STATE-OF-THE-ART REVIEW

The Multifunctional Protein BAG3

A Novel Therapeutic Target in Cardiovascular Disease

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SUMMARY

The B-cell lymphoma 2–associated anthanogene (BAG3) protein is expressed most prominently in the heart, the skeletal muscle, and in many forms of cancer. In the heart, it serves as a co-chaperone with heat shock proteins in facilitating autophagy; binds to B-cell lymphoma 2, resulting in inhibition of apoptosis; attaches actin to the Z disk, providing structural support for the sarcomere; and links the α-adrenergic receptor with the L-type Ca^{2+} channel. When BAG3 is overexpressed in cancer cells, it facilitates prosurvival pathways that lead to insensitivity to chemotherapy, metastasis, cell migration, and invasiveness. In contrast, in the heart, mutations in BAG3 have been associated with a variety of phenotypes, including both hypertrophic/restrictive and dilated cardiomyopathy. In murine skeletal muscle and vasculature, a mutation in BAG3 leads to critical limb ischemia after femoral artery ligation. An understanding of the biology of BAG3 is relevant because it may provide a therapeutic target in patients with both cardiac and skeletal muscle disease. (J Am Coll Cardiol Basic Trans Science 2018;3:122–31) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

WHAT IS B-CELL LYMPHOMA 2–ASSOCIATED ATHANOGENE 3?

B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG3) is a 575 amino acid member of the BAG family of proteins. Each of the members of the family share at least 1 BAG domain that is located near the carboxy-terminal end of the protein and binds to the ATPase domain of the constitutively expressed (Hsc) and the inducible (Hsp) heat shock protein-70 (Hsc70/Hsp70), which is a chaperone protein that facilitates protein quality control. The biological importance of BAG3 is evidenced by the fact that it is highly conserved in nature with homologues being found in reptiles, fruit flies, invertebrates, silkworms, nematodes, yeast, and even flowering plants (1–6). First cloned and sequenced in 1999 (1,7), BAG3 is present in all mammalian tissues

but is expressed most prominently in the heart, the skeletal muscle, and in many cancers (8,9). Early studies in cancer found that tumors over-expressing BAG3 were insensitive to chemotherapy (10), whereas knockdown of BAG3 led to enhanced chemosensitivity (11). BAG3 regulates cancer cell migration, invasiveness, adhesion, metastasis, and proliferation (12). Studies in cancer cells also showed that BAG3 expression is regulated by stress conditions, including ischemia, viral infection, heat shock, oxidants, serum starvation, and exposure to high temperature (13–21). However, the regulation of BAG3 by stress seems to be tissue specific (22–24).

BAG3 PROTEIN–BINDING DOMAINS PROVIDE FUNCTIONAL PLEIOTROPY

The unique ability of BAG3 to perform a wide array of functions within the cell (12) is due to the presence of multiple protein-binding domains within its structure (**Figure 1, Central Illustration**). For example, by binding to Hsc70/Hsp70, BAG3 is able to mediate chaperone-assisted autophagy by shuttling client proteins along the cellular microtubular apparatus that are dedicated to eliminating misfolded proteins (25). The BAG domain also couples with the antiapoptotic protein Bcl-2, resulting in inhibition of apoptosis (26). Two highly conserved IPV (Ile-Pro-Val) motifs allow BAG3 to interact with the small heat shock proteins HspB8 and HspB6 to support macroautophagy, a process in which misfolded or damaged proteins and organelles (e.g., mitochondria) are sequestered in autophagosomes and degraded (27). The second IPV domain binds to αB-crystallin, a member of the small heat shock protein family (Hsp20), with the subsequent inhibition of protein aggregation (28). A WW domain plays a pivotal role in chaperone-assisted selective autophagy, a tension-induced autophagy pathway that is essential for mechanotransduction in muscle and immune cells (29,30). The WW region also couples with the PXXP region to modify the three-dimensional structure of the protein. The proline-rich PXXP region also facilitates binding to the Src homology 3-containing protein phospholipase C-γ with subsequent stimulation of invasion, adhesion, and migration of cancer cells (14,31) and the retrograde transport of misfolded proteins to perinuclear aggresomes (12).

BAG3 PLAYS A CRITICAL ROLE IN MAINTAINING CARDIAC HOMEOSTASIS

The first evidence that BAG3 plays an important role in maintaining myocardial homeostasis came less than a decade ago when Homma et al. (32) showed that homozygous deletion of BAG3 in mice led to profound myofibrillar disorganization and death by 4 weeks. Fang et al. (33) recently confirmed this observation; however, they were able to mitigate the early lethality of BAG3 deletion by generating a mouse in which the BAG3 deletion was cardiac specific. It is noteworthy that Homma et al. also reported that mice with a heterozygous BAG3 deletion had a normal phenotype at 4 weeks. We recently found that by 10 weeks of age, BAG3 mice with heterozygous deficiency have a substantial reduction in ventricular function (34). To study the mechanisms responsible for the marked pathology seen in BAG3-deficient mice, Hishiya et al. (35) used a small interfering ribonucleic acid (RNA) to knockdown BAG3 in neonatal myocytes. They reported that after BAG3 knockdown, mechanical stretch led to the rapid disruption of the myofibril structures, whereas BAG3 overexpression facilitated the re-distribution of CAPZβ1 to the proper cellular location.

