The Significance of HSP70 Co-Chaperones in Modulating Sarcomeric Proteostasis

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Project Summary

Background: Hypertrophic cardiomyopathy (HCM) is a cardiovascular disease that affects one in 500 people.[1] A known cause of heart failure and sudden cardiac death, HCM is characterized by left ventricular (LV) hypertrophy (>13 mm LV end diastolic wall thickness). HCM is a genetically inherited disease in which 50% of HCM cases arise from allelic variances.[2] Approximately, 50% of familial HCM cases arise from variants in sarcomeric proteins.[3] *MYBPC3*, the gene that codes for MyBP-C protein, is the leading sarcomeric gene that harbors pathogenic variants. The majority of *MYBPC3* variants result in premature termination codons, triggering nonsense-mediated mRNA decay or degradation of truncated protein through the ubiquitin-proteasome system (UPS).[4] Accordingly, a reduction in transcript leads to reduced MyBP-C levels in hearts from patients with, suggesting haploinsufficiency as a pathogenic mechanism.[5] Heat shock protein 70 kDa (HSP70) directs client proteins including MyBP-C toward stabilization or UPS-mediated degradation depending on the presence of certain co-chaperone proteins that bind to HSP70 (**Fig 1**).[6] Common variants in 3 of these co-chaperones have been shown to be among the top risk alleles associated with HCM: BAG3, DNAJC18, and HSPB7.[7,8,9,10]

Aim: Identify whether co-chaperones of HSP70 (BAG3, DNAJC18, and HSPB7) modulate sarcomeric protein expression, specifically MyBP-C.

Hypothesis: I hypothesize that the knockdown (KD) of HSP70 co-chaperones BAG3 and HSPB7 compromise MyBP-C expression, while DNAJC18 KD will preserve MyBP-C expression.

Methods: Human induced pluripotent stem cell cardiomyocytes (hiPSC-CMs) were transduced with GFP-tagged adenovirus (AdV) expressing shRNA targeted against BAG3, DNAJC18, HSPB7, with scrambled shRNA as a negative control at an MOI5. Transduction efficiency was measured via fluorescence microscopy and flow cytometry for GFP+ expression. Cellular toxicity following viral transduction was assessed via the CyQUANT™ LDH Cytotoxicity Assay per manufacturer's protocol.[11] Protein from the hiPC-CMs was isolated 4 days post viral transduction and quantified using the Bio-Rad DC™ Protein Assay.[12] Protein expression was assessed via western blots to GAPDH. Fold change was determined by comparing expression under knockdown conditions compared to scramble. Student's t-test was used to determine statistical significance with a p≤0.05 deemed significant.

Results: KD of HSP70 co-chaperone BAG3 markedly reduced expression of multiple sarcomeric and Z-disc proteins, most markedly MyBP-C. HSPB7 KD increased MyBP-C and myosin expression while DNAJC18 had minimal to no effects on sarcomere protein content. I also observed that the co-chaperones regulated each other's expression.

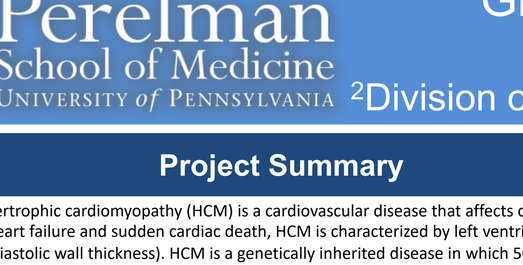

Figure 1: The HSP70 co-chaperone network.[13] BAG3 is a nucleotide exchange factor (NEF), DNAJC18 is a J-domain protein (JDP), and HSPB7 is a small heat shock protein (sHSP).

