Minireview *Highlight article*

MicroRNA regulation of BAG3

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Impact Statement

BAG3 is an important antiapoptotic molecule that plays a crucial role in development of cancer. The role of BAG3 in other disease pathology, including peripheral artery disease, is increasingly becoming apparent. Managing expression of BAG3 in diseased tissue is a potential means of intervention. Since microRNAs (miRs) are important therapeutic agents that can be delivered in a targeted manner, it is pertinent to identify and evaluate the miRs that regulate expression of BAG3 in various tissue and cell types. In this review, the authors have searched the literature for miRs that target BAG3 expression in tissue- and cell-specific manners and also have performed analyses of miR databases to identify potentially new miRs that may target BAG3 mRNA.

Abstract

B-cell lymphoma 2 (Bcl-2)-associated athanogene 3 (BAG3) protein is a member of BAG family of co-chaperones that modulates major biological processes, including apoptosis, autophagy, and development to promote cellular adaptive responses to stress stimuli. Although BAG3 is constitutively expressed in several cell types, its expression is also inducible and is regulated by microRNAs (miRNAs). miRNAs are small non-coding RNAs that mostly bind to the 3′-UTR (untranslated region) of mRNAs to inhibit their translation or to promote their degradation. miRNAs can potentially regulate over 50% of the protein-coding genes in a cell and therefore are involved in the regulation of all major functions, including cell differentiation, growth, proliferation, apoptosis, and autophagy. Dysregulation of miRNA expression is associated with pathogenesis of numerous diseases, including peripheral artery disease (PAD). BAG3 plays a critical role in regulating the response of skeletal muscle cells to ischemia by its ability to regulate autophagy. However, the biological role of miRNAs in the regulation of BAG3 in biological processes has only been elucidated recently. In this review, we discuss how miRNA may play a key role in regulating BAG3 expression under normal and pathological conditions.

Keywords: MicroRNA, BAG3, apoptosis, autophagy, gene regulation, skeletal muscle, peripheral artery disease

Experimental Biology and Medicine **2022; 247: 617–623. DOI: 10.1177/15353702211066908**

MicroRNAs and BAG3 at a glance

B-cell lymphoma 2 (Bcl-2)-associated athanogene 3 (BAG3) protein is a member of BAG family of co-chaperones that modulates major biological processes, including apoptosis, autophagy, and development to promote cellular adaptive responses to stress stimuli.[1,2] BAG family derives the name from the BAG protein domain that all six members (BAG1-6) of the family share. BAG1 was the first member of the family initially identified as a Bcl-2-interacting antiapoptotic protein.[3] Later, other members of the family were identified.[4] These proteins participate in diverse cellular functions owing to the interactions with many different proteins through its multiple domains. BAG3 plays important roles in cancer cell survival and proliferation, neuronal degeneration, cardiomyopathy, and viral propagation. At the cellular and biochemical levels, BAG3 functions in signal transduction,[5] ciliogenesis,[6] organization of the contractile protein apparatus in cardiomyocytes,[7] inhibition of HSP70-mediated trafficking of proteins for proteasomal degradation,[8] mRNA stability,[9] and promoting autophagy[1] (Figure 1). There are excellent reviews that discuss the varied functions of BAG3 protein.[10–13] Various cancer cells have increased expression of BAG3 that is associated with increased cell proliferation and decreased apoptosis by interacting with several cell cycle–related proteins, stabilization of mRNA, and regulates expression of microRNAs. BAG3 associates with several proteins involved in striated myocyte function ranging from Ca^{2+} channels, gap junctions, signaling receptors to proteins that are required for structural integrity of the myofibrils. In neurons, BAG3 facilitates proteasomal clearance of stress-induced protein aggregates that may lead to neuronal damage in diseases like Alzheimer's, amyotrophic lateral sclerosis (ALS), Huntington's, and Parkinson's diseases.

