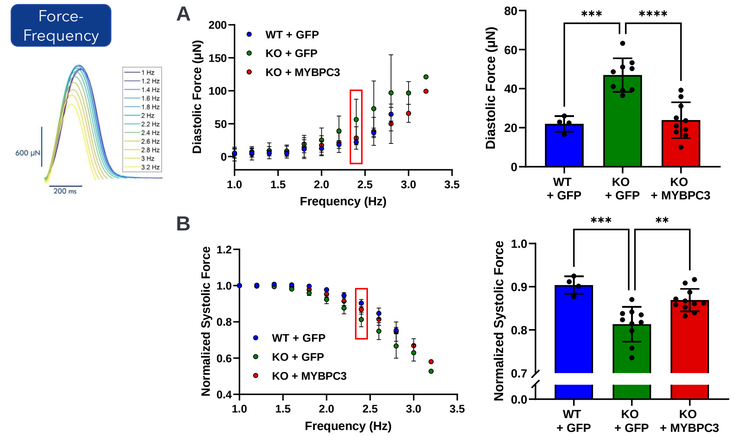


Figure 4. Impaired relaxation also reflected in increase in diastolic force, and decreased systolic force, at higher frequencies. (A) Diastolic force, measured as the force generated by the EHT at the end of each contraction cycle, is significantly greater as twitch frequency increases in *MYBPC3*$^{-/-}$ tissues than in WT and AAV-MYBPC3 treated tissues. (B) Similarly, systolic force as a function of frequency decreases significantly in untreated knockout tissues. This contractile dysfunction is again reversed with the administration of AAV-MYBPC3. ****p<0.0001; ***p<0.001; **p<0.005.

Impaired Length-Dependent Activation (LDA) Reversed with AAV Treatment

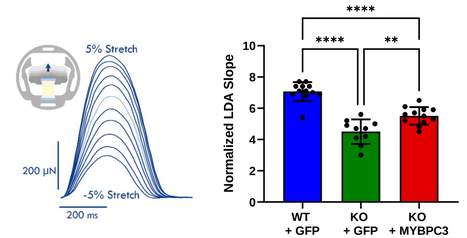


Figure 5. AAV treatment partially restored LDA in KO tissues. Reduced LDA observed in *MYBPC3*$^{-/-}$ tissues is consistent with reported HCM pathology in mice and patient samples. The subsequent increase in LDA with AAV treatment potentially indicates ability of gene replacement to restore control over the super-relaxed state.
****p<0.0001; **p<0.005.

Summary of Results

- *MYBPC3*$^{-/-}$ EHTs exhibited impaired relaxation and diastolic contractile function relative to WT
- Knockout EHTs also displayed increased contraction velocities in conjunction with deficits in systolic force-frequency
- Treatment of *MYBPC3*$^{-/-}$ EHTs with AAV-MYBPC3 reversed these contractile dysfunctions

Correspondence: jtsui@tenayathera.com