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.[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 directly modulate sarcomeric protein turnover and HCM pathophysiology is unknown.

Hypothesis: I hypothesize that HSP70 co-chaperones identified by recent GWAS regulate contractility by affecting 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) 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.[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.

Conclusions/Future Directions: GWAS-identified HSP70 co-chaperones BAG3, DNAJC18, and HSPB7 regulate the steady state expression of sarcomeric and Z-disc proteins. Further studies will be needed to elucidate whether regulation is occurring at the transcription, translational, or posttranslational level, and 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.

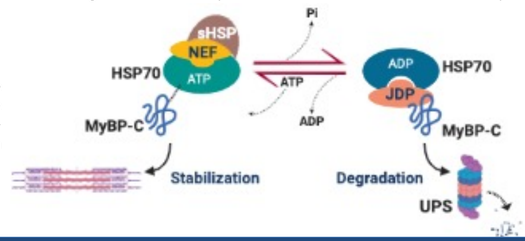

Figure 1: The HSP70 co-chaperone network.[13] BAG3 is a nucleotide exchange factor (NEF), DNAJC18 is a J-domain protein (JDP), and HSPB7 is a small heat shock protein (sHSP).

AdV Transduction of iPSC-CMs

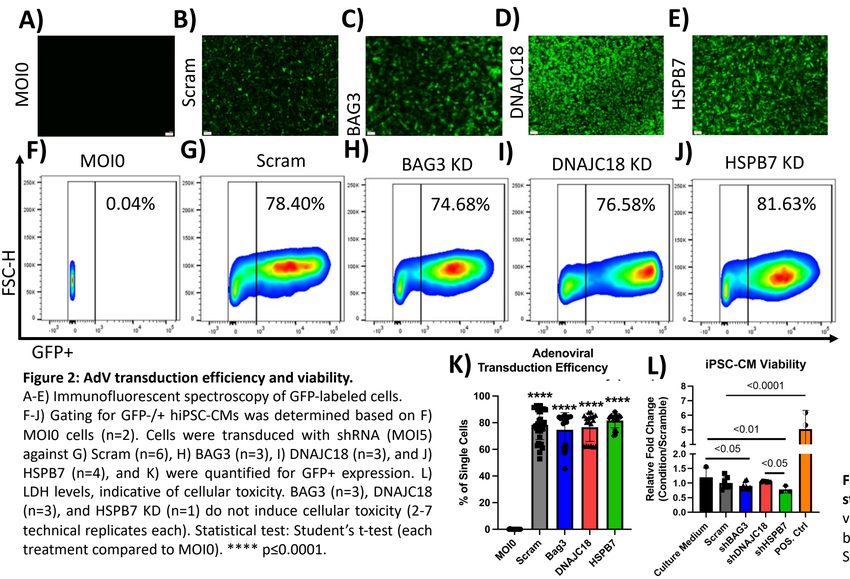

Figure 2: AdV transduction efficiency and viability.
A-E) Immunofluorescent spectroscopy of GFP-labeled cells.
F-J) Gating for GFP-/+ hiPSC-CMs was determined based on F) MOI0 cells (n=2). Cells were transduced with shRNA (MOI5) against G) Scram (n=6), H) BAG3 (n=3), I) DNAJC18 (n=3), and J) HSPB7 (n=4), and K) were quantified for GFP+ expression. L) LDH levels, indicative of cellular toxicity. BAG3 (n=3), DNAJC18 (n=3), and HSPB7 KD (n=1) do 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

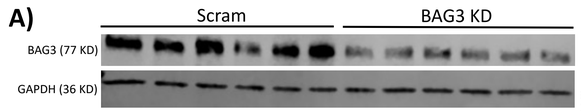

Figure 3: shRNA mediated knockdown of BAG3 on the protein and transcript levels in iPSC-CMs. A) Representative western blot image of scramble control (MOI5) vs BAG3 KD (MOI5). B) BAG3 protein levels (n=3). 3-6 technical replicates each. C) *BAG3* transcript levels (n=3). 1-2 technical replicates each.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

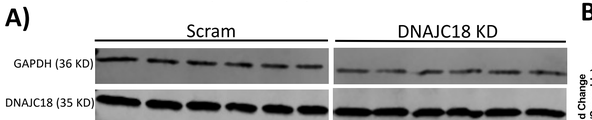

Figure 4: shRNA mediated knockdown of DNAJC18 on the protein and transcript levels in iPSC-CMs. A) Representative western blot image of scramble control (MOI5) vs DNAJC18 KD (MOI5). B) DNJAC18 protein levels (n=3). 3-6 technical replicates each). C) *DNAJC18* transcript levels (n=3). 2 technical replicates each.
Statistical test: Student's t-test. p values displayed between bars.

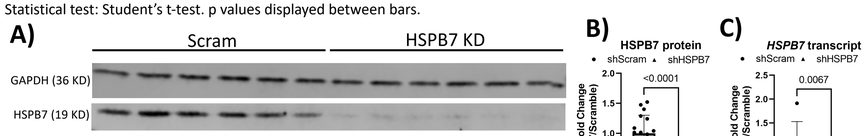

Figure 5: shRNA mediated knockdown of HSPB7 on the protein and transcript levels in iPSC-CMs. A) Representative western blot image of scramble control (MOI5) vs HSPB7 KD (MOI5). B) HSPB7 protein levels (n=3). 3-6 technical replicates each). C) *HSPB7* transcript levels (n=3). 2 technical replicates each.
Statistical test: Student's t-test. p values displayed between bars.