Human BAG3 is a 575 amino acid long protein (mouse BAG3 protein is 577 amino acids long) that has an N-terminal WW domain and a C-terminal BAG domain (Figure 2). The WW domain in coordination with the PXXP domain allows interaction of BAG3 protein with SH3 (Src homology 3) domain–containing proteins, such as phospholipase C gamma and motor protein dynein, to regulate cell adhesion

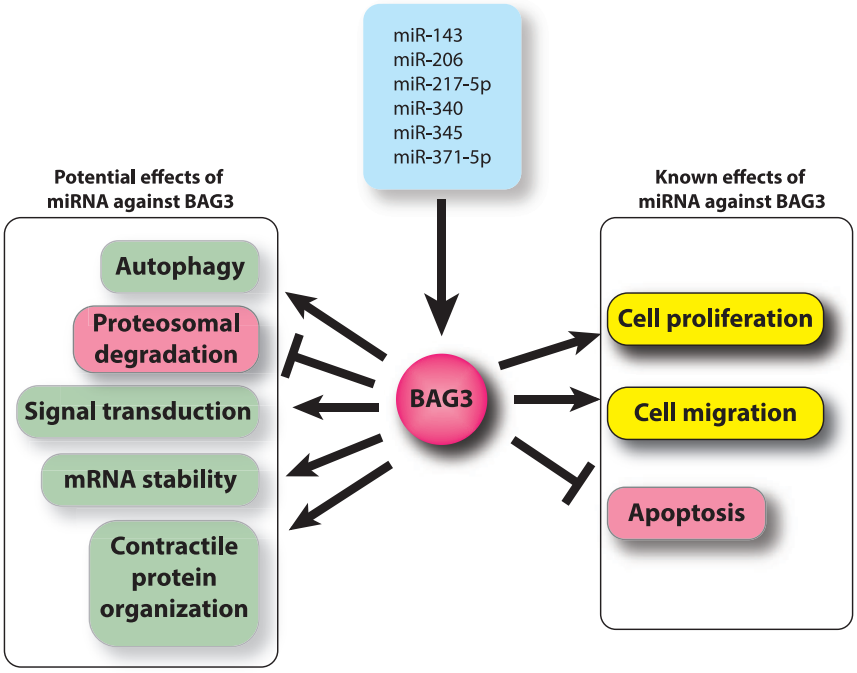

Figure 1. Known and potential biological pathways regulated by BAG3-targeting miRNA. The known miRNA-mediated regulation of BAG3 has been studied primarily in context of cell proliferation, apoptosis, and cell migration in cancer cells. BAG3 has potential roles in pathologies of other tissues that remain unexplored. (A color version of this figure is available in the online journal.)