In contrast to the localization of BAG3 in the cytoplasm of neonatal myocytes, in the adult myocyte, BAG3 is localized in the sarcolemma and transverse tubules, and co-immunoprecipitates with the β_1-adrenergic receptor and the L-type Ca^{2+} channels (36). These results suggest that BAG3 plays a significant role in excitation–contraction coupling. This concept was supported by the findings that: 1) reducing BAG3 levels with short hairpin RNA decreased contraction and $[Ca^{2+}]_i$ transient amplitudes in the presence of isoproterenol (ISO); 2) L-type Ca^{2+} current (I_{Ca}) and sarcoplasmic reticulum Ca^{2+} content but not Na^+/Ca^{2+} exchange current (I_{NaCa}) or sarcoplasmic reticulum Ca^{2+} uptake were reduced in ISO-treated shBAG3 myocytes; 3) forskolin or dibutyryl cyclic adenosine monophosphate restored I_{Ca} amplitude in shBAG3 myocytes to that observed in ISO-stimulated wild-type myocytes, consistent with BAG3 having effects upstream and at the level of the receptor; and 4) protein levels affecting Ca^{2+} homeostasis were unchanged after BAG3 knockdown (shBAG3).

BAG3 IS IMPORTANT IN THE PATHOBIOLOGY OF ISCHEMIA/REPERFUSION INJURY

In view of the literature suggesting that BAG3 is an adaptive mechanism to maintain cellular homeostasis under stress, we wondered whether BAG3 played a role during the stress of ischemia/reperfusion injury. When neonatal mouse ventricular cardiomyocytes

