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The Role of BAG3 in Health and Disease: A "Magic BAG of Tricks"

Heng Lin, **Shon A. Koren**, **Gregor Cvetojevic**, **Peter Girardi**, **Gail V.W. Johnson***

Department of Anesthesiology and Perioperative Medicine, University of Rochester Medical Center, Rochester NY 14642 USA

Abstract

The multi-domain structure of BAG3 facilitates its interaction with many different proteins that participate in regulating a variety of biological pathways. After revisiting the BAG3 literature published over the past ten years with Citespace software, we classified the BAG3 research into several clusters, including cancer, cardiomyopathy, neurodegeneration and viral propagation. We then highlighted recent key findings in each cluster. To gain greater insight into the roles of BAG3, we analyzed 5 different published mass spectrometry data sets of proteins that co-immunoprecipitate with BAG3. These data gave us insight into universal, as well as cell-type specific BAG3 interactors in cancer cells, cardiomyocytes, and neurons. Finally, we mapped variable BAG3 SNPs and also mutation data from previous publications to further explore the link between the domains and function of BAG3. We believe this review will provide a better understanding of BAG3 and direct future studies towards understanding BAG3 function in physiological and pathological conditions.

Keywords

BAG3; interactome; cancer; myopathy; neurodegeneration; viral replication

Introduction:

In 1999, Bcl-2-associated athanogene 3 (BAG3) was first identified as a member of a family of BAG-1-related proteins. These proteins all have a conserved region near their C termini (the BAG domain) that binds the ATPase domain of heat shock protein 70 (Hsp/Hsc70) and regulates its chaperone activity (Takayama, Xie, & Reed, 1999). In the same year, another group of researchers identified a protein they named Bis (alias BAG3) that interacted with Bcl-2 to synergistically inhibit apoptosis (J. H. Lee et al., 1999). In the following year, BAG3 also got another alias, CAI stressed-1 (CAIR-1) as it is upregulated in response to

*corresponding author: Gail V.W. Johnson, PhD, Professor, Department of Anesthesiology and Perioperative Medicine, University of Rochester, 601 Elmwood Ave, Box 604 (room 4-6314 for deliveries), Rochester, NY 14642, gail_johnsonvoll@urmc.rochester.edu, +1-585-276-3740 (voice).

Contributions

HL and GVWJ conceived and organized the structure of the review, HL wrote the majority of the review and made the figures, SAK assisted with the bioinformatic analyses, and GC and PG wrote sections. All authors contributed to the final editing of the review.

Conflict of interest

The authors declare that there is no conflict of interest.

stress conditions (Doong et al., 2000). This brief history of the initial studies on BAG3 indicates that the diverse functions of BAG3 were evident from the very beginning. In the following 20+ years, over 400 research papers have been published, which have greatly expanded our knowledge about BAG3, although there is still much to be learned.

Human BAG3 is 575 amino acids in length and has a molecular mass of 74 kDa. Besides the BAG domain located at the C terminus, BAG3 also contains a tryptophan-tryptophan (WW) domain at the N-terminus, two conserved Ile-Pro-Val (IPV) domains, a proline-rich region (PXXP), and two phosphoserine-containing 14-3-3 binding motifs (Sturner & Behl, 2017). This multi-domain structure of BAG3 facilitates its interaction with many proteins and regulation of many biological pathways. Perhaps the best studied role of BAG3 is that of a co-chaperone acting to monitor cellular protein quality control through its BAG domain association with Hsp/Hsc70 (Doong et al., 2000). BAG3 also associates with many small heat shock proteins such as HspB8, HspB6, HspB5, and HspB1 through its IPV domains, which further enables BAG3 maintenance of proteostasis (Carra, Seguin, & Landry, 2008; Fuchs et al., 2009; Rauch et al., 2017). However, as we will discuss, the BAG3 interactome contains conserved and cell-specific partners that facilitate the involvement of BAG3 in a variety of cellular pathways beyond its co-chaperone activity.

BAG3 is expressed in most cell types and tissues, but to date most of the research on BAG3 has been driven by clinical associations and focused on numerous cancer cells (Kogel, Linder, Brunschweiger, Chines, & Behl, 2020), skeletal muscle, cardiomyocytes (Doong et al., 2000; Homma et al., 2006), and nervous system studies (Santoro et al., 2017), with few over-lapping studies. Inter-publication citation analysis (CiteSpace) of the 347 research articles about BAG3 in the past decade revealed that the studies are mainly distributed into 5 major clusters: cancer, cardiomyopathy, actin dynamics, protein aggregation (involving neurodegeneration), and viral production (Figure 1). This analysis depicts the relatively isolated clusters of BAG3 research, with the largest being cancer and cardiomyopathy related studies. As BAG3 mutations mostly involve cardiomyopathy (discussed later), the BAG3 mutation cluster stems from the cardiomyopathy cluster with little inter-report citation with the other clusters. The cluster of neurodegenerative disease research, as a relatively new sub-field on BAG3, appears to be in the initial stages of forming a new cluster among actin dynamics and protein aggregation research. As BAG3 has many putative interacting partners and functions across numerous cell types, considering inter-field studies and hypotheses may be beneficial in investigating BAG3-mediated cellular function.

