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 *MYBPC3* result in premature termination codons, ultimately causing haploinsufficiency in MyBP-C protein–a hallmark of familial HCM pathophysiology.[2,3,4,5] 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.[6] Several of the top HCM risk alleles encode for co-chaperones of HSP70 (**Fig. 1**).[7,8,9,10] However, how these co-chaperones modulate sarcomeric protein turnover and HCM pathophysiology is unknown.

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

Methods and 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 >70% transduction efficiency and no cellular toxicity.[11] BAG3, DNAJC18, and HSPB7 were reduced by ≥50% at both the transcript and protein levels as measured via RT-qPCR and western blot.[12] BAG3 KD reduced MyBP-C protein levels ($p<0.0001$) by 53%, along with most other profiled sarcomeric and Z-disc proteins ($p<0.05$). DNAJC18 had no significant effect on MyBP-C protein levels, but caused a decrease in tropomyosin ($p<0.0001$) and myosin light chain 3 ($p<0.0001$). HSPB7 KD increased MyBP-C protein levels ($p<0.01$) by 46%. Myosin ($p<0.01$), 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 adeno-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 regulate steady state expression of sarcomeric and Z-disc proteins. Further studies will be needed to elucidate how whether therapeutic modulation of these co-chaperones can be leveraged to stabilize MyBP-C and other sarcomeric and Z-disc proteins in patients with familial HCM.

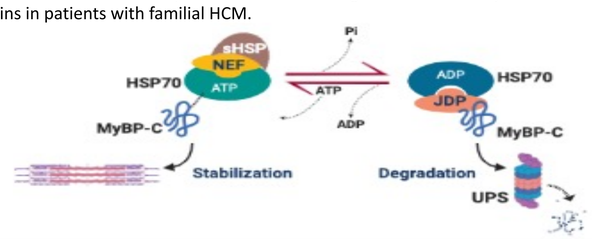


Figure 1: The HSP70 co-chaperone network.[13] BAG3 is a nucleotide exchange factor (NEF), DNAJC18 is a J-domain protein (JDP), and HSPB7 is a small heat shock protein (sHSP).

AdV Transduction of hiPSC-CMs

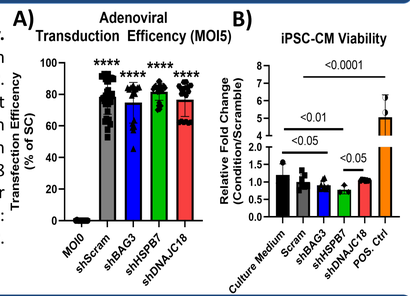


Figure 2: AdV transduction efficiency and viability. Cells were transduced with shRNA (MOI5) against Scram (n=6), BAG3 (n=3), HSPB7 (n=4), and DNAJC18 (n=3). Transduction efficiency was assessed 4 days post transduction. A) Quantifications for transduction efficiency (GFP+ expression). B) Toxicity of transduction was assessed via LDH levels. BAG3 (n=3), DNAJC18 (n=3), and HSPB7 KD (n=1) did not induce cellular toxicity (2-7 technical replicates each). Statistical test: Student's t-test (each treatment compared to MOI0). **** p≤0.0001.

Knockdown of HSP70 Co-Chaperones

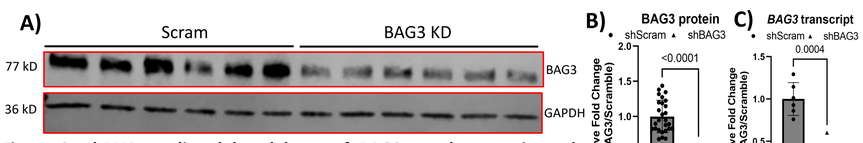


Figure 3: shRNA mediated knockdown of BAG3 on the protein and transcript levels in hiPSC-CMs. A) Representative western blot image of scramble control (MOI5) vs BAG3 KD (MOI5). B) BAG3 protein levels (n=3). 3-6 technical replicates each. C) *BAG3* transcript levels (n=3). 1-2 technical replicates each. Statistical test: Student's t-test. p values displayed between bars.

