HSP70 Co-Chaperones Modulate Sarcomeric Proteostasis in Hypertrophic Cardiomyopathy

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Project Summary

Background: Hypertrophic cardiomyopathy (HCM) is the most common genetically inherited cardiovascular disease that affects one in 500 people. 1 Variants in MYH7 leads to premature termination codons, ultimately causing nonsense-mediated decay in MyBP-C protein-a hallmark of familial HCM cardiomyopathy.1,2,3 Recent GWAS reports identify risk alleles for HCM that are concordant with left ventricular (LV) functional traits of decreased LV ejection fraction and increased ejection fraction, and in contrast, are protective alleles for dilated cardiomyopathy.4 Several of the top HCM risk alleles encode for co-chaperones of HSP70 (Fig. 1).1,4,5 However, how these co-chaperones modulate sarcomeric protein turnover and HCM cardiomyopathy is unknown.

Hypothesis: I hypothesize that HSP70 co-chaperones identified by recent GWAS can modulate cardiac contractility by regulating sarcemic and 2-disc steady state expression.

Methods/Results: Human induced pluripotent stem cell cardiomyocytes (hiPSC-CMs) were transduced with GFP-tagged adenovirus (AdV) expressing shRNA targeted against BAG3, DNAJC18, HSPB7, or scrambled shRNA (control) with high transduction efficiency and no cellular toxicity.1,5 BAG3 KD and DNAJC18 KD were reduced by 95% or both the transcript and protein levels as measured via qRT-PCR and Western blot,1,5 BAG3 KD reduced MyBP-C protein levels (p<0.001) by 53%, along with most other profibrotic sarcomeric and 2-disc proteins (p<0.05). DNAJC18 had no significant effect on MyBP-C protein levels, but caused a decrease in tropomyosin (p=0.001), and myosin light chain 3 (p=0.001). HSPB7 KD increased MyBP-C protein levels (p=0.01) by 46%. Myosin (p=0.05), tropomyosin (p=0.05), and myosin light chain 2 (p=0.05) also showed increased expression upon HSPB7 knockdown. Co-chaperone KD also caused transcriptome-wide changes identified by RNA-seq. BAG3 was overexpressed (OE) via an adenovirus-associated virus (AAV) resulted in an increase in MyBP-C (p=0.01).

Conclusions/Future Directions: GWAS identified HSP70 co-chaperones BAG3, DNAJC18, and HSPB7 and regulate steady state expression of sarcomeric and 2-disc proteins. Further studies will be needed to elucidate how therapeutic modulation of these co-chaperones can be leveraged to stabilize MyBP-C and other sarcomeric and 2-disc proteins in patients with familial HCM.

IPSC-CM Transcriptome is Altered Upon Co-Chaperone KD

Figure 1: The HSP70 co-chaperone network.1,5 BAG3 is a nuclear stress exchange factor (NIEF), DNAJC18 is a J-domain protein (JDP), and HSPB7 is a small heat shock protein (HSP).

Adv Transduction of hiPSC-CMs

Figure 2: Adv transduction efficiency and viability. Cells were transduced with shRNA (MOIs) against Scram (n=4), BAG3 (n=3), HSPB7 (n=4), and DNAJC18 (n=3). Transduction efficiency was assayed via flow cytometry 4 days post transduction. A) Quantifications for transduction efficiency (GFp+ expression). B) Toxicity of transduction was assessed via LDH levels. BAG3 (n=3), DNAJC18 (n=3), and HSPB7 (n=4) did not induce cellular toxicity (2-7 technical replicates each). Statistical test: Student’s t-test (each treatment compared to MOI = 0). p≤0.001.

Figure 3: shRNA mediated knockdown of BAG3 on the protein and transcript levels in hiPSC-CMs. A) Representative western blot image of scramble control (MOIs) vs BAG3 KD (MOIs). B) BAG3 protein levels (n=3). 3-6 technical replicates each. C) BAG3 transcript levels (n=3). 1-2 technical replicates each. Statistical test: Student’s t-test. p values displayed between bars.

Conclusions/Acknowledgments

Data Summary:

1. BAG3 KD decreases MyBP-C expression and most other sarcomeric and 2-disc protein, while HSPB7 knockdown increases MyBP-C expression. Demonstrating the HSP70 system is essential in regulating sarcomeric and 2-disc protein expression.
2. BAG3 OE increases MyBP-C expression, providing a proof of concept for therapeutic approaches that leverage HSP70 co-chaperone network to regulate sarcomeric protein expression.

Figure 4: Knockdown of HSP70 Co-Chaperones

Figure 5: shRNA mediated knockdown of DNAJC18 on the protein and transcript levels in hiPSC-CMs. A) Representative western blot image of scramble control (MOIs) vs DNAJC18 KD (MOIs). B) DNAJC18 protein levels (n=3). 3-6 technical replicates each. C) DNAJC18 transcript levels (n=3). 2 technical replicates each. Statistical test: Student’s t-test. p values displayed between bars.

Figure 6: BAG3 KD reduces steady state levels of MyBP-C, whereas HSPB7 KD increases steady state levels of MyBP-C. A-C) Representative western blot images of scramble control (MOIs) vs KD (MOIs). D-F) Western blot quantification, average of 3 technical replicates for each biological replicate. A-B) BAG3 KD (n=3), C-D) HSPB7 KD (n=4), and E-F) DNAJC18 KD (n=4). Statistical test: Student’s t-test. **** p<0.0001, ** p<0.01, ns p>0.05.

References