Identifying the Role of HSP70 Co-Chaperones in Modulating Sarcomeric Proteostasis

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Project Summary

Background: Hypertrophic cardiomyopathy (HCM) is the most common genetically inherited cardiovascular disease that affects one in 500 people. Variants in MYH7 produce in premature termination codons, ultimately causing haploinsufficiency in MYH7 protein—a hallmark of familial HCM pathophysiology.1,2,3 Recent GWAS reports identify risk alleles for HCM that are concordant with left ventricular (LV) functional traits of decreased left ventricular chamber volumes 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 (BJ, BL).4,5,6,7 However, how these co-chaperones directly modulate sarcomeric protein turnover and HCM pathophysiology is unknown.

Hypothesis: 1. We hypothesize that HSP70 co-chaperones identified by recent GWAS regulate activity affecting myocardial contractility and 2-Disc steady state expression.

Methods and Results: Human induced pluripotent stem cell cardiomyocytes (iPSC-CMs) were transduced with lentiviral vectors as follows: A) empty vector (Scram), engineered shRNA targeting against BAG3, DNAJC18, HSP70, or scrambled shRNA (control) and transduction efficiency was assessed by flow cytometry for GFP expression. A >70% transduction efficiency was achieved with each viral construct (p<0.0001). Viral transduction and knockdown of HSP70 co-chaperone did not induce cellular toxicity.8,9 BAG3, DNAJC18, and HSP70 were reduced by ≥50% with the transcript and protein levels as measured via RT-qPCR and western blot.10 BAG3 KD reduced MyBP-C protein levels (p<0.0001) by 53%, along with MyBP-C mRNA and codon variants (p=0.05). DNAJC18 KD MyBP-C protein levels (p<0.01) by 46%, Myosin ATPase (p<0.01), troponin I (p<0.05), and myosin light chain 3 (p<0.05) also showed increased expression upon HSP70 knockdown.

Conclusions/Future Directions: GWAS-identified HSP70 co-chaperones BAG3, DNAJC18, and HSP70 regulate the steady state expression of sarcomeric 2-Disc proteins. Further studies will be needed to elucidate whether regulation is occurring at the transcriptional, translational, or posttranslational level, and whether 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.

Figure 1: The HSP70 co-chaperone network.11 BAG3 is a nucleolar exchange factor (NEF), DNAJC18 is in a domain protein (DLP), and HSP70 is a small heat shock protein (sHSP).

Adv Transduction of iPSC-CMs

Figure 2: Adv transduction efficiency and viability. A-E) Immunofluorescent spectroscopy of GFP-labeled cells. F) Sorting for GFP+: iPSC-CM was determined based on F) M04 cells [n=2]. Cells were transduced with shRNA [M05] against BAG3 [n=6], BAG3 [n=5], DNAJC18 [n=5], and DNAJC18 [n=5, T4]. B) HSP70 [n=4], and K] were quantified for GFP expression. C) LDH levels, indicative of cellular toxicity to lethal levels BAG3 (n=2), DNAJC18 (n=3), and HSP70 (n=1) do not induce cellular toxicity [2.7 technical replicates each]. Statistical test: Student’s t-test each treatment compared to M04. *** p<0.001.

Knockdown of HSP70 Co-Chaperones

Figure 3: shRNA-mediated knockdown of BAG3 on the protein and transcription levels in iPSC-CMs. A) Representative western blot image of scramble control (M04) in BAG3 KD (p<0.01). B) BAG3 KD protein levels (n=3). 3-6 technical replicates each. C) BAG3 KD protein levels (n=3). 3-6 technical replicates each. D) BAG3 KD protein levels (n=3). 3-6 technical replicates each. E) Statistical test: Student’s t-test: p<0.001.

Figure 4: shRNA-mediated knockdown of DNAJC18 on the protein and transcription levels in iPSC-CMs. A) Representative western blot image of scramble control (M04) in DNAJC18 KD (M05). B) DNAJC18 KD protein levels (n=3). 3-6 technical replicates each. C) DNAJC18 KD protein levels (n=2). 2 technical replicates each. D) Western blot quantification, average of 3 technical replicates for each biological replicate (n=3-4). Statistical test: Student’s t-test: *** p<0.001. ** p<0.01, * p<0.05.

Figure 5: shRNA-mediated knockdown of HSP70 on the protein and transcription levels in iPSC-CMs. A) Representative western blot image of scramble control (M04) in HSP70 KD (M06). B) HSP70 KD protein levels (n=3). 3-6 technical replicates each. C) HSP70 KD protein levels (n=3). 3-6 technical replicates each. D) Western blot quantification, average of 3 technical replicates for each biological replicate (n=3-4). Statistical test: Student’s t-test: *** p<0.001. ** p<0.01, * p<0.05.

HSP70 Co-Chaperones Modulate Turnover of Other Sarcomeric Proteins

Figure 7: BAG3, DNAJC18, and HSP70 KD alter sarcomeric and Z-disc protein expression. A) Western blot quantification (M07) in BAG3 KD, B) DNAJC18 KD, and C) HSP70 KD. Average of 3 technical replicates for each biological replicate (n=3-4). Statistical test: Student’s t-test: *** p<0.001. ** p<0.01, * p<0.05.

Figure 8: Individual KD of HSP70 co-chaperones BAG3, DNAJC18, and HSP70 are associated with changes in the expression of other co-chaperones, suggesting inter-regulation. A) BAG3 KD, B) DNAJC18 KD, C) HSP70 KD. Average of 3 technical replicates for each biological replicate (n=3-4). Statistical test: Student’s t-test: *** p<0.001, ** p<0.01, * p<0.05.

Conclusions/Acknowledgments

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References