BAG3 and HSPB7 KD Modulate MyBP-C Expression

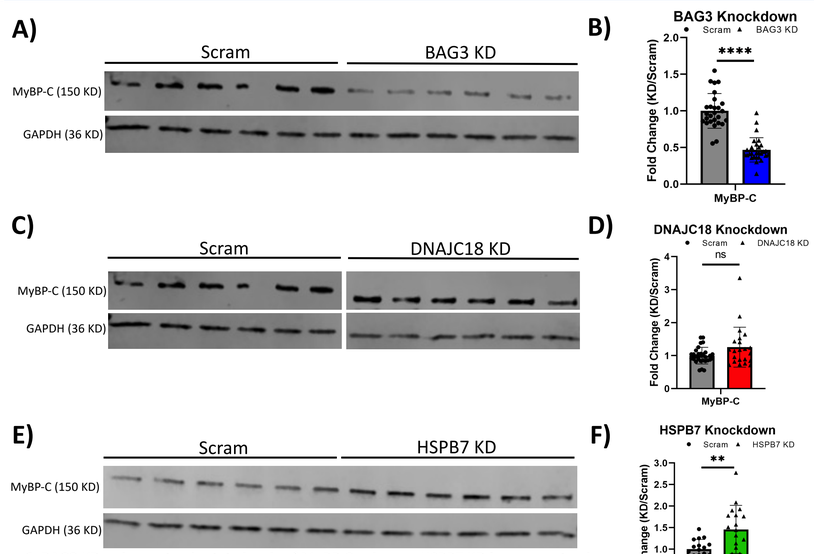

Figure 6: BAG3 KD reduces steady state levels of MyBP-C, whereas HSPB7 KD increases steady state levels of MyBP-C. A,C,E) Representative western blot images of scramble control (MOI5) vs KD (MOI5). B,D,F) Western blot quantification, average of 3 technical replicates for each biological replicate. A-B) BAG3 KD (n=5), C-D) DNAJC18 KD (n=4), and E-F) HSPB7 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

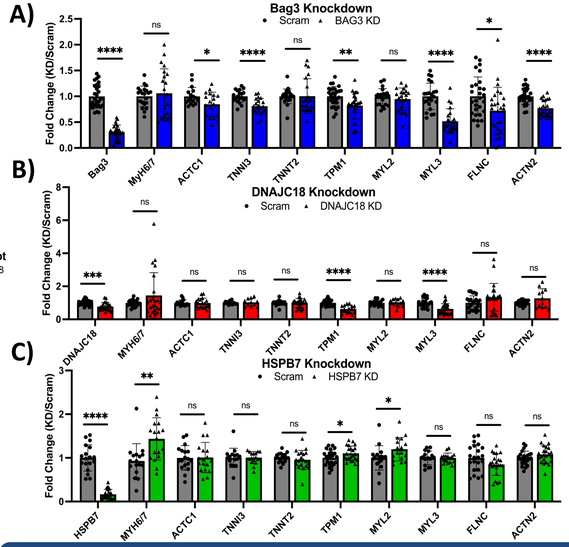

Figure 7: BAG3, DNAJC18, and HSPB7 KD alter sarcomeric and Z-disc protein expression. A) BAG3 KD, B) DNAJC18 KD, and C) HSPB7 KD. Average of 3 technical replicates for each biological replicate (n=3-4).
Statistical test: Student's t-test. **** p≤0.0001, *** p ≤0.001, ** p≤0.01, * p≤0.05, ns p>0.05.

HSP70 Co-Chaperones Regulate Each Other's Expression

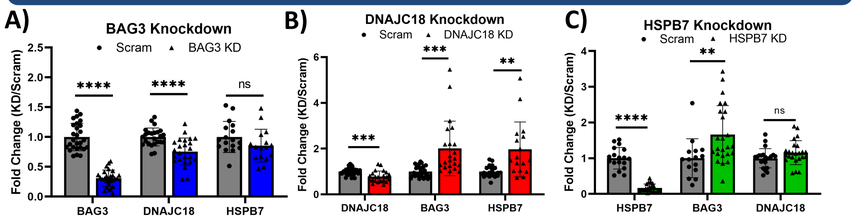

Figure 8: Individual KD of HSP70 co-chaperones BAG3, DNAJC18, and HSPB7 are associated with changes in the expression of other co-chaperones, suggesting inter-regulation. A) BAG3 KD, B) DNAJC18 KD C) HSPB7 KD. Average of 3 technical replicates for each biological replicate (n=3-4). Statistical test: Student's t-test. **** p≤0.0001, *** p ≤0.001, ** p≤0.01, ns p>0.05.

Conclusions/Acknowledgments

Data Summary:

1. BAG3 KD decreases MyBP-C expression, while HSPB7 knockdown increases MyBP-C expression.
2. BAG3 KD markedly decreases most other profiled sarcomeric and Z-disc proteins.
3. HSP70 co-chaperones BAG3, DNAJC18, and HSPB7 can co-regulate each other's expression

Future Directions:

1. To differentiate transcriptional vs post-translational modifications, transcript levels of the sarcomeric and Z-disc proteins in Fig. 8 will be assessed via RNA sequencing.
2. Cycloheximide chase assays, which inhibit translation of new protein in cells, will be used to determine protein half life to assess stability of presumed client proteins.

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