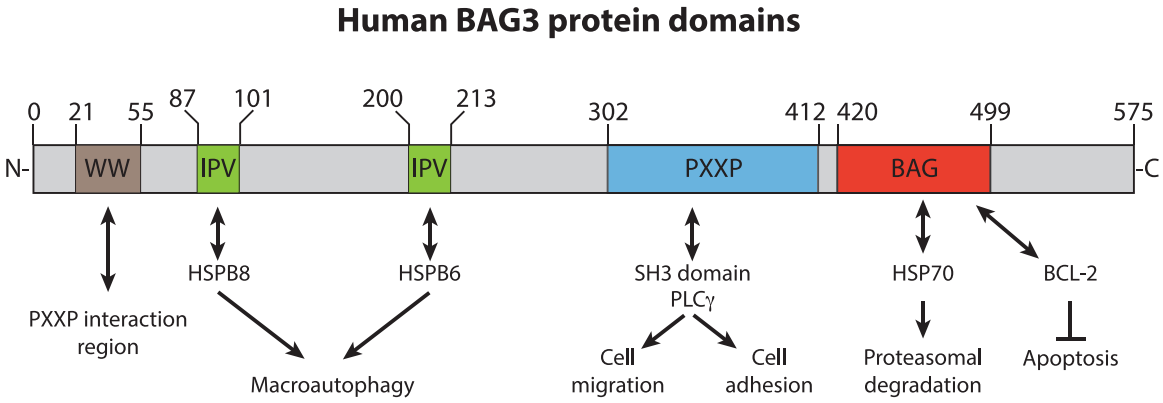

Figure 2. The schematic representation of BAG3 protein and its interaction sites. B-cell lymphoma 2 (Bcl-2)-associated athanogene 3 (BAG3) protein is a 575 amino acid long protein that has an N-terminal WW domain that allows interactions with SH3 (Src homology 3) domain–containing proteins such as phospholipase C gamma and motor protein dynein to regulate cell adhesion and migration. The BAG domain interacts with BCL2 to facilitate antiapoptotic processes in the cells. In addition, it also interacts with the heat shock protein (HSP70) to regulate its proteasome degradation. An IPV (Ile-Pro-Val) motif interacts with HSPB6 and/or HSPB8. HSPB8 is responsible for recognizing the misfolded proteins, whereas BAG3 recruits and activates the chaperone-assisted macroautophagy. TargetScan, miRDB, and miRWalk algorithms were queried and compared for miRNAs that could target BAG3. TargetScan resulted in 118 entries, miRDB predicted 23 miRNAs, and miRWalk listed 1611 miRNAs. Ten miRNAs were present when comparing all bioinformatic scans together. (A color version of this figure is available in the online journal.)

and migration, respectively.[13] The BAG domain interacts with BCL2 to facilitate antiapoptotic processes in the cells. In addition, it also interacts with the heat shock protein (HSP70) as a co-chaperone to deliver proteins for proteasomal degradation.[13] Two IPV (Ile-Pro-Val) motifs interact with HSPB6 alone or HSPB6 and HSPB8. HspB8 is responsible for recognizing the misfolded proteins, whereas BAG3 recruits and activates chaperone-assisted macroautophagy, a process of removal of damaged organelles and proteins to maintain cellular homeostasis.[1] Thus, BAG3 is involved in a multifaceted interaction with other proteins to modulate cellular events (Figure 2).

BAG3 is constitutively expressed in several cell types, including cancer cells, cardiomyocytes, and skeletal muscle cells. Moreover, BAG3 is the only member of the BAG family of proteins that is induced by stress signals.[13] BAG3 plays a critical role in regulating the response of skeletal muscle cells to ischemia by autophagy.[2] Indeed, BAG3 is involved in ischemia- induced autophagy in a murine hindlimb ischemia model of peripheral artery disease (PAD, also Dhanabalan and Dokun, unpublished results). PAD is characterized by atherosclerotic blockage of blood flow to tissues of the lower extremities, including skeletal muscles. Thus, cell survival under many stress conditions

Table 1. Known miRNA that regulates BAG3 in human cells.

miRNA	Cell/tissue	Species	Method	Effect on BAG3 expression	Mechanism of miR action	Reference
miR-143	Glioblastoma	Human	Knockdown by miR transfection	Decreased BAG3	—	PMID: 26541455
miR-206	Cervical cancer cells	Human	Knockdown by miR transfection	Decreased BAG3	Binding to the 3′-UTR	PMID: 29295729
miR-217-5p	Colorectal cancer cells	Human	Knockdown by miR transfection	Decreased BAG3	Binding to the 3′-UTR	PMID: 28905214
miR-340	Ovarian cancer cells	Human	Knockdown by miR transfection	Decreased BAG3	—	PMID: 29441908
miR-345	Colorectal cancer cells	Human	Knockdown by miR transfection	Decreased BAG3	Binding to the 3′-UTR	PMID: 21665895
miR-371-5p	Cardiomyocytes	Human	Co-immunoprecipitation	Increased BAG3	Binding to the 3′-UTR	PMID: 26512958

may depend on regulation of BAG3 expression. The expression of BAG3 is regulated both at the transcription and post-transcription levels, including by miRNAs.[14,15] In the following sections, we discuss how miRNAs may play a key role in regulating BAG3 expression under pathological conditions.

miRNA regulation of BAG3

miRNAs are small non-coding RNA (20–25 nucleotides) that binds to the 3′-UTR (untranslated region) of mRNA to inhibit their translation or promote their degradation.[16] However, miRNAs may also bind to other regions of mRNAs.[17] miRNAs can potentially regulate expression of more than 50% of protein-coding genes and, therefore, are involved in regulation of all major cellular processes, including cell differentiation, growth, proliferation, migration, apoptosis, and autophagy.[18,19] Genomic sequences of miRNAs are either embedded in the introns of a host gene or may be encoded by independent genes. Thus, their expression may depend on transcription of the host genes or may be independently regulated. Dysregulation of miRNA expression results in the pathogenesis of numerous diseases, including peripheral artery disease (PAD).[20,21]

Although a large number of studies have been done on identifying miRNA and their targets, relatively few miRNAs have been shown to regulate BAG3 expression in cancer,[22,23] apoptosis,[22,24,25] and cardiomyopathy[26] (Table 1 and Figure 1). All these studies have been performed on human primary cells, cell lines, or tissue. Moreover, not all miRNAs have been shown to directly regulate BAG3 expression. Based on the predictive algorithms, three major miRNA databases miRWalk, TargetScan, and miRDB report 116, 158, and 39 miRNA that may potentially target mouse *Bag3* mRNA.[27–29] Of these predicted miRNAs, only one miRNA (hsa-miR-221) was common to all three algorithms (Table 2). Interestingly, despite being a valuable preclinical model for numerous human diseases, studies on miRNA-mediated regulation of BAG3 in mice are lacking. Similar analysis as for human miRNA above yielded 14 potential mouse miRNAs by all three algorithms. A comparison of the predicted BAG3-targeting miRNAs in human and mice identified 37 miRNAs that are common to both human and mouse. Of these miRNA, miR-217 is a predicted miRNA in humans and mouse that has been associated with BAG3 regulation.[24] More studies are needed to identify miRNAs that directly bind to regulate BAG3 expression in different tissues to understand the mechanism of BAG3 gene regulation and for potential therapeutic use of miRNAs in BAG3-mediated diseases.

Regulation of BAG3 in muscle development

BAG3 plays a critical role in muscle development.[30,31] A number of miRNAs are specifically expressed in striated muscles and are called myomiRs; typically, they control myogenic precursor fate and muscle tissue homeostasis.[32] A myomiR, miR-206, has predicted target sequence in the 3′-UTR of the BAG3 mRNA. Overexpression of miR-206 downregulates BAG3 expression.[23] The expression of miR-206 in muscles is developmentally regulated.[33,34] In addition, the expression of miR-206 is upregulated in the diaphragm of dystrophin-deficient (mdx) mice, a model of muscular dystrophy.[35] Interestingly, experimental downregulation of miR-206 improves motor function in these mdx mice.[36] Another study demonstrated that miR-206 is upregulated in the skeletal muscle of mdx mice and also upon injection of cardiotoxin, a potent inducer of muscle injury with subsequent adaptive muscle regeneration.[37] Although these studies did not investigate the expression of BAG3, it is likely that miR-206 effects are mediated through BAG3 regulation. Another miRNA, miR-29, is essential for normal myoblast development since the loss of miR-29 in myoblasts contributes to muscle dystrophy.[38,39] miR-29 regulates myoblast differentiation through a positive feed-forward mechanism involving genetic interactions between the transcription factors NF-κB and YY1. In PAD, miR-29a modulation improves perfusion recovery,[20] whereas BAG3 expression regulates miR-29b exprssion.[40] Collectively, these studies demonstrate that the expression patterns of BAG3-targetting miRNAs are distinctly altered during various types of cardiac and skeletal muscle disease, and that the manipulation of disease-associated miRNAs represents a potentially powerful therapeutic approach to treat muscle disease. However, despite the demonstrated roles of these miRNAs in regulation of BAG3 expression, a direct role of these miRNAs and several other miRNAs in regulation BAG3 mRNA has not been shown.

Table 2. Predicted human miRNA regulators of BAG3 by three algorithms. TargetScan, miRDB, and miRWalk algorithms were queried and compared for miRNAs that could target human BAG3. TargetScan resulted in 158 entries, miRDB predicted 39 miRNAs, and miRWalk listed 116 miRNAs. One miRNAs (has-miR-221) was present in all three predictions.