BAG3 interactome

The multi-domain structure of BAG3 aids in an extensive interactome and participation in a wide variety of cellular functions. Beyond the well-established Hsp70/Hsc70 and chaperone binding activity of BAG3, many other discoveries have suggested that BAG3 coordinates vital cellular functions. BAG3 reportedly regulates protein synthesis, metabolism, and nutrient sensing by associating with regulators of the mTOR axis (Kathage et al., 2017), cell proliferation and apoptosis (via PLCγ1 (Doong et al., 2000)), development- and disease-associated kinases (such as Src (Colvin et al., 2014)), cytoskeletal active transport (via

dynein subunits (Gamerdinger, Kaya, Wolfrum, Clement, & Behl, 2011)), GTPase activity (via RAPGEF2 (Iwasaki et al., 2010)), signal transduction (via 14-3-3γ (Xu et al., 2013)), dendritic spine morphology and plasticity (via synaptopodin (Ji, Tang, Zeidler, Hohfeld, & Johnson, 2019)) and viral particle recognition (Gout, Gutkowska, Takayama, Reed, & Chroboczek, 2010), among others. These validated interaction partners suggest that BAG3 participates in central and cell-type specific cellular pathways, such as synaptic plasticity, but few studies have compared the BAG3 interactome across cell types.

Several higher-throughput experiments using co-immunoprecipitation coupled mass spectrometry (IP-MS) have identified hundreds of interaction partners with BAG3. A meta-analysis of five separate IP-MS reports identified common proteins associated with BAG3 in cancer cell lines (HeLa (Y. Chen et al., 2013), MCF7 (Pasillas et al., 2015)), induced pluripotent stem cell cardiomyocytes (Judge et al., 2017), neurons (Zhou et al., 2020), and human embryonic kidney cells (HEK293T (Hiebel et al., 2020)). Since reports varied in experimental methods, BAG3 tag and immunoprecipitation protocols, controls, and statistical analyses, the same cutoffs for positive association hits were used as reported in the original papers. However, the p value cutoff between a protein's intensity in control IP (e.g. IgG) to BAG3 IP was raised to 0.10 to match the highest p- value used among all reports. Positive hit proteins from each report were analyzed for KEGG and Reactome pathway enrichment via g.Profiler (Raudvere et al., 2019). Significantly enriched KEGG and Reactome pathways were compared for conserved and cell-type specific pathway analysis (Figure 2). As expected, cellular responses to stress and heat shock responses were found conserved in all cell types, supporting the role of BAG3 in mediating stress responses involving HSF1 activation. Interestingly, two novel pathways were also significantly enriched among all cell types: signaling by ROBO receptors, which regulate axonal guidance and cell migration, as well as influenza infection.

Many pathways uniquely presented per cell type, suggesting BAG3 may regulate differing cellular functions depending on the tissue or cell investigated. In cancer HeLa and MCF7 cells, BAG3 interacted with proteins that participate in cancer-related pathways, such as cell cycle regulation, glycolysis and gluconeogenesis, and metabolism and catabolism. Interestingly, many neurodegenerative disease and viral pathways were also over-represented in the HeLa BAG3 interactome, such as Alzheimer's disease (AD) and Epstein-Barr virus infection (Y. Chen et al., 2013). In cardiomyocytes, BAG3 associates with proteins involved in RNA metabolism and protein synthesis, the spliceosome, and COVID-19 infection (Judge et al., 2017). In HEK293T cells, BAG3-associated proteins over-represented pathways involving neurodegenerative disease, such as Amyotrophic lateral sclerosis (ALS), viral infection, cell cycle and apoptosis control, and RNA metabolism, such as transport, translation, and splicing (Y. Chen et al., 2013). In neurons, BAG3 associated with proteins involved in synaptic plasticity, such as vesicle cycling and long-term potential, neurodegenerative diseases in particular ALS and AD, and metabolism (Zhou et al., 2020).

Overall, few pathways are conserved across all reports, though this is likely at least partly due to differences in experimental condition and statistical analysis. Many pathways are represented in at least one report, such as the large 139 pathways overlap between BAG3-associated pathways in HeLa and HEK293T cells (Y. Chen et al., 2013; Hiebel et

al., 2020). This overlap includes cell cycle regulation pathways, apoptosis, p53-dependent repair mechanisms, and central kinase signaling cascades by MAPK and AKT, suggesting similar roles of BAG3 among these cell types. Other BAG3-associated pathways are found over-represented in multiple reports, such as RNA metabolism and translation related pathways shared in cardiomyocytes, HEK293T, and MCF7 cancer cells (Hiebel et al., 2020; Pasillas et al., 2015). BAG3 in neurons shares fewer over-represented associated pathways with other cell types. The greatest overlap exists between neurons and HeLa cells, where BAG3-associated proteins in both cell types significantly over-represented neurodegenerative pathways relating to Parkinson's disease (PD), AD, and Huntington's disease (HD), as well as metabolism pathways that include glycolysis, gluconeogenesis, and interleukin signaling (Y. Chen et al., 2013; Zhou et al., 2020). Altogether, the considerable number of shared pathways beyond HSF1-mediated heat shock responses and autophagy suggest a broad role of BAG3 in cells (Klimek, Kathage, Wordehoff, & Hohfeld, 2017). We will discuss these alternative roles of BAG3 further in this review.