FIGURE 1 The Structure of BAG3 and its Protein-Binding Domains

The consensus protein-binding domains in B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG3) (WW, IPV, PXXP, and BAG) and the putative signaling pathways that are regulated in cancer cells through each binding domain are shown. Numbers are amino acids. αB-crystallin = small heat shock protein B5 (HSPB5); CASA = chaperone-assisted selective autophagy; Hsc70/Hsp70 = constitutively expressed and the inducible heat shock protein 70; Hsp = heat shock protein; PLC-γ = phospholipase C-gamma; SH3 = SRC homology 3 domain.
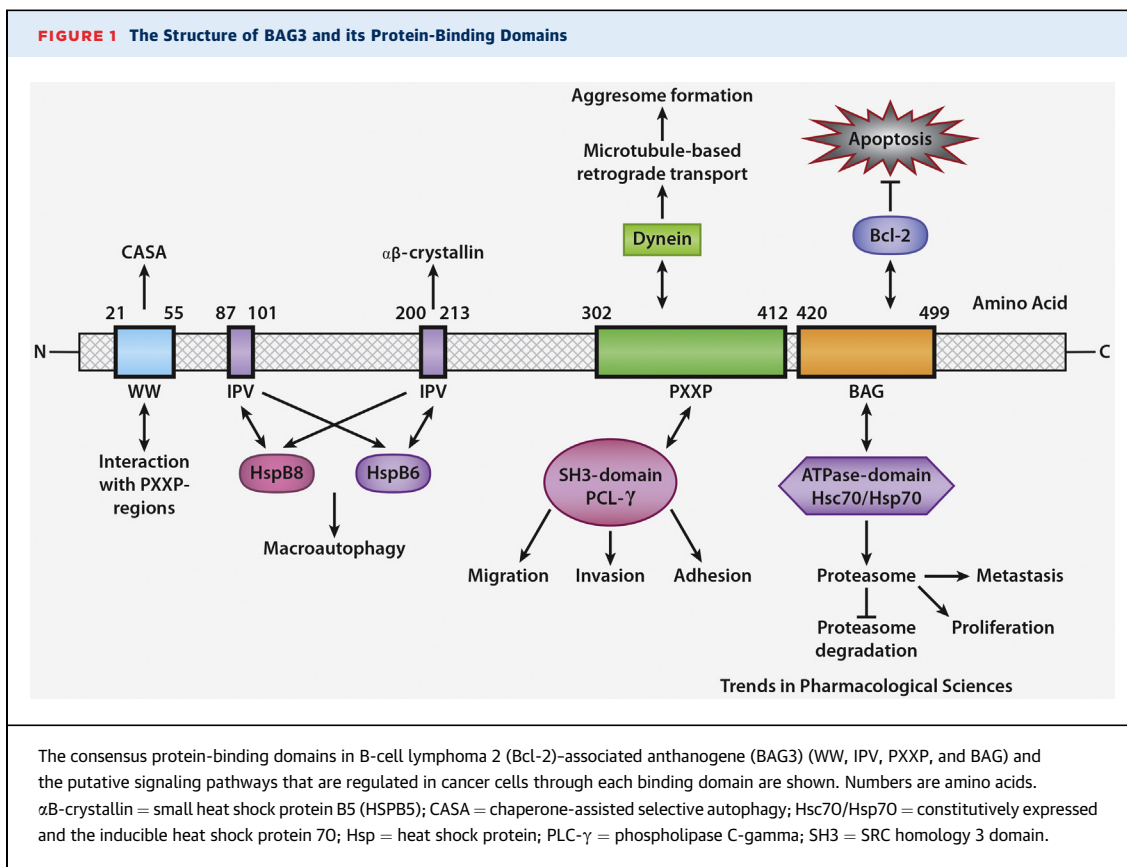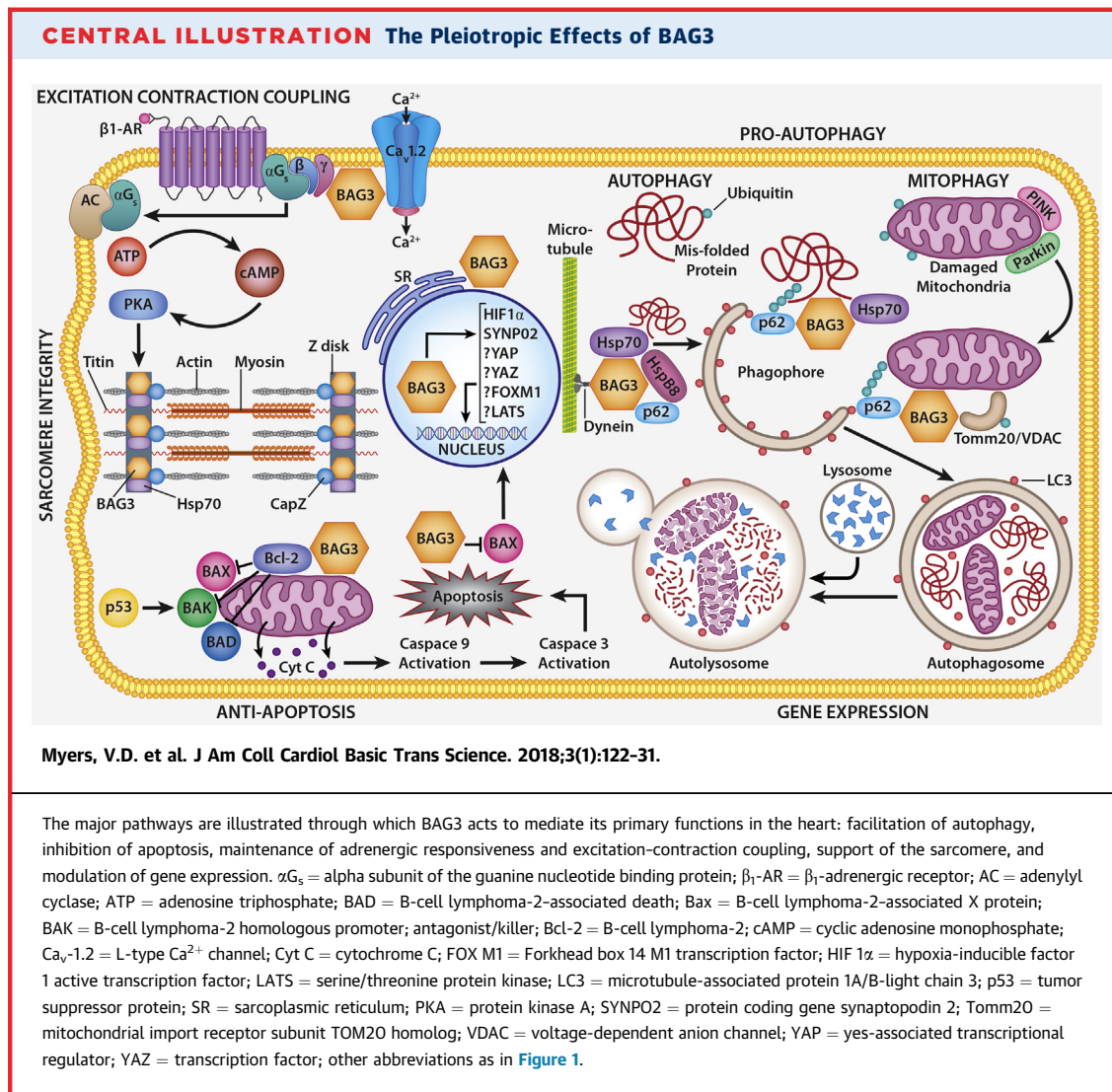
were exposed to hypoxia/re-oxygenation, BAG3 levels were reduced by >50%, which was accompanied by enhanced expression of markers of apoptosis and decreased expression of markers of autophagy, as well as reduced autophagy flux (23). Autophagy flux was measured by transfecting cells with a green fluorescent protein (GFP)-red fluorescent protein (RFP)-LC3 reporter construct. When the confocal image of the green (GRP) and red (RFP) fluorescence of the puncta merge, the LC3 appear green-yellow; however, when LC3 is incorporated into autophagasomes and lysosomal fusion takes place, the resulting acidity of the autophagalysosome quenches the GFP (green) signal and LC3 appear red-orange. If autophagy flux does not occur, puncta do not accumulate (**Figure 2**).