Database	Predicted miRNA
TargetScan	hsa-miR-6769b-5p hsa-miR-3938 hsa-miR-8081 hsa-miR-5197-3p hsa-miR-3921 hsa-miR-298 hsa-miR-8055 hsa-miR-509-3-5p hsa-miR-656-3p hsa-miR-3192-3p hsa-miR-6751-5p hsa-miR-548ae-3p hsa-miR-4255 hsa-miR-889-5p hsa-miR-6842-5p hsa-miR-520f-5p hsa-miR-4712-3p hsa-miR-6835-5p hsa-miR-6778-3p hsa-miR-548aj-3p hsa-miR-6769a-5p hsa-miR-4524b-3p hsa-miR-555 hsa-miR-4795-3p hsa-miR-5007-3p hsa-miR-6807-3p hsa-miR-6803-5p hsa-miR-129-1-3p hsa-miR-1279 hsa-miR-4664-5p hsa-miR-6791-3p hsa-miR-135a-5p hsa-miR-4509 hsa-miR-6817-3p hsa-miR-142-3p.2 hsa-miR-6779-3p hsa-miR-411-5p.1 hsa-miR-371b-5p hsa-miR-654-5p hsa-miR-5195-5p hsa-miR-4653-5p hsa-miR-4501 hsa-miR-4797-5p hsa-miR-1283 hsa-miR-4282 hsa-miR-548aa hsa-miR-5689 hsa-miR-337-3p hsa-miR-4774-3p hsa-miR-146a-3p hsa-miR-548z hsa-miR-143-3p hsa-miR-135b-5p hsa-miR-4744 hsa-miR-548j-3p hsa-miR-3925-3p hsa-miR-6507-3p hsa-miR-1250-3p hsa-miR-6887-3p hsa-miR-520a-5p hsa-let-7a-2-3p hsa-miR-196a-3p hsa-miR-5197-5p hsa-miR-29a-5p hsa-miR-7110-3p hsa-miR-3662 hsa-miR-4447 hsa-miR-6752-5p hsa-miR-548d-3p hsa-miR-198 hsa-miR-4770 hsa-miR-1179 hsa-miR-646 hsa-miR-19b-1-5p hsa-miR-525-5p hsa-miR-541-3p hsa-miR-514b-5p hsa-miR-4424 hsa-miR-6835-3p hsa-miR-126-5p hsa-miR-148a-3p hsa-miR-6883-3p hsa-miR-548bb-3p hsa-miR-6507-5p hsa-miR-6873-3p hsa-miR-4766-5p hsa-miR-3591-5p hsa-miR-556-3p hsa-miR-373-5p hsa-miR-202-5p hsa-miR-4762-3p hsa-miR-548h-3p hsa-miR-129-2-3p hsa-miR-548am-3p hsa-miR-548aq-3p hsa-miR-371a-5p hsa-miR-548e-5p hsa-miR-301b-5p hsa-miR-3920 hsa-miR-589-3p hsa-miR-153-5p hsa-miR-548t-3p hsa-miR-593-3p hsa-miR-509-5p hsa-miR-944 hsa-miR-3184-3p hsa-miR-7110-5p hsa-miR-33a-3p hsa-miR-548ac hsa-miR-4418 hsa-miR-3162-3p hsa-miR-6836-3p hsa-miR-203a-3p.1 hsa-miR-455-3p.1 hsa-miR-5683 hsa-miR-493-5p hsa-miR-19b-2-5p hsa-miR-221-5p hsa-miR-4678 hsa-miR-548ah-3p hsa-miR-2052 hsa-miR-6832-3p hsa-miR-301a-5p hsa-miR-4422 hsa-miR-548x-3p hsa-miR-6070 hsa-miR-616-5p hsa-miR-6829-5p hsa-miR-513c-5p hsa-miR-548ap-3p hsa-miR-6829-3p hsa-miR-3158-5p hsa-miR-3529-3p hsa-miR-6758-3p hsa-miR-6088 hsa-miR-4446-3p hsa-miR-148b-3p hsa-miR-140-3p.2 hsa-miR-4708-5p hsa-miR-217 hsa-miR-5697 hsa-miR-552-3p hsa-let-7c-3p hsa-miR-4472 hsa-miR-891b hsa-miR-342-5p hsa-miR-187-5p hsa-let-7g-3p hsa-miR-5700 hsa-miR-19a-5p hsa-miR-372-5p hsa-miR-3152-5p hsa-miR-3185 hsa-miR-92a-2-5p hsa-miR-152-3p hsa-miR-8073 hsa-miR-1273f hsa-miR-4687-5p
miRWalk	hsa-miR-6516-5p hsa-miR-376a-5p hsa-miR-6883-3p hsa-miR-6501-3p hsa-miR-7108-3p hsa-miR-4305 hsa-miR-4743-3p hsa-miR-6873-3p hsa-miR-4433a-3p hsa-miR-8054 hsa-miR-6851-3p hsa-miR-5587-3p hsa-miR-5193 hsa-miR-761 hsa-miR-149-5p hsa-miR-6736-3p hsa-miR-4638-5p hsa-miR-1910-3p hsa-miR-3689b-5p hsa-miR-6771-3p hsa-miR-3918 hsa-miR-4671-5p hsa-miR-3667-3p hsa-miR-4999-5p hsa-miR-6742-3p hsa-miR-4724-3p hsa-miR-4700-5p hsa-miR-3127-5p hsa-miR-5001-3p hsa-miR-4524b-3p hsa-miR-4438 hsa-miR-183-5p hsa-miR-4793-5p hsa-miR-6867-3p hsa-miR-7109-3p hsa-miR-3609 hsa-miR-3184-3p hsa-miR-6515-5p hsa-miR-6871-3p hsa-miR-1251-3p hsa-miR-585-5p hsa-miR-6820-3p hsa-miR-449b-3p hsa-miR-221-5p hsa-miR-4675 hsa-miR-3692-3p hsa-miR-23b-5p hsa-miR-4701-5p hsa-miR-6780b-3p hsa-miR-573 hsa-miR-2681-5p hsa-miR-132-3p hsa-miR-1238-3p hsa-miR-4695-3p hsa-miR-216a-5p hsa-miR-3160-5p hsa-miR-450a-2-3p hsa-miR-1236-5p hsa-miR-5196-3p hsa-miR-9902 hsa-miR-4666b hsa-miR-200b-5p hsa-miR-18b-3p hsa-miR-6511b-3p hsa-miR-5047 hsa-miR-4659b-3p hsa-miR-96-5p hsa-miR-4448 hsa-miR-492 hsa-miR-6729-3p hsa-miR-3689a-5p hsa-miR-4769-5p hsa-miR-4720-5p hsa-miR-6758-3p hsa-miR-1237-3p hsa-miR-6782-5p hsa-miR-449c-5p hsa-miR-4685-3p hsa-miR-6864-3p hsa-miR-5004-5p hsa-miR-6760-3p hsa-miR-9903 hsa-miR-6870-3p hsa-miR-552-3p hsa-miR-660-5p hsa-miR-6790-3p hsa-miR-3972 hsa-miR-378e hsa-miR-483-3p hsa-miR-548at-5p hsa-miR-6891-3p hsa-miR-345-5p hsa-miR-4659a-3p hsa-miR-6894-3p hsa-miR-6841-3p hsa-miR-604 hsa-miR-6505-3p hsa-miR-28-3p hsa-miR-7111-3p hsa-miR-4749-3p hsa-miR-5002-5p hsa-miR-4715-5p hsa-miR-6834-3p hsa-miR-6759-3p hsa-miR-493-3p hsa-miR-3689e hsa-miR-6789-3p hsa-miR-664b-3p hsa-miR-744-5p hsa-miR-532-3p hsa-miR-1281 hsa-miR-32-5p hsa-miR-6125 hsa-miR-4697-3p hsa-miR-1256 hsa-miR-4687-5p
miRDB	hsa-miR-126-5p hsa-miR-6832-3p hsa-miR-548bb-3p hsa-miR-6507-5p hsa-miR-656-3p hsa-miR-4762-3p hsa-miR-548h-3p hsa-miR-4797-5p hsa-miR-129-2-3p hsa-miR-196a-1-3p hsa-miR-371a-5p hsa-miR-520f-5p hsa-miR-12132 hsa-miR-548z hsa-miR-6088 hsa-miR-146a-3p hsa-miR-143-3p hsa-miR-6507-3p hsa-miR-593-3p hsa-miR-1250-3p hsa-miR-944 hsa-let-7a-2-3p hsa-miR-196a-3p hsa-miR-548ac hsa-miR-4795-3p hsa-miR-29a-5p hsa-miR-891b hsa-miR-217-5p hsa-miR-7110-3p hsa-let-7g-3p hsa-miR-6807-3p hsa-miR-548d-3p hsa-miR-129-1-3p hsa-miR-4770 hsa-miR-1279 hsa-miR-221-5p hsa-miR-646 hsa-miR-8073 hsa-miR-6835-3p
Common	hsa-miR-221-5p

miRNAs regulation of BAG3 in cancer and apoptosis

The major understanding of the role of BAG3 protein has been obtained from studies on apoptosis and cancer. BAG3 is a potent antiapoptotic protein.[1,10,41] Apoptosis is a natural process of programmed cell death in metazoans that plays an important role during tissue and organ development and aging. Impairment in apoptosis or increased antiapoptotic proteins may lead to cancer progression.[42] As an antiapoptotic protein, increased BAG3 expression has been shown to be involved in acute and chronic leukemias.[43] In addition, BAG3 can retain BAX (BCL2-like protein 4) in cytosol and prevent mitochondrial translocation, thereby protecting the cells from apoptosis.[44] Several miRNAs have been shown to affect tumor growth and metastasis by regulating BAG3 expression. For example, miR-340 is well known to be a proapoptotic and antimetastasis miRNA in several type of cancers.[22] This study showed that miR-340 negatively regulates BAG3 and inactivates PI3K/AKT pathway.[22] Defects in PI3K/AKT signaling pathway increase the release of cytochrome c and caspase activity, thereby increasing cellular apoptosis.[45] In addition, miR-340-mediated negative regulation of BAG3 exerted a tumor-suppressive role in an ovarian cancer cell model, SKOV3 cells, by inhibiting cell viability and improved apoptosis.[22] miR-21-5p increased S-phase kinase-associated protein (Skp2) expression via BAG3, thereby facilitating the proliferation of cancer cells.[46] The pro-apoptotic role of miR-217-5p also has been studied in carcinogenesis of various types of cancer, including colorectal cancer, and its apoptotic activity has been linked to its negative effects on BAG3 expression. miR-217-5p-mediated BAG3 downregulation stimulates BAX translocation into mitochondria, thereby promoting cellular apoptosis.[24]

miR-143 is another tumor suppressor miRNA whose overexpression significantly increased apoptosis through inhibition of BAG3 expression. BAG3 overexpression reversed the miR-143-mediated cytotoxic activity of shikonin (natural naphthoquinone) in glioblastoma stem cells (GSCs) suggesting that the miR-143 tumor-suppressive (or pro-apoptotic) effects could be through the negative regulation of BAG3.[47] miR-345 targets the 3′-UTR of BAG3 and likely plays a role in pancreatic cancer as enhanced expression of BAG3 suppressed the apoptosis effects of miR-345 in pancreatic cancer cells.[48] miR-206 has been shown to affect BAG3 by targeting its 3′-UTR.[23] Interestingly, deletion of BAG3 increased miR-206-mediated inhibition of the cell cycle. Moreover, tumor engraftment studies in athymic nude mice showed that miR-206 injection reduced tumor growth in a dose-dependent manner by targeting BAG3.[23] Interestingly, downregulation of BAG3 increases miR-29b expression to promote cellular apoptosis by decreasing the activity of Mcl-1 (induced myeloid leukemia cell differentiation protein), an antiapoptotic BCL2 family member.[40] It is evident from the above discussion that dysregulation in miRNA expression has been associated with cancer progression, but their direct interaction with BAG3 has not been confirmed.

miRNAs regulation of BAG3 in cardiomyopathy. BAG3 supports cell survival and reduces apoptosis induced by stressors through its BAG-domain-mediated interactions with BCL2.[49] Under physiological conditions, BAG3 is expressed in cardiomyocytes to maintain cellular homeostasis by autophagy.[50] A variety of stressors such as heat and ischemia can induce BAG3 expression.[2,51–53] Moreover, deletion of BAG3 results in reduced myogenin expression, a protein involved in myogenesis, thus suggesting a critical role for BAG3 in differentiating myocytes.[54] Several BAG3 mutations are associated with cardiomyopathy.[55] A 3′-UTR mutation of BAG3 mRNA is reported in Takotsubo cardiomyopathy that results in loss of binding for miRNA-371a-5p.[26] Interestingly, in this case, the binding of miR-371a-5p to 3′-UTR enhances BAG3 expression through increased translation of the protein.[56,57]

miRNAs regulation of BAG3 in skeletal muscle

Despite recent research related to the role of miRNA regulation on BAG3 expression in cancer and cardiomyopathy, little is known about miRNA regulation of BAG3 in ischemic skeletal muscle. A link between the regulation of BAG3 by miRNAs and ischemic muscle injury has not been studied. Since the loss of BAG3 expression in the experimental models of hind limb ischemia in mice results in poor perfusion recovery and increased skeletal muscle injury,[2] understanding the regulatory effect of miRNAs on BAG3 expression could provide insight into mechanisms to improve tissue regeneration and blood flow following skeletal muscle ischemic injury.

Although the myomiRs miR-217-5p and miR-143 affect BAG3 expression to regulate apoptosis in cancer cells[24,47] and in muscle cells, miR-217 inhibits the transforming growth factor (TGF)-β1-induced proliferation and extracellular matrix (ECM) deposition, thereby promoting apoptosis.[47] It is not known whether miR-217 regulates BAG3 in ischemic skeletal muscle cells. Moreover, miR-143 is highly expressed in skeletal muscle tissue.[58] Downregulation of miR-143 caused cell cycle block and restrained cell proliferation.[59] Given these findings, it is possible that these effects of miR-143 may occur through regulation of BAG3 expression in skeletal muscle. Interestingly, BAG3 is also a predicted target of miR-29. *In vivo* and *in vitro* studies have shown that miR-29 induces skeletal muscle atrophy in response to atrophic stimuli and inhibition of miR-29 alleviates denervation and immobilization-induced atrophy.[60] It is possible that the protective effects seen following miR-29 inhibition occurs through modulation of BAG3. However, there are no studies directly linking miR-29 or any other miRNA to BAG3 regulation in skeletal muscles. Similarly, the role of miRNA regulation of BAG3 in other cell types within the mouse or human limb has not been studied. Moreover, in addition to the known functions of miR-217, miR-143, and miR-29 in BAG3 regulation, their role in the skeletal muscle injury may provide valuable insight into the mechanism of miRNA regulation of tissue injury. In addition, whether these miRNAs regulate BAG3 through direct or indirect interaction could provide additional understanding that may be invaluable in designing therapy for diseases involving skeletal muscle injury. Given the known protective effects of BAG3 following skeletal muscle injury, a systematic study to examine the role of miRNAs as therapeutic agents in regulation of BAG3 in this context would be highly valuable.

Therapeutic potential of miRNAs regulation of BAG3 expression

A role of miRNAs as therapeutic agents has been contemplated. For example, the use of miRNAs to target tumor suppressor mRNAs encoding oncoproteins can be highly effective. However, the therapeutic potential of miRNAs largely depends on their stability in the body and an optimal delivery method.[61] Despite being a prominent therapeutic target for cancer and cardiomyopathy, the ubiquitous expression of BAG3 has posed the challenge of site-specific targeting of BAG3 regulation in the body.[62] Although targeted regulation of BAG3 expression by miRNAs could be highly effective against cancers, the delivery of miRNA in a specific manner is a well-known challenge. However, with the advent of new vehicles for miRNA delivery, the goal of using miRNAs as specific and effective therapeutic agent in multiple diseases, including those related to BAG3, seems to be attainable.

AUTHORS' CONTRIBUTIONS

AOD contributed to conceptualization, project administration, and funding acquisition; MVS, KD, and JV contributed to literature search and analysis; KD, JV, and AOD contributed to writing—original draft preparation; and MVS and AOD contributed to writing—revision, review, and editing. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

We thank Thomas Wong for technical assistant on this manuscript preparation.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by National Heart, Lung, and Blood Institute (R01 HL130399) to AOD.

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