AdV Transduction of iPSC-CMs

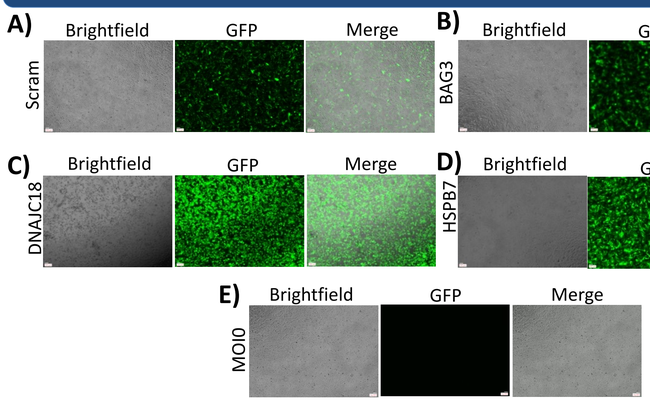

Figure 2: GFP-tagged AdV transduction of hiPSC-CMs was confirmed using fluorescence microscopy. hiPSC-CMs transduced with AdV containing shRNA (MOI 5) against B) BAG3, C) DNAJC18, and D) HSPB7 compared to A) cells transduced with a scrambled shRNA control (MOI5) and E) non-transduced cells (MOI 0).

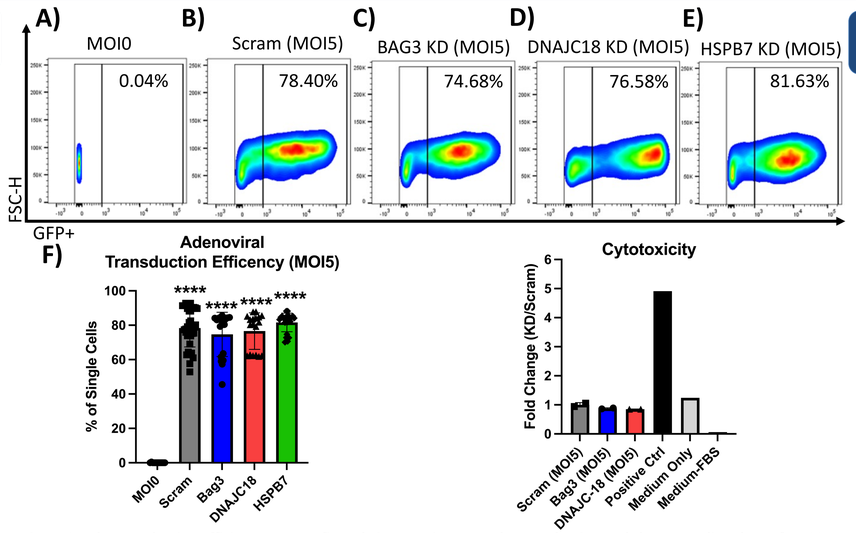

Figure 3: AdV transduction efficiency was confirmed via flow cytometry. A) Gating for GFP-/+ hiPSC-CMs was determined based on the MOI0 cells (n=2). Cells transduced with shRNA against B) Scram (n=6), C) BAG3 (n=3), D) DNAJC18 (n=3), and E) HSPB7 (n=4) were quantified for GFP+ expression. F) Quantification of A-E. Statistical test: Student's t-test (each treatment compared to MOI0) **** p≤0.0001.

Figure 4: AdV transduction and shRNA-mediated KD of BAG3 and DNAJC18 does not cause cellular toxicity. LDH levels, indicative of cellular toxicity, indicate BAG3 (n=2) or DNAJC18 (n=2) KD does not induce cellular toxicity.