These deleterious effects of hypoxia/re-oxygenation on apoptosis and autophagy were recapitulated by knockdown of BAG3 and were rescued by BAG3 overexpression. The in vitro findings were relevant to the whole organism as mice in which BAG3 was overexpressed by infecting the mice with a recombinant adeno-associated virus (rAAV9-BAG3) under the control of a cytomegalovirus promoter before ischemia/reperfusion had significantly decreased infarct size, improved left ventricular ejection fraction, decreased markers of apoptosis, and enhanced autophagy flux compared with noninfected controls. Recent studies suggest that diminished levels of BAG3 during ischemia/reperfusion might also result in pathological changes due to abnormal mitophagy, which has been reported in patients with myofibrillar myopathies (37,38).

A NONSYNONYMOUS MUTATION IN BAG3 LEADS TO THE DEVELOPMENT OF A MYOFIBRILLAR MYOPATHY

In 2009, Selcen et al. (39,40) described 3 unrelated children with myofibrillar myopathy who harbored a single nucleotide polymorphism in exon 3 of *BAG3* that resulted in a substitution of a leucine for a proline at amino acid position 209 (P209L). All 3 children presented in childhood with progressive and severe muscle weakness, respiratory insufficiency, a restrictive/hypertrophic cardiomyopathy, and elevation of serum creatine kinase levels. Electron microscopy of muscle biopsy specimens revealed Z disk streaming in minimally affected myofibrils and apoptotic nuclei in more affected fibers. Since the

CENTRAL ILLUSTRATION The Pleiotropic Effects of BAG3

Myers, V.D. et al. J Am Coll Cardiol Basic Trans Science. 2018;3(1):122–31.