Function of BAG3 in Cancer

The role of BAG3 in cancer has been studied intensively over the past ten years with 139 of the 347 papers on BAG3 being cancer-related based by CiteSpace analysis (Figure 1). BAG3 plays a role in the development, prognosis and resistance to treatment of over 10 different types of cancer including melanomas (Doong et al., 2000), glioblastomas (Festa et al., 2011; Gentilella & Khalili, 2011), thyroid carcinomas (Chiappetta et al., 2007; Meng et al., 2014), lung cancers (Chiappetta et al., 2014), breast cancers (Das et al., 2018; Liu et al., 2017), hepatocellular carcinomas (Xiao et al., 2014), pancreatic ductal adenocarcinomas (PDACs) (Rosati et al., 2012), colorectal carcinomas (Yang et al., 2013), endometrioid endometrial adenocarcinomas (Esposito et al., 2017), leukemia (Romano et al., 2003; Zhu et al., 2014), prostate cancers (Ammirante, De Laurenzi, Graziano, Turco, & Rosati, 2011; Iwasaki et al., 2007), chondrosarcoma (Shi et al., 2018) and colon cancer (N. Li et al., 2018) (Figure 3). The elevated levels of BAG3 in these cancer cells is often associated with increased cell survival, proliferation, migration, invasion and stemness of cancer cells (Figure 3). Further, increased expression of BAG3 in these cancer cells correlates with increased resistance to chemotherapeutics compared to drug-responsive cancer cells, such as chronic lymphocytic leukemia (H. Y. Chen et al., 2010), chronic myelogenous leukemia, adult acute myeloid leukemia (Valdez et al., 2008), thyroid cancer (Du, Meng, Zhang, Guan, & Wang, 2008), and colon cancer cells (Wang et al., 2018), where BAG3 expression is not significantly elevated.

BAG3 inhibits apoptosis and promotes cell proliferation

As a Bcl-2 binding protein (J. H. Lee et al., 1999), BAG3 plays a role in stabilizing Bcl-2 family proteins, including Bcl-xL, and Mcl-1, which inhibit cancer cell apoptosis (Karpel-Massler et al., 2015; Wang et al., 2018). Besides regulating the protein level of Bcl-2 family proteins, BAG3 retains the pro-apoptotic BAX protein in the cytosol, preventing its mitochondrial translocation (Festa et al., 2011). Moreover, depletion of BAG3 results in inhibition of the phosphorylation of focal adhesion kinase (FAK), significant loss of cell-matrix adhesion, and matrix detachment-induced cell death, which is inhibited by Bcl-2 (Antonietti et al., 2017). As an Hsp70 binding partner, BAG3 competes with another

BAG protein family member, BAG1, which directs its client to the proteasome, leading to stabilization of targeted cargo proteins. In melanoma cells, BAG3 stabilizes IKK-γ from proteasome degradation by competing with BAG1, which normally directs this client to the proteasome for degradation (Luders, Demand, & Hohfeld, 2000). This stabilization of IKK-γ leads to sustained NF-kB activation and cell survival (Ammirante et al., 2010).

BAG3 also plays an essential role in promoting the proliferation of cancer cells. Microarray-based transcriptome analysis of wild type and BAG3 knockout HeLa cells revealed that "cellular growth and proliferation" and "cell death and survival" were the major pathways regulated by BAG3 in these cancer cells (Furusawa et al., 2018). In neuroblastoma cells, BAG3 modulates the degradation of cyclin B1 expression, which regulates cell-cycle progression (Gentilella, Passiatore, Deshmane, Turco, & Khalili, 2008). In addition, BAG3 stabilizes the mRNA of S-phase kinase-associated protein 2 (Skp2), a cell cycle regulator, in ovarian cancer cells (Yan et al., 2017). BAG3 also stabilizes glutaminase via interacting with SIRT5, and thus promotes glutaminolysis (Zhao et al., 2019), which plays an essential role in maintaining bioenergetics and promoting proliferation of cancer cells (Wise & Thompson, 2010; Y. Xiang et al., 2015). These findings support the role of BAG3 in regulating the cell cycle and cell proliferation in cancer cells.