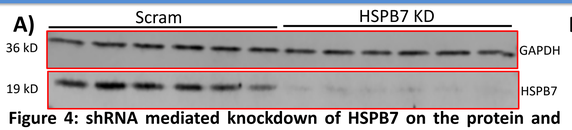
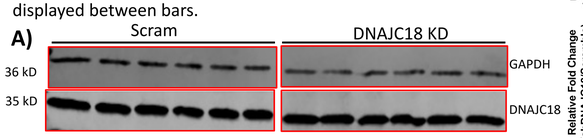


Figure 4: shRNA mediated knockdown of HSPB7 on the protein and transcript levels in hiPSC-CMs. A) Representative western blot image of scramble control (MOI5) vs HSPB7 KD (MOI5). B) HSPB7 protein levels (n=3). 3-6 technical replicates each. C) *HSPB7* transcript levels (n=3). 2 technical replicates each. Statistical test: Student's t-test. p values displayed between bars.



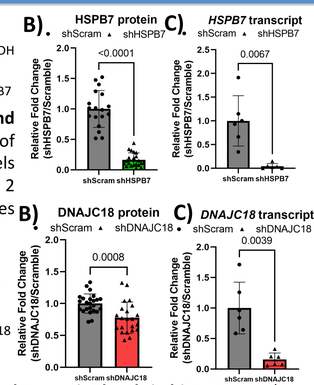
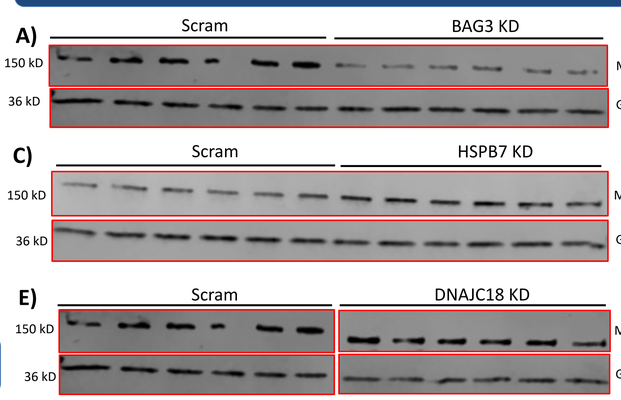


Figure 5: shRNA mediated knockdown of DNAJC18 on the protein and transcript levels in hiPSC-CMs. A) Representative western blot image of scramble control (MOI5) vs DNAJC18 KD (MOI5). B) DNJAC18 protein levels (n=3). 3-6 technical replicates each. C) *DNAJC18* transcript levels (n=3). 2 technical replicates each. Statistical test: Student's t-test. p values displayed between bars.

BAG3 and HSPB7 KD Modulate MyBP-C Expression



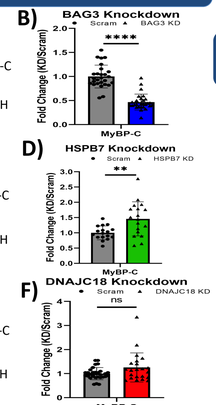


Figure 6: BAG3 KD reduces steady state levels of MyBP-C, whereas HSPB7 KD increases steady state levels of MyBP-C. A,C,E) Representative western blot images of scramble control (MOI5) vs KD (MOI5). B,D,F) Western blot quantification, average of 3 technical replicates for each biological replicate. A-B) BAG3 KD (n=5), 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.