The major pathways are illustrated through which BAG3 acts to mediate its primary functions in the heart: facilitation of autophagy, inhibition of apoptosis, maintenance of adrenergic responsiveness and excitation–contraction coupling, support of the sarcomere, and modulation of gene expression. αG_s = alpha subunit of the guanine nucleotide binding protein; β_1-AR = β_1-adrenergic receptor; AC = adenylyl cyclase; ATP = adenosine triphosphate; BAD = B-cell lymphoma-2-associated death; Bax = B-cell lymphoma-2-associated X protein; BAK = B-cell lymphoma-2 homologous promoter; antagonist/killer; Bcl-2 = B-cell lymphoma-2; cAMP = cyclic adenosine monophosphate; Ca_v-1.2 = L-type Ca^{2+} channel; Cyt C = cytochrome C; FOX M1 = Forkhead box 14 M1 transcription factor; HIF 1α = hypoxia-inducible factor 1 active transcription factor; LATS = serine/threonine protein kinase; LC3 = microtubule-associated protein 1A/B-light chain 3; p53 = tumor suppressor protein; SR = sarcoplasmic reticulum; PKA = protein kinase A; SYNPO2 = protein coding gene synaptopodin 2; Tomm20 = mitochondrial import receptor subunit TOM20 homolog; VDAC = voltage-dependent anion channel; YAP = yes-associated transcriptional regulator; YAZ = transcription factor; other abbreviations as in **Figure 1**.
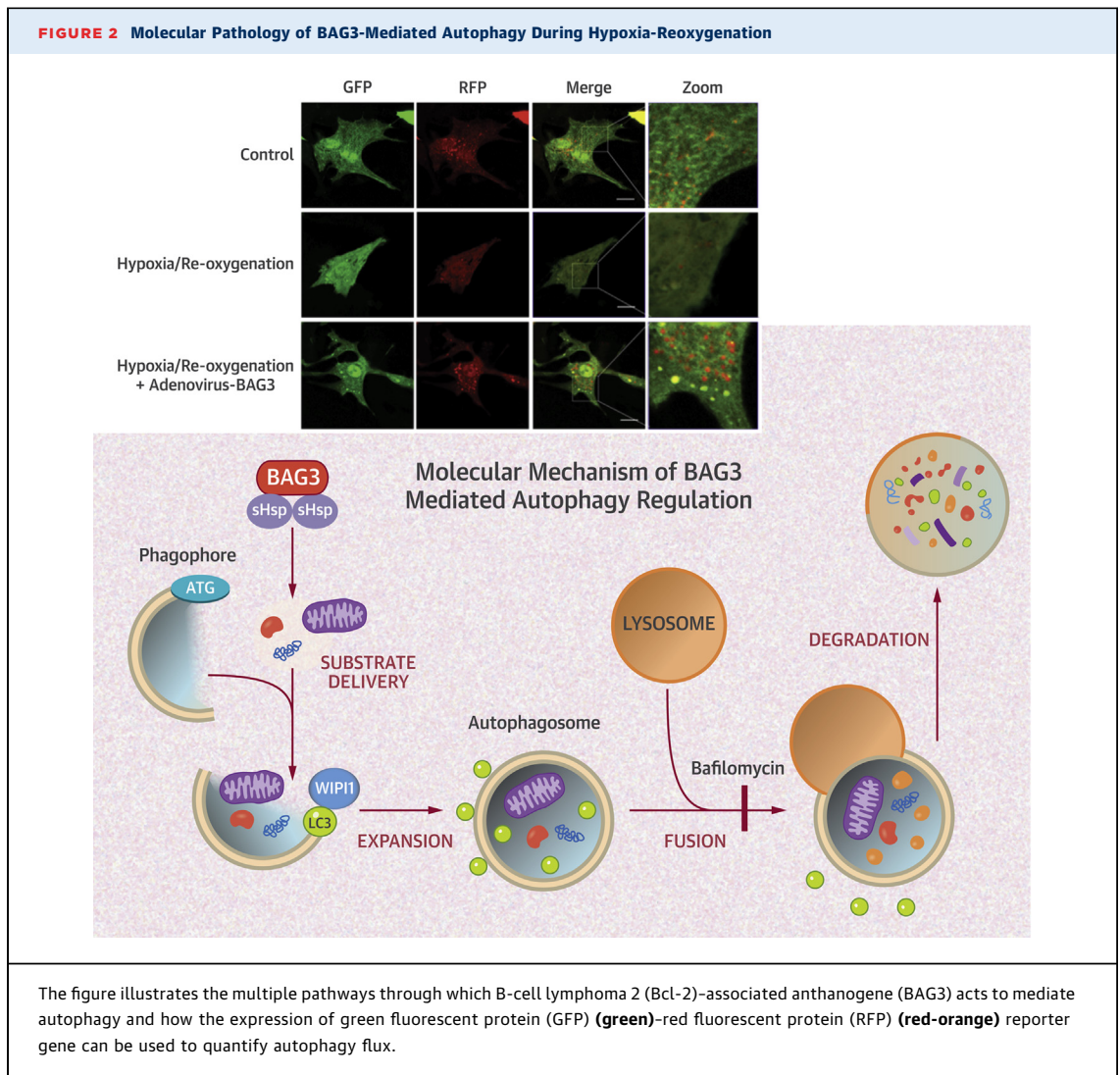
original report by Selcen et al., additional reports have documented the same phenotype in individuals with sporadic P209L mutations (41-45).

Studies were then conducted in zebrafish to identify the mechanisms of action of P209L BAG3-P209L localized to the Z disk and were associated with the accumulation of small granular protein aggregates containing wild-type BAG3 within the striations (46). Surprisingly, the formation of these protein aggregates was not attributable to impaired autophagy. By contrast, BAG3 knockdown in the zebrafish resulted in myofibrillar disintegration but not in the formation of protein aggregates. However, the myofibrillar disintegration was rescued by overexpression of BAG3-P209L, leading investigators to suggest that the formation of BAG3-P209L aggregates in the zebrafish harboring the P209L mutation reduced the pool of

available BAG3. The reduction in available BAG3 then led to myofibrillar disintegration. Interestingly, when the P209L mutation was transfected into skeletal myoblasts, the multinucleation that occurs during the differentiation of myoblasts into skeletal muscle myotubes was disturbed. In contrast with the studies of Hishiya et al. (35), in which BAG3 knockdown caused myofibrillar disorganization, the P209L mutation did not disturb the assembly or integrity of the Z disk or nuclear localization of BAG3 when transfected into rat neonatal cardiomyocytes (46).