BAG3 enhances EMT, invasiveness, and stemness of cancer cells

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells lose their polarity and cellular adhesion properties and acquire invasive characteristics to become mesenchymal stem cells (Grigore, Jolly, Jia, Farach-Carson, & Levine, 2016). In cancer cells, EMT contributes to the acquisition of properties that are essential for metastasis (Y. Zhang & Weinberg, 2018). Increasing evidence indicates that BAG3 is involved in EMT and invasive properties of tumors. For example, phosphorylation of BAG3 regulates EMT and invasiveness of thyroid cancer cells (N. Li et al., 2013), and suppresses miR-29b expression enhancing MMP2 expression, which in turn increases cell motility and invasiveness of uterine corpus carcinoma and glioblastoma cells (Habata et al., 2015; Im et al., 2016). In addition, BAG3 positive pancreatic stellate cells release multiple cytokines enhancing migration and invasion of pancreatic ductal adenocarcinoma cells (Yuan et al., 2019). Moreover, BAG3 promotes the stemness of cancer cells (Liu et al., 2017). Cancer stem cells are a group of cells within tumors that retain stemness properties including self-renewal cloning, growing, and the ability to metastasize (Overton, Ihara, & Bishop, 1987); removal of cancer stem cells is a key to the success of cancer treatment. For example, BAG3 stabilizes STAT3, a master regulator of stemness, in tumor spheres of glioblastoma stem cell-like cells (Im et al., 2016).

BAG3 in regulating mRNA stability

Interestingly, studies of BAG3 in pancreatic cancer have illuminated a novel function for BAG3. BAG3 is highly expressed in PDAC, one of the deadliest cancers (Rosati et al., 2012). BAG3 interacts with a constitutive decay degradation element (CDE) of the 3'-UTR regions of hexokinase 2 (HK2) mRNA and alters recruitment of the RNA-binding proteins Roquin and IMP3 to the HK2 mRNA. Ultimately, this leads to the stabilization of the HK2 mRNA and a corresponding increase in HK2 expression which promotes aerobic glycolysis

(An et al., 2017). Later, the same research team demonstrated that BAG3 regulates the recruitment of Argonaute 2 (Ago2) to IL-6 mRNA (C. Li et al., 2019) and HuR to IL-8 mRNA (C. Li et al., 2018), which results in the mRNA transcript stabilization of both genes. Beyond PDAC, BAG3 has also been reported to promote the stemness of breast cancer stem cells through interaction with CXCR4 mRNA, resulting in increased expression (Liu et al., 2017).

Secreted BAG3 in PDAC

Falco et al. were the first to show that BAG3 is detectable in serum from PDAC patients (Falco et al., 2013). Later, the same group identified BAG3 in both the exosome and soluble fractions of five human pancreatic carcinoma cell lines (Rosati et al., 2015). Interestingly, serum BAG3 levels are significantly higher in samples from PDAC patients compared to patients with the inflammatory disease or other types of cancer, such as colon and stomach carcinomas (De Marco et al., 2018). This finding suggests BAG3 may be a potential biomarker for the diagnosis of PDAC. Without a signal peptide at the N terminal, BAG3 is likely released from the pancreatic carcinoma cells (donor cells) through an unconventional protein secretion (UPS) route (Nickel & Rabouille, 2009). Secreted BAG3 binds to induced transmembrane 2 (IFITM 2) on the plasma membrane of macrophages (recipient cells), thereby promoting the release of IL-6 from macrophages, which in turn facilitates the growth of pancreatic tumors (Rosati et al., 2015). These findings demonstrating that there is a secreted form of BAG3 are indicative of a remote regulatory function of BAG3 in cancer cells.

BAG3 promotes resistance to chemotherapy

With the growing knowledge about the function of BAG3 in regulating apoptosis, proliferation, stemness, EMT, and invasiveness in a variety of cancers, an increasing number of studies have reported a role for BAG3 in chemotherapy resistance. Mcl-1 is an antiapoptotic Bcl-2 family member which is highly expressed in various human tumors (Shamas-Din, Kale, Leber, & Andrews, 2013). Mcl-1 has been shown to be a resistance factor that limits the efficacy of various antitumor agents (W. Xiang, Yang, & Bai, 2018). BAG3 stabilizes Mcl-1 and promotes AUY922 (Hsp90 inhibitor) resistance in colon cancer cells (Wang et al., 2018). Knockdown of BAG3 sensitizes ovarian cancer cells to apoptosis (Sugio et al., 2014) and significantly increased the efficacy of paclitaxel, in combination with the Mcl-1 antagonist MIM1, in inducing cell death (Habata et al., 2016). In urothelial cancer cells, knockdown of BAG3 significantly downregulated Mcl-1 protein levels and increased their susceptibility to ABT-737 (Bcl-2 inhibitor) (Mani et al., 2016). Depletion of BAG3 sensitizes a lung carcinoma cell line (A549) and ovarian cancer cells to treatment with cisplatin (Qiu et al., 2017; Qiu, Sun, Zhang, & Han, 2019). Moreover, BAG3 promotes resistance to 5-FU chemotherapy treatment in colorectal cancer (N. Li et al., 2018).

In summary, BAG3 may be a suitable biomarker for certain types of cancer. Inhibition or neutralization of BAG3 may be a potentially promising strategy for anticancer therapies. It should be noted that certain cancers such as small cell carcinomas and lung cancer with neuroendocrine differentiation were negative for BAG3 expression (Yeo et al., 2015) and in a specific case, BAG3 was reported to inhibit cell growth in carcinomas (Kong et al., 2016).

These contradictory data indicate the need for further investigation of the functions of BAG3 in cancer cells.