BAG3 and HSPB7 KD Modulate MyBP-C Expression

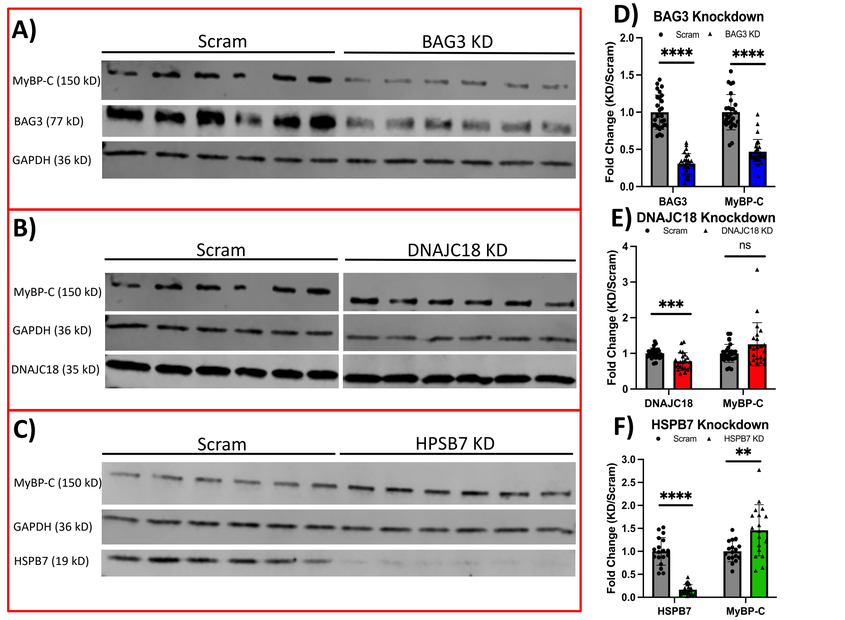

Figure 5: BAG3 KD reduces MyBP-C expression, whereas HSPB7 KD increases MyBP-C expression. A-C) Representative western blot images of scramble control (MOI5) vs KD (MOI5). D-F) Western blot quantification, average of 3 technical replicates for each biological replicate. D) BAG3 KD (n=5), E) DNAJC18 KD (n=4) F) HSPB7 KD (n=4). Statistical test: Student's t-test **** p≤0.0001, *** p ≤0.001, ** p≤0.01, ns p>0.05.

HSP70 Co-Chaperones Modulate Turnover of Other Sarcomere Proteins

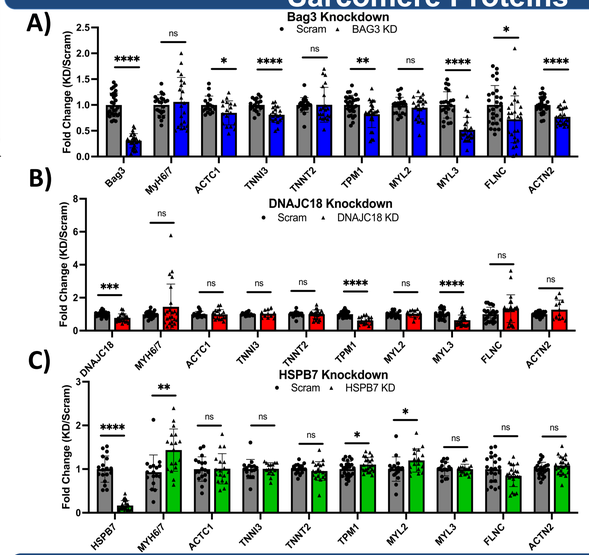

Figure 6: BAG3, DNAJC18, and HSPB7 KD alter sarcomeric and z-disk protein expression. Average of 3 technical replicates for each biological replicate (n=3-4). A) BAG3 KD, B) DNAJC18 KD, and C) HSPB7 KD. Statistical test: Student's t-test **** p≤0.0001, *** p ≤0.001, ** p≤0.01, * p≤0.05, ns p>0.05.

HSP70 Co-Chaperones Regulate Each Other's Expression

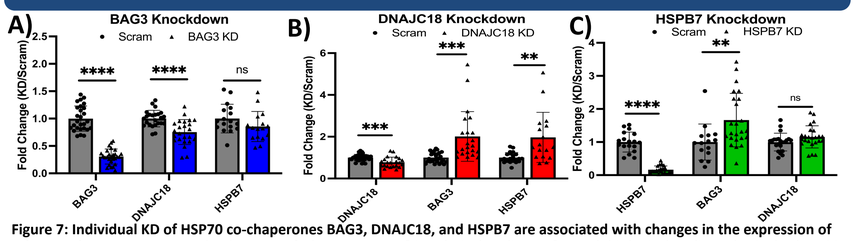

Figure 7: Individual KD of HSP70 co-chaperones BAG3, DNAJC18, and HSPB7 are associated with changes in the expression of other co-chaperones, suggesting inter-regulation. Average of 3 technical replicates for each biological replicate (n=3-4). A) BAG3 KD, B) DNAJC18 KD C) HSPB7 KD. Statistical test: 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 sarcomere protein content, demonstrating its importance in modulating sarcomeric proteostasis.
3. HSP70 co-chaperones that are associated with HCM (BAG3, DNAJC18, and HSPB7) co-regulate each other's expression, suggesting that they may directly interact in complex with each other.

Future Directions:

1. In order to differentiate transcriptional vs post-transcriptional modifications, levels of co-chaperones and sarcomeric proteins will be assessed using RT-qPCR.
2. Cycloheximide chase assays, which inhibit translation of new protein in cells, will be used to determine direct client interactions that regulate protein turnover.

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