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EDITORIAL COMMENT

Going Further With Half as Much

It's in the BAG*

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Variants in sarcomere genes are a major cause of inherited cardiomyopathies, primarily hypertrophic cardiomyopathy (HCM), but others as well. There is a striking diversity of biophysical mechanisms through which these variants can induce sarcomere dysfunction, but a frequent pathway is via protein haploinsufficiency. Through either the introduction of a stop codon (also known as a truncating variant), a destabilizing mutation that leads to degradation, or some structural alteration such that the protein can no longer be incorporated into the sarcomere lattice, only the wild-type allele contributes useful protein to the cardiomyocyte.

The haploinsufficiency mechanism of disease occurs with variants in several sarcomere proteins, such as titin variants leading to dilated cardiomyopathy, but perhaps the best studied is cardiac myosin-binding protein C (cMyBPC). Indeed, variants in the *MYBPC3* gene are detected in approximately one-half of all patients with familial HCM. One appealing strategy to treat these patients is to somehow boost the amount of protein contributed by the wild-type allele. This approach would hypothetically allow the cardiomyocyte to do more with only half the genomic contribution for the affected gene. Furthermore, because physiology is remarkable adaptive, especially in the younger age range at which these carriers are affected, even a modest increase in cMyBPC protein levels could have significant ameliorative properties.

This is the approach that Thompson et al[1] have taken in this issue of *JACC: Basic to Translational Science*. The goal of this study was to identify a small molecule that could enhance the level of cMyBPC protein in a model of haploinsufficiency. Toward this end, they developed a high-throughput assay using human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) carrying a *MYBPC3* truncation variant (+/c.2373dupG) that leads to HCM. Levels of cMyBPC and alpha and beta myosin heavy chain were assessed with the use of homogeneous immunoassay technology after exposure to a library of 2,426 compounds. Unfortunately, although this screen was well designed and an impressive achievement, it did not yield any compounds that reproducibly increased cMyBPC protein levels. However, if you do not find what you are looking for, sometimes the next best thing is the exact opposite of what you were looking for, because it can provide valuable insight. Two compounds were identified that *decreased* cMyBPC levels. One of these was JG-98, an allosteric inhibitor of the interaction between heat shock protein 70 (Hsp70) and the proautophagy co-chaperone Bcl-2-associated athanogene 3 (BAG3).

The BAG3 protein binds to Hsp70 via its BAG domain, to act as a nucleotide exchange factor, although it has several other domains that allow it to bind other molecules, including small heat shock proteins. This complex plays an important anti-apoptotic role in striated muscle (both cardiac and skeletal) and the central nervous system, although it is also highly expressed in tumors. Indeed, inhibiting the Hsp70-BAG3 interaction has been shown to be a viable target for treating a handful of cancer types, and it was for this purpose that JG-98 was investigated and found to inhibit proliferation in various

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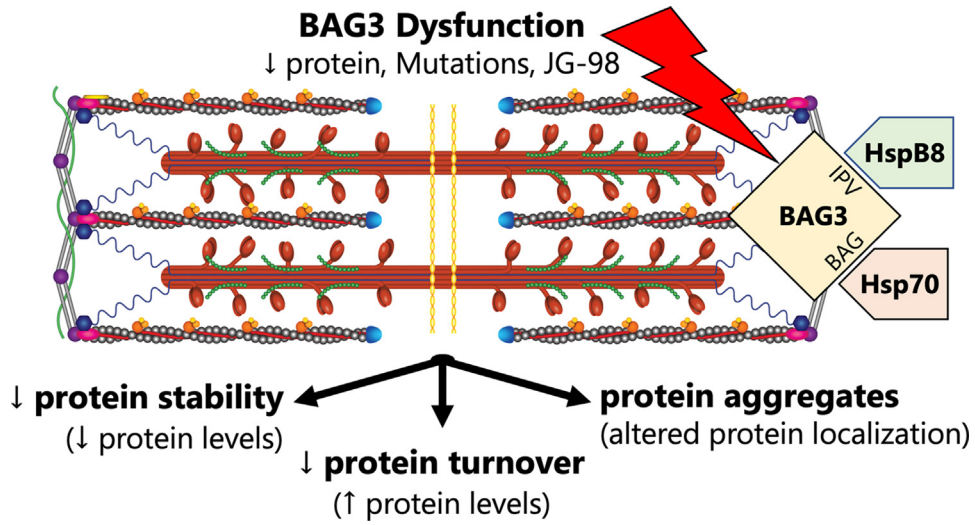
FIGURE 1 Diversity of Phenotypes Arising From BAG3 Dysfunction



Bcl-2–associated athanogene 3 (BAG3) and heat shock protein (Hsp) 70 have diverse functions at the cardiac sarcomere, such that dysfunction could result in multiple possible effects on their protein clients.

cancer cell lines.[2] As is frequently the case, however, therapies that are effective against tumors are often cardiotoxic, and BAG3 modulation appears to fall into that category as well.[3]

That JG-98 was a "hit" for modulating cMyBPC protein levels was not entirely unexpected, considering previous studies from the Day group showing that Hsp70 is a chaperone for cMyBPC,[4] and our own work showing that cMyBPC is a client for the BAG3 complex, which localizes to the cardiac sarcomere.[5] Although it may have been possible to predict de novo that modulating the BAG3-Hsp70 interaction would be capable of altering cMyBPC protein levels, it would have been challenging to predict the direction of this change. That is because previous studies in which BAG3 has been dysregulated, through either decreased protein levels or BAG3 mutations, have shown opposing effects on sarcomere proteins. These possibilities include: increased sarcomere client levels due to decreased protein turnover[5]; decreased sarcomere client levels due to loss of protein stability[6]; and sarcomere client aggregation into an insoluble fraction[7] (**Figure 1**). This range of possible outcomes highlights the multifaceted roles of BAG3 in the cardiomyocyte and the complexity involved with targeting it for modulation. The findings of Thompson et al possibly support a protein stability mechanism of BAG3-Hsp70, as they found that JG-98 depressed cMyBPC stability via a cycloheximide chase experiment.

One possible complicating aspect of this study was the reliance on iPSC models, which was necessary for the execution of the high-throughput drug screen. However, iPSC-CMs are an immature model, and this may be a particular limitation when studying the role of BAG3 in the sarcomere. Our work has suggested that BAG3 may have slightly different roles in immature vs adult cardiomyocytes, specifically in the adult sarcomere, which is under significantly greater mechanical stress that requires a more active protein quality control system. Translating these important findings into an in vivo model should be of high priority in the future.

We and others have shown that BAG3 protein levels are decreased in end-stage failing hearts (both human and nonhuman models). In fact, the magnitude of this decrease in BAG3 is approximately 50%, which quantitatively matches the inhibitory effect of JG-98 on the BAG3-Hsp70 interaction in cardiomyocytes. If patients with cMyBPC-truncating variants had worsening HCM it may eventually lead to a similar reduction in BAG3 protein levels that could lead to destabilization of cMyBPC and further decreased levels of the protein (similar to what was observed with JG-98 exposure). This would potentially accelerate the deterioration of function

and progression of disease. As suggested by Thompson et al[1], approaches that increased BAG3 protein levels or increased its activity could be therapeutically beneficial in these patients. Supporting this hypothesis, we previously found that adeno-associated virus-mediated overexpression of BAG3 was able to rescue sarcomere function in a mouse heart failure model.[5]

Although gene therapy is a therapeutic possibility, small molecules are a more appealing opportunity (for the same reason the investigators performed a drug screen rather than overexpressed cMyBPC directly). Unfortunately, no direct activators of BAG3 or enhances of the Hsp70-BAG3 interaction have been identified thus far. Furthermore, progress on this front is hampered by a lack of mechanistic insight into sarcomere protein quality control in the adult heart. Despite progress in recent years, many steps in these processes remain black boxes, making informed drug development impossible at the moment. Studies such as Thompson et al's[1] are critical as they reveal some of these mechanistic aspects that bring us closer to such goals, but more work is necessary.

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