Impact of BAG3 on Myopathies

BAG3 is highly expressed in muscle tissue, particularly in cardiac muscle. BAG3 co-localizes with Z-disks proteins such as α-actinin and desmin, which show a repeating and regular stripe-like pattern in longitudinal sections of muscle (Homma et al., 2006). In 2006, Homma and colleagues were the first to generate a homozygous BAG3 knockout mouse (Homma et al., 2006). These mice showed neonatal noninflammatory myofibrillar myopathy in skeletal and cardiac muscle, disruption of Z-disk architecture, and myofibrillar degeneration with apoptotic features, leading to death at an early postnatal stage (Homma et al., 2006). This was the first direct demonstration of the role of BAG3 in regulating myofibrillar maintenance in vivo.

Follow-up studies further explored the role of BAG3 in regulating the function of cardiomyocytes. BAG3 was found to regulate the stability of F-actin through the actin capping protein, CapZβ1, which plays an important role in regulating the stability and proper distribution of actin at the Z-disk in the myocyte (Lin, Li, Swanson, & Russell, 2013; Pappas, Bhattacharya, Cooper, & Gregorio, 2008). BAG3 deficiency lead to the degradation of CapZβ1 through the ubiquitin-proteasome system (Hishiya, Kitazawa, & Takayama, 2010), and overexpression of CapZβ1 in cardiomyocytes attenuated the myofibrillar disruption caused by BAG3 depletion (Hishiya et al., 2010). Additionally, BAG3 regulates chaperone-assisted selective autophagy mediated degradation of the Z-disk protein, filamin, which is necessary to maintain structure and function of myocytes (Arndt et al., 2010). Besides regulating the Z-disk, BAG3 also associates with the gap junction protein connexin 43 (Cx43) in primary neonatal rat ventricular cardiomyocytes (Ghasemi Tahrir et al., 2019). BAG3 regulates the phosphorylation and turnover of Cx43 (Ghasemi Tahrir et al., 2019), which promotes cardiac tissue conductance (Saffitz, Laing, & Yamada, 2000). Moreover, BAG3 also modulates myocyte contraction and action potential duration through association with the β1-adrenergic receptor, L-type Ca^{2+} channels and phospholamban. Knockdown of BAG3 caused reduced myocyte contraction amplitudes and lower calcium transient amplitudes in cardiomyocytes in reponse to isoproterenol treatment (Feldman et al., 2016).

In 2009, Selcen et al. first reported 3 unrelated myofibrillar myopathy cases in children with a missense mutation in BAG3, leading to a replacement of leucine with proline at amino acid 209 position (P209L) (Selcen et al., 2009). All 3 cases developed progressive and severe muscle weakness, respiratory insufficiency, and cardiomyopathy in their childhood (Selcen et al., 2009). These cardiomyopathy cases in humans were consistent with Homma's findings (Homma et al., 2006) in BAG3 knockout mice and suggested the important role of BAG3 in maintaining cardiomyocyte function. Subsequently, additional clinical evidence supported the connection between the P209L BAG3 mutation and cardiomyopathy (Konersman et al., 2015; H. C. Lee et al., 2012; Odgerel et al., 2010). Clinical muscle biopsies of patients harboring the P209L BAG3 mutation showed disintegration of Z-discs with an extensive accumulation of granular debris and large inclusions within fibers.

The P209L mutation of BAG3 lead to decreased BAG3 and Hsp70 levels together with α-actinin desmin, filamin, and fast myosin heavy chain, which compromised the muscle fiber morphology and its function (Kostera-Pruszczyk et al., 2015). In zebrafish, loss of BAG3 resulted in myofibrillar disintegration, but didn't have an effect on the formation of protein aggregates. Interestingly, overexpression of P209L BAG3 rescued this myofibrillar disintegration phenotype in the zebrafish. These data suggest P209L BAG3 tends to aggregate, gradually reducing the pool of existing BAG3, which leads to BAG3 insufficiency and myofibrillar disintegration (Ruparelia, Oorschot, Vaz, Ramm, & Bryson-Richardson, 2014). This hypothesis was further supported in a mouse model where cardiac-specific α MHC-human BAG3 P209L was expressed in mice (Quintana et al., 2016). These mice showed progressive heart failure with the presence of pre-amyloid oligomers, abnormal mitochondrial dynamics, and activation of p38 signaling (Quintana et al., 2016). It should be noted that in the α MHC-human BAG3 P209L transgenic mice, P209L BAG3 is overexpressed in the presence of wild type BAG3 but still leads to haploinsufficiency of wild-type BAG3 (Quintana et al., 2016), which is consistent with the findings of Ruparelia et al (Ruparelia et al., 2014). Recently, Ruparelia and colleagues screened autophagy-promoting compounds in P209L BAG3 mutant zebrafish and human myoblasts and identified nine, including metformin, that could reduce aggregates. Further evaluation of histology and behavior shows that metformin is also able to rescue the fiber disintegration and swimming deficit observed in the BAG3 −/− fish (Ruparelia et al., 2020). These studies suggest a mechanism that P209L BAG3 tends to aggregate with the available BAG3 pool leading to BAG3 insufficiency, which results in impaired autophagic activity.