Quintana et al. (47) recently showed that cardiac-restricted overexpression of P209L in mice led to myocardial deposition of pre-amyloid oligomers, abnormal mitochondrial dynamics, modest diminution in left ventricular function and diameter, and activation of p38 signaling (47). BAG3 P209L hearts

FIGURE 2   Molecular Pathology of BAG3-Mediated Autophagy During Hypoxia-Reoxygenation

The figure illustrates the multiple pathways through which B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG) acts to mediate autophagy and how the expression of green fluorescent protein (GFP) **(green)**-red fluorescent protein (RFP) **(red-orange)** reporter gene can be used to quantify autophagy flux.

also demonstrated increased numbers of activated cardiac fibroblasts but without an appreciable increase in fibrosis. Overexpression of P209L in the presence of 2 normal *BAG3* alleles also resulted in haploinsufficiency of wild-type *BAG3*, which might have accounted in part for the resulting phenotype. It is noteworthy that because P209L expression was cardiac specific, the P209L mice did not develop skeletal muscle paralysis or hypoxia, characteristic features of children with P209L mutations.

MUTATIONS IN BAG3 CAN LEAD TO A DILATED CARDIOMYOPATHY

In 2011, Norton et al. (48) first reported a large deletion in exon 4 of *BAG3* that segregated as an autosomal dominant mutation with affected members of a family with dilated cardiomyopathy. In contrast to

the subjects reported by Selcen et al. (39), Norton et al. (48) found no evidence of skeletal muscle weakness or neurological dysfunction in the family. Subsequent sequencing of the coding exons in 311 unrelated probands with familial dilated cardiomyopathy identified additional variants in *BAG3* that also segregated with affected family members. In the same year, Villard et al. (49) also identified a causative variant, albeit unique ones, in BAG3 in a genome-wide association study using DNA from 1,199 subjects with dilated cardiomyopathy and a similar number of control subjects. Further sequencing of DNA obtained from a large number of probands identified additional BAG3 variants. Since then, investigators have reported a large number of BAG3 mutations that were associated with development of a dilated cardiomyopathy (48-54) that, in aggregate, resulted in BAG3 being included on commercial panels that can

be used by physicians to screen patients with familial dilated cardiomyopathy. In fact, BAG3 mutations are a not- uncommon cause of dilated cardiomyopathy: between 2.3% and 15% of randomly selected probands harbored BAG3 variations, leading Franaszczyk et al. (53) to posit that "the BAG3 gene emerges as a major dilated cardiomyopathy locus."

We reported a 10-nucleotide deletion in *BAG3* that predicted truncation of 135 amino acids from the carboxyl-terminal end of the protein that segregated as an autosomal dominant with all affected family members (50). We obtained tissue from the left ventricular myocardium of 1 affected family member who underwent heart transplantation, and we found that the deletion resulted in haploinsufficiency because BAG3 protein levels were reduced by more than one-half in the affected heart compared with normal control subjects. This finding was confirmed by Toro et al. (51) in a large family with a novel frame-shift variant in BAG3. Interestingly, we also found that BAG3 levels were reduced in the hearts of patients with heart failure who did not harbor a mutation in BAG3, suggesting for the first time that BAG3 may also play a role in nonfamilial dilated cardiomyopathy. However, the molecular mechanisms that regulate BAG3 expression remain unknown.

A common feature in patients with BAG3-related familial disease is genetic heterogeneity, which can often confound scientific interpretation. For example, in the Spanish family reported by Toro et al. (51), 50 of 100 family members carried the mutation; however, only 35 of the 50 carriers were symptomatic. This phenomenon is important for cardiologists to recognize, as genetic testing in patients and family members with familial disease becomes more common because an individual harboring a BAG3 mutation who is asymptomatic may still pass the gene on to his or her children. In addition, because individuals carrying a BAG3 mutation can present in the fourth or fifth decade of life, the number of affected family members and the number of carriers may often not be in synch.

CAN NONSYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISMS IN BAG3 CAUSE DILATED CARDIOMYOPATHY?

The clinical observation of marked differences between the myofibrillar myopathy phenotype of individuals harboring the BAG3 P209L genetic variant and nonsynonymous single nucleotide polymorphisms that have been associated with dilated cardiomyopathies raises a common conundrum: how

can we determine the biological importance of coding region variants that change a single amino acid of BAG3? To date, determination of causality is based, in large part, on the fact that the mutation segregates with family members who have the phenotype (and not with family members who do not) and use of an amino acid scoring matrix designed to compare the evolutionary conservation of amino acid substitutions between related proteins. We would argue that as additional mutations are identified, it will become increasingly important to assess their biological effect. For example, when mutations that had been associated with the development of cardiac dilatation (Arg218Trp and Leu462Pro) were transfected into neonatal rat cardiomyocytes, there was impaired localization with α-actinin and desmin as well as enhanced localization within the nuclei; this targeting was not seen with wild-type BAG3 or with a BAG3 mutant that was associated with myofibrillar myopathy (Pro209Leu) (46). Similarly, when dilated cardiomyopathy-associated mutations (Arg218Met and Leu462Pro) were transfected into rat neonatal cardiomyocytes and exposed to serum starvation, apoptosis was seen in approximately 90% of cells, but apoptosis was not observed in cells that were transfected with either the P209L-BAG3 variant or a nonpathogenic control mutation (Arg258Met). More recently, Fang et al. (34) showed that when the Glu455Lys mutation found in patients with dilated cardiomyopathy was knocked-in to mice, there was a disruption of the interaction between BAG3 and Hsc70/Hsp70, suggesting the interaction is essential for BAG3 to stabilize the heat shock proteins and maintain cardiomyocyte protein homeostasis.