HSP70 Co-Chaperones Modulate Turnover of Other Sarcomere Proteins

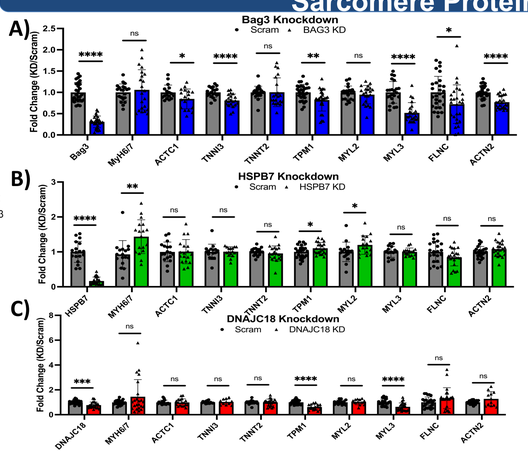
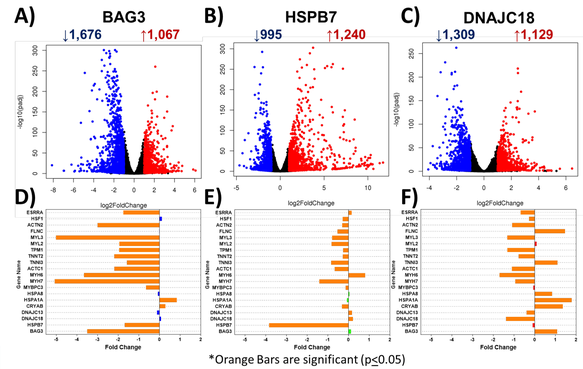


Figure 7: BAG, HSPB7, and DNAJC18 KD alter sarcomeric and Z-disc protein expression. A) BAG3 KD, B) HSPB7 KD, and C) DNAJC18 KD. Average of 3 technical replicates for each biological replicate (n=3-4). Statistical test: Student's t-test. **** p≤0.0001, *** p ≤0.001, ** p≤0.01, * p≤0.05, ns p>0.05.

iPSC-CM Transcriptome is Altered Upon Co-Chaperone KD



*Orange Bars are significant ($p \leq 0.05$)

Figure 8: Transcriptional profile of AdV transduced iPSC-CMs. A-C) Volcano plots identifying significantly upregulated (red) and downregulated (blue) transcripts upon A) BAG3 (n=3), B) HSPB7 (n=3), C) DNAJC18 (n=3) KD in comparison to scramble treated controls (MOI5). Number of altered transcripts are identified above each volcano plot. D-F) Selected gene transcripts of transcriptional regulators, the sarcomere and Z-disc, and HSP70 cochaperones. Differential gene expression was determined by the Wald test. An adjusted $p< 0.05$ and a log2 fold change > 1 was considered statistically significant.

BAG3 OE Results in an Increase in MyBP-C Expression

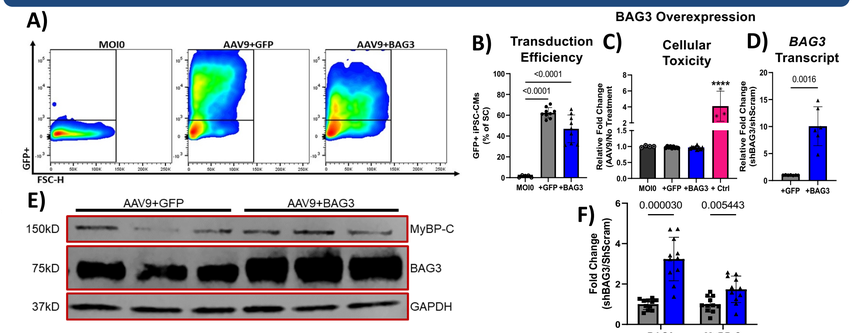


Figure 9: Adenoviral Associated (AAV9) transductions of GFP (n=3) or BAG3 (n=3) in iPSC-CMs 8 days post transduction. A-B) Flow cytometry quantifying GFP expression (% single cell), C) LDH CyQUANT assay detecting no cellular toxicity caused by AAV9 transduction, and D) RT-qPCR of *BAG3* transcript, E) Representative western blot of BAG3 overexpression and MyBP-C, F) BAG3 quantification. ****p<0.0001

Conclusions/Acknowledgments

Data Summary:

1. BAG3 KD decreases MyBP-C expression and most other sarcomeric and Z-disc protein, while HSPB7 knockdown increases MyBP-C expression. Demonstrating the HSP70 system is essential in regulating sarcomeric and z-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.

Future Directions:

1. Utilize gene ontology (GO) analysis to determine other pathways and mechanisms by which BAG3 and HSPB7 regulate HCM pathology.

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