In addition to the P209L mutation, more than 20 BAG3 variants or SNPs have been found to be related to cardiomyopathy. The reported BAG3 variants and the related diseases are listed in Table 1. These variants include missense mutations, truncations, and frameshift mutations (Figure 4A). The cited papers include both research studies and case reports, which reflect a combination of prevalence of a certain variant and the focus of research interests. With this analysis, we can find some interesting patterns: 1. P209 mutation of BAG3, including P209L, P209S, and P209Q, are the most extensively reported, which indicates the prevalence of the mutation at this site. 2. The same mutation could lead to multiple phenotypes. For example, P209L BAG3 was found in a variety of cardiomyopathies and neuropathies, including myofibrillar myopathy (MFM) (D'Avila et al., 2016; Konersman et al., 2015; H. C. Lee et al., 2012; Odgerel et al., 2010; Selcen et al., 2009), myopathy (Andersen et al., 2018), axonal neuropathy (D'Avila et al., 2016; Kostera-Pruszczyk et al., 2015; Odgerel et al., 2010), and Charcot-Marie-Tooth disease (CMT) (Noury et al., 2018). 3. The different mutations on the same site could show a tendency for different pathologies. For example, R71W is correlated with DCM (Norton et al., 2011), while R71Q is non-syndromic (Citro et al., 2013; Villard et al., 2011); P209L showed a tendency of development of MFM, while P209S is associated with CMT (Adriaenssens et al., 2020; J. Fu et al., 2020; Shy et al., 2018). 4. It should be noted that the cardiomyopathy caused by BAG3 mutations at P209 is sometimes present with neuropathy or even present as a neuropathy only, which indicates the important role of BAG3 in regulating neuronal function. 5. Interestingly, ignoring the citation number of the different mutations, about one third (6/16) of the pathogenic missense mutations are in the α2 and α3 helix of the BAG

domain. This domain regulates the association of BAG3 with Hsp70 (Brockmann et al., 2004). Moreover, if the truncations and frameshift mutations are taken into consideration, the longest truncation product (T451X) of BAG3 mutant protein lacks this region. It seems that the mutation which affects the region of E455~R477 is highly correlated with cardiomyopathy. It will be intriguing to examine the 3D structure of the E455~R477 region that is located at α2 and α3 helix of the BAG domain. However, there is only one report available about the 3D structure of the BAG domain in human BAG1 (Sondermann et al., 2001). Since the BAG domain is highly conserved (over 70% consensus) between BAG1 and BAG3 (Figure 4B), we use the interaction between the BAG domain from BAG1 and Hsp/Hsc70 as a model for analysis. After mapping the E455~R477 region (colored in red) to the 3D model, we are surprised to see that this region is most proximal to Hsp70 (Figure 4C). It should be noted that the well-studied P209L mutation is located in an IPV motif, which is important for the association of BAG3 with HspB8. These findings indicate that disruption of the association of BAG3 with small heat shock proteins may be important for the development of cardiomyopathy. However, a recent study has shown that disrupted binding of BAG3 mutants (BAG3 P209 and P470S) to either HspBs or Hsp70 is not the cause of the dramatic collapse of protein quality control (Meister-Broekema et al., 2018). On the contrary, for both the BAG3P209 and BAG3P470 mutants, binding to Hsp70 was found to be required for the phenotypic effects. These BAG3 mutations do not affect Hsp70 binding, but the stimulation of Hsp70-dependent client processing is impaired. Genetic and pharmaceutical disruption of the association of BAG3 with Hsp70 completely reverses stress-induced protein aggregation for both BAG3 mutations. These mutant BAG3 proteins showed a dominant gain of function mutation, leading to aggregation of itself, Hsp70, and Hsp70 clients, which results in aggregates observed in clinical biopsy and development of cardiomyopathy (Meister-Broekema et al., 2018).

BAG3 in the nervous system:

The fact that cardiomyopathy caused by BAG3 mutations sometimes arises along with neuropathy (D'Avila et al., 2016; Kostera-Pruszczyk et al., 2015; Odgerel et al., 2010) indicates a role for BAG3 in the nervous system. An increasing number of reports support the importance of BAG3 in neural function and neurodegenerative disease (Figure 1). The expression of BAG3 shows an interesting bi-phasic pattern during the development of the central nervous system in rats. In the embryonic stage, BAG3 is expressed in the medial telencephalic wall (lateral ventricle) and in the marginal zone of the cortex in Cajal-retzius cells in developing neurons. This is followed by an abrupt increase in expression of BAG3 in the cortical and hippocampal neurons during the first postnatal week, which declined thereafter, suggesting a role in regulating neuronal development (Choi et al., 2006). Additionally, BAG3 localized to radial glial cells in the midbrain. More specifically, it is localized to the dorsal midline glia, both within the brain stem and spinal cord (Choi et al., 2009). However, unlike the early postnatal stages, BAG3 increases in the mouse hippocampus from 4 to 12 months of age, which indicates BAG3 may play a role maintaining protein homeostasis during aging and that dysregulation could be a contributing factor in age-related neurodegenerative conditions (Tang, Ji, Pallo, Rahman, & Johnson, 2018).