BAG3 MUTATIONS MAY PLAY A ROLE IN THE OCCURRENCE OF TAKOTSUBO CARDIOMYOPATHY

Takotsubo cardiomyopathy is characterized by transient and reversible myocardial stunning and left ventricular apical ballooning in the absence of obstructive coronary disease. It usually occurs in postmenopausal women and is triggered by emotional or physical stress (55). Citro et al. (56) reported finding a nonsynonymous single nucleotide polymorphism in *BAG3* in 2 patients with documented Takotsubo cardiomyopathy that was not found in 1,043 control subjects. Because the missense mutation is close to the IPV domain that is necessary for binding the small heat shock proteins, the investigators suggested that an absence in the protective effects of the small heat shock

proteins might play a role in the altered stress response seen in patients with Takotsubo cardiomyopathy.

In a subsequent study, the same group sequenced *BAG3* in 70 patients with Takotsubo cardiomyopathy and in 81 healthy control subjects (57). They found a mutation in the 3′-untranslated region of the BAG3 gene that resulted in a loss of binding of a specific micro-RNA. Because the micro-RNA (miR-37 1a-5p) is upregulated on exposure to epinephrine through an extracellular signal-regulated kinase–dependent pathway, the investigators proposed that the presence of the polymorphism could abrogate miR-37 binding and result in an altered response to epinephrine. These findings are intriguing but require confirmation in larger populations of patients with Takotsubo cardiomyopathy.

BAG3 PLAYS A ROLE IN HUMAN IMMUNODEFICIENCY VIRUS–ASSOCIATED CARDIOMYOPATHY

BAG3 levels increase in tissues infected with human immunodeficiency virus (HIV)-1 and appear to suppress HIV-1 gene expression (58,59). However, the relationship between the heart, BAG3, and HIV-1 derives from the interaction between BAG3 and the transactivator of transcription protein Tat. Tat is a protein that is transcribed from the HIV-1 virus and regulates the host cell's response to a viral infection. We hypothesized that when the heart is infected with HIV-1, Tat binds to and silences BAG3, resulting in increased apoptosis and decreased autophagy flux (60). This theory is supported by the finding that transgenic mice, in which Tat is overexpressed (Tg26), exhibit normal contractile function at baseline but are unable to tolerate stress (61). Furthermore, BAG3 knockdown results in cardiac dysfunction in Tg26 mice, whereas BAG3 overexpression rescued contractile abnormalities in myocytes overexpressing Tat.

A BAG3 VARIANT IS ASSOCIATED WITH CRITICAL LIMB ISCHEMIA IN MICE

Evidence suggests that BAG3 also plays an important role in the development of critical limb ischemia. In 2008, Annex et al. (62) identified a quantitative trait locus, Lsq-1, that was responsible for the differential response to surgical hindlimb ischemia in C57BL-6 mice and BALB/c mice. Although BALB/c mice quickly lose their limbs after surgical hindlimb ischemia, there was an absence of tissue loss in C57Bl-6 mice, leading investigators to suspect that differences

in the clinical course of critical limb ischemia were due to genetic determinants of susceptibility rather than simply a generalized manifestation of peripheral artery disease (62,63). Recent research has shown that BAG3 is found in the Lsq-1 locus and that variations in BAG3 at amino acid 81 (Ile81Met) can explain the phenotypic differences between the response to femoral artery occlusion in C57BL6 and BALB/c mice. In fact, the BAG3^{Ile81} genotype segregates with tissue protection from hindlimb ischemia in C57BL6 mice, and treating BALB/c mice that are BAG3^{Met81} with AAV9-BAG3^{Ile81} increased limb tissue perfusion and improved ischemic muscle myopathy and muscle precursor cell differentiation while also improving muscle regeneration (64). Consistent with this observation, AAV9-BAG3^{Ile81} but not AAV9-BAG3^{Met81} improved ischemic limb blood flow and limb muscle histology, and restored muscle function. In addition, AAV9-BAG3^{Ile81} infection improved binding to HSB8 in ischemic skeletal muscle cells and enhanced ischemic muscle autophagy flux. Thus, there is considerable synergism between the effects of BAG3 variants in the heart and the skeletal muscle.

BAG3 MUTATIONS ARE A RISK FACTOR FOR MYOCARDITIS

A question that has perplexed heart failure specialists for many years has been: Why is the incidence of acute myocarditis in children in the United States low (1 per 100,000 children) when the incidence of infections with viruses (e.g., Coxsackievirus) is very high? Belkaya et al. (65) tested the hypothesis that previously silent genetic risk factors might exist in children who develop acute myocarditis. They found that 7 of 42 children with acute myocarditis carried rare biallelic (homozygous or compound heterozygous) nonsynonymous or splice site variations in 6 cardiomyopathy-associated genes, including BAG3, which presumably put them at risk. Thus, the presence of a mutation in BAG3 might not alter the cardiac phenotype under normal circumstances but could place an individual at risk of developing left ventricular dysfunction after a stressful event.

CAN BAG3 SERVE AS A THERAPEUTIC TARGET IN CARDIAC AND SKELETAL MUSCLE DISEASE?

BAG3 levels are reduced in multiple animal models of heart failure, including post-transaortic constriction in mice, post-ligation of the left coronary artery in both mice and pigs, and, most importantly, in human failing heart (24,34). In addition, BAG3 levels are

reduced in the skeletal muscle of mice with femoral artery occlusion. The finding that BAG3 levels are significantly reduced in murine models of heart failure was confirmed by Fang et al. (34). A growing body of evidence suggests that replenishing normal levels of BAG3 can have salutary effects in these animal models. For example, the injection of rAAV9-BAG3 into mice with left ventricular dysfunction and diminished levels of BAG3 after a myocardial infarction significantly improved left ventricular function 3 weeks after injection but had no effect on sham-operated controls (66). Infection with rAAV9-BAG3 did not change markers of apoptosis or autophagy at 3 weeks, suggesting that the early effects of BAG3 evolve from improving excitation-contraction coupling, whereas the long-term effects are attributable to enhanced protein quality control (34,67). Similar benefits were seen when skeletal muscle in mice harboring a disadvantageous BAG3 genotype were injected with an AAV9 driving expression of the wild-type BAG3 (64). Taken together, these studies suggest that the restitution of normal levels of BAG3 in the context of BAG3 depletion may be a viable therapy for patients with disease that is attributable to diminished levels of BAG3.

Although BAG3 seems to be a rational therapeutic target in many forms of cardiac and skeletal muscle disease, several important questions remain. For example, we need to understand the long-term effects of BAG3 overexpression on the heart. Does chronic overexpression adversely influence the balance between autophagy and apoptosis or does the heart and skeletal muscle re-set to accommodate an increase in 1 of the 2 pathways? In the absence of BAG3 agonists, this question could be answered by generating transgenic mice with both controlled and inducible overexpression of BAG3.

Gene therapy using an AAV vector would seem to be an obvious means of increasing BAG3 levels in hearts or skeletal muscle with haploinsufficiency due to disease or a genetic mutation; however, important questions need to be addressed. First, as noted earlier, BAG3 facilitates adhesion, migration, and, in many cases, the survival of cancerous cells. Successful gene therapy, therefore, requires that BAG3 is only expressed in the heart or skeletal muscle. The possibility of off-target effects could be mitigated by the use of a cardiac-specific promoter; however, to date, cardiac-specific promoters have not been carefully evaluated for their degree of "leakiness." Second, it

will be important to identify genetic variants in which gene therapy might have a dominant-negative effect, suppressing expression of the wild-type allele and/or enhancing the expression of the allele containing the variant. A dominant-negative effect is most likely to be seen in the case of single nucleotide polymorphisms, and rapid high-throughput screening will be useful for identifying genotypes that raise this concern. CRISPR-Cas9 gene editing may provide an exciting opportunity to correct mutations in BAG3 in situ, which would obviate the potential concerns regarding dominant-negative effects with gene therapy.

Another therapeutic option is the development of small peptides that could provide the same biological benefit as the intact protein but could be delivered directly into the tissue or administered systemically if they prove to be non-oncogenic. Analysis of the specific roles played by each of the protein-protein binding domains of BAG3 may identify candidate regions that could then be evaluated in experimental animals for their ability to adequately reverse the effects of haploinsufficiency. The growing field of nanotechnology might provide an ideal method for delivering these small peptides to their targets.

Finally, the possibility that the genetic heterogeneity seen in individuals with BAG3 mutations is due to variable exposure to epigenetic factors, including viruses that cause stress, needs to be carefully evaluated. Would treating patients with BAG3 mutations and a normal phenotype with an α-adrenergic receptor antagonist mitigate the effects of stress? It is encouraging to see the enormous advances that have occurred in our understanding of this interesting protein in the relatively brief period of time since its role in the heart and skeletal muscle was first recognized. Hopefully, as BAG3 gains greater interest in the cardiac research community, we can address the fundamental questions that need to be answered to translate the research in the laboratory into therapeutic interventions for patients with heart and skeletal muscle disease.

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