Background: Hypertrophic cardiomyopathy (HCM) is a cardiovascular disease that affects one in 500 people. A known cause of heart failure and sudden cardiac death, HCM is characterized by left ventricular (LV) hypertrophy (>13 mm in the diastolic wall thickness). HCM is a genetically inherited disease in which 50% of HCM cases arise from allelic variances. Approximately, 50% of familial HCM cases arise from variants in sarcomeric genes. MyBP-C, the gene that codes for MyBP-C protein, is the leading sarcomeric gene that harbors pathogenic variants. The majority of MyBP-C variants result in premature termination codons, triggering nonsense-mediated mRNA decay or degradation of truncated protein through the ubiquitin-proteasome system (UPS). Accordingly, a reduction in transcript levels leads to MyBP-C levels in hearts from patients with, suggesting haplosufficiency as a pathogenic mechanism. Heat shock protein 70kDa (HSP70) directs client proteins including MyBP-C toward stabilization, or UPS-mediated degradation depending on the presence of certain co-chaperones that bind to HSP70 (Fig. 1). 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 modulate sarcomeric protein expression, specifically MyBP-C.

Hypothesis: We hypothesize that the knockdown (KD) of HSP70 co-chaperones BAG3 and HSP7 modulate 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 (AV) expressing shRNA targeted against BAG3, DNAJC18, HSPB7, with scrambled shRNA as a negative control at an MOI of 5. Transduction efficiency was measured via fluorescence microscopy and flow cytometry for GFP expression. Cellular toxicity following knockdown transfection was assessed via the CyQUANT™ LDH cytotoxicity Assay in per manufacturer’s protocol. Protein from the hiPSC-CMs was isolated 4 days post viral transduction and quantified using the Bio-Rad DC™ Protein Assay. Protein expression was assessed via western blots to GAPDH. Fold change was determined by comparing expression under knockdown conditions compared to controls. Student’s t-test was used to determine statistical significance with a p<0.05 deemed significant.

Results: KD of HSP70 co-chaperones BAG3 markedly decreased expression of multiple sarcomeric and 2-disc proteins, mostly markedly MyBP-C. HSP70 KD increased MyBP-C and mAPP expression while DNAJC18 had minimal to no effects on sarcomere protein content. We also observed that the co-chaperones regulated each other’s expression.

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