Major characteristics of many neurodegenerative diseases involve the aggregation and accumulation of misfolded proteins associated with cytotoxic effects in neurons. These misfolded proteins include tau protein that is found in neurofibrillary tangles (H. Fu et al., 2019; Sturner & Behl, 2017; Tapia-Rojas et al., 2019), mutant huntingtin with an expanded polyQ tract (Hipp, Park, & Hartl, 2014; Ogen-Shtern, Ben David, & Lederkremer, 2016) and mutant superoxide dismutase 1 (SOD1) (Crippa et al., 2010). Even though neurodegenerative diseases are characterized by different mutant/misfolded protein aggregates, they present a unifying attribute; an inability of the protein clearance machinery to efficiently degrade the misfolded proteins and maintain proteostasis. In this context, BAG3 has been shown to promote the clearance of disease-relevant aggregation-prone proteins including tau (Lei, Brizzee, & Johnson, 2015), α-synuclein (Cao et al., 2017), mutant SOD1 (Gamerdinger et al., 2011), and mutant huntingtin (Carra, Seguin, Lambert, & Landry, 2008a), which are related to AD, ALS, HD and PD, respectively. These findings point to the importance of BAG3 in maintaining proteostasis and how dysregulation of BAG3 may contribute to the development of age-related neurodegenerative disease (Figure 5).

BAG3 functions in Alzheimer's disease:

Dysregulation of proteostasis in neurons will lead to the accumulation of pathologic proteins, with phosphorylated tau species being a defining characteristic of AD (Kruger & Mandelkow, 2016). Our lab was the first to demonstrate a role for BAG3 in clearing tau species in neurons. Proteasome inhibition increased BAG3 expression and promoted phosphorylated tau clearance in neurons, and knockdown of BAG3 attenuated autophagy measures and increased the accumulation of phosphorylated tau in neurons (Lei et al., 2015). Recently, we have provided evidence that BAG3 regulates autophagic flux in the neurites through interaction with the post-synaptic cytoskeleton protein synaptopodin. Knockdown of either BAG3 or synaptopodin disrupts the fusion of autophagosomes and lysosomes in the post-synaptic compartment, which leads to the accumulation of phosphorylated tau in autophagosomes at post-synaptic densities (Ji et al., 2019). This finding suggests a crucial role of BAG3 in regulating proteostasis and synaptic function in neurons. Additional data on the importance of BAG3 in aberrant tau clearance was shown when comparing inhibitory and excitatory neurons in the entorhinal cortex of EC-tau mice (expressing pathological human tau in the entorhinal cortex), human AD, and human non-AD brain samples (H. Fu et al., 2019). The levels of BAG3 were increased in non-neuronal cells and inhibitory neurons in both AD and non-AD samples, which are relatively resistant to tau pathology compared to excitatory neurons (H. Fu et al., 2019). Furthermore, in excitatory neurons the knockdown of BAG3 increased tau levels, while BAG3 overexpression attenuated tau accumulation(H. Fu et al., 2019). Together, these findings indicate that BAG3 may have a neuroprotective role by selectively targeting aberrant tau protein for degradation, thereby restoring proteostasis. Another study found that BAG3 protein levels were lower in human AD brains compared with the aged-matched controls (Zhou et al., 2020). These investigators also overexpressed GFP-BAG3 in neurons followed by immunoprecipitation with a GFP antibody and mass spectrometric analyses of co-precipitating proteins. In this study, BAG3 was found to interact with post-synaptic proteins and regulated glutamatergic synaptic protein turnover through its association with Hsp70. When AAV-shBAG3 was injected into

the brains of newborn mice and analyses were carried out 1–3 months later, post-synaptic proteins were decreased, Golgi staining showed fewer dendritic spines in the hippocampus, and prefrontal cortex at this early postnatal stage compared to neonates injected with a control AAV, indicating that BAG3 levels influence these post-synaptic proteins (Zhou et al., 2020). Our latest findings have identified a group of neuronal BAG3 interactors that are involved in the endocytic pathway. Our data demonstrate that a BAG3-Hsp70-TBC1D10B complex attenuates the ability of the GTPase activating protein, TBC1D10B, to inactivate Rab35. BAG3, through its interaction with TBC1D10B supports the activation of Rab35 and recruitment of Hrs, which initiates ESCRT-mediated endosomal tau clearance. Intrahippocampal expression of BAG3 in P301S mice increased the co-localization of phospho-tau with the ESCRT III protein CHMP2B and reduced the levels of the mutant human tau (Lin et al., 2021). In summary, these studies indicate a close link between the protective function of BAG3 and tau clearance in an excitatory neuron preferential manner. These findings highlight an important role of BAG3 in neuronal proteostasis and pathogenesis of AD, as well as other neurodegenerative diseases.

BAG3 in Amyotrophic Lateral Sclerosis:

ALS is a progressive nervous system disease that affects motor neurons in the brain and spinal cord. One of the genes that is mutated in familial forms of the disease is SOD1, which results in its misfolding, aggregation, and proteasome inhibition (Crippa et al., 2010). Overexpression of $SOD1^{G93A}$ in immortalized motor neurons (NSC34 cells) induced the expression of BAG3, HspB8, and Hsp70 expression and consequently an enhanced removal of $SOD1^{G93A}$. When autophagy was blocked and $SOD1^{G93A}$ was not degraded, $SOD1^{G93A}$ was found to immunoprecipitated with BAG3, HspB8, Hsc70, and CHIP (Crippa et al., 2010). This finding indicates the importance of BAG3-mediated autophagy in clearing aggregated, misfolded proteins in another neurodegenerative disorder. HspB8 is upregulated in spinal cord motor neurons in the mutant SOD1 transgenic $SOD1^{G93A}$ mice (Crippa et al., 2010), and the mutant SOD1 interacts with the HspB8/BAG3/Hsc70/CHIP complex. Overexpression of HspB8 increases the solubility and clearance of mutant SOD1 without affecting the wild type SOD1. This can be blocked by an autophagy inhibitor (Crippa et al., 2010). In the spinal cords of SOD^{G93A} and SOD^{G85R} transgenic mouse models, BAG3 showed a higher expression level compared to control mice, and BAG3 co-localized with the mutant SOD1 in the perinuclear inclusions (Gamerdinger et al., 2009). Further studies showed that BAG3, which associates with cytoplasmic dynein, collaborates with Hsp70 to direct $SOD1^{G85R}$ (an Hsp70 substrate) to the aggresome for degradation. It should be noted that the PXXP domain is needed for the association of BAG3 with dynein for perinuclear transportation, while the BAG domain is necessary for the association of Hsp70 and its substrate (Gamerdinger et al., 2009). Besides, $SOD1^{G85R}$ was shown to co-localize with autophagosomal and lysosomal markers, including p62, NBR1, LC3B, and Lamp1, indicating an autophagic degradation mechanism (Gamerdinger et al., 2009).

BAG3 in Huntington's disease:

A characteristic of HD is the accumulation of the aggregation-prone mutant huntingtin protein which has an elongated poly-glutamine tract. A polyglutamine tract >35Q in huntingtin is considered pathological (Rubinsztein et al., 1996). Carra et al. found that

BAG3 promotes the clearance of Htt43Q, human huntingtin protein with 43 glutamines (Q), through a selective macroautophagy pathway (Carra, Seguin, Lambert, & Landry, 2008b). BAG3 associates with HspB8 and regulates its activity. Overexpression of BAG3, HspB8 or both decreased levels of Htt43Q, whereas BAG3 knockdown prevented HspB8-induced Htt43Q degradation (Carra, Seguin, et al., 2008b). Another line of study showed that deletion of the IPV domain, which is needed for the association of BAG3 with HspB8, would disrupt the effect of BAG3 on the degradation of Htt43Q (Fuchs et al., 2009). These findings suggest a protective role of BAG3 in regulating the clearance of mutant huntingtin through selective macroautophagy, and likely plays a role in regulating the clearance of other pathological polyQ proteins (Paulson, Shakkottai, Clark, & Orr, 2017).

BAG3 in Parkinson's disease

PD is a disorder causing motor deficiencies due to the neurodegeneration of dopaminergic neurons. It is characterized by Lewy bodies in the substantia nigra pars compacta. It has been shown that these inclusion bodies are mostly composed of misfolded α-synuclein(Henderson, Trojanowski, & Lee, 2019). Increased expression and co-localization of BAG3 and α-synuclein were found upon immunoblotting and histochemical staining in $SNCA^{A35T}$ mice (overexpress mutant α-synuclein in the brain), relative to non-transgenic controls (Cao et al., 2017). BAG3 knockdown decreased LC3B-II in MG132 treated and untreated cells, while BAG3 overexpression showed the opposite effects even upon treatment with the vacuolar H+-ATPase inhibitor, bafilomycin A1 (Cao et al., 2017). SQSTM1 and BAG3 binding was increased in $SNCA^{A35T}$ mice, along with the expression of SQSTM1 and LC3B-II, while SQSTM1 co-localized to the substantia nigra compacta neurons (Cao et al., 2017). BAG3 knockdown leads to accumulation of α-synuclein, while BAG3 overexpression decreased α-synuclein levels. Furthermore, inhibition of autophagy through Atg5 knockdown attenuates the effects of BAG3, indicating the role of BAG3 in regulating α-synuclein by modulating autophagy. Furthermore, a recent Genome-wide association study (GWAS) in microglia showed that BAG3 is one of the PD risk SNPs within active regulatory regions of DNA, suggesting its potential role in the development of PD (Boom, Pierce, & Coetzee, 2020).

BAG3 in viral replication and function.

BAG3 also participates in the regulation of replication, persistence, and survival of viruses through interaction with viral proteins. Multiple BAG3 domains are involved in the interaction between viral proteins that will either promote or inhibit their function. One domain in particular, the WW domain, has been shown to interact with the PPxY motif of VP40, a crucial protein involved in viral assembly and budding (Harty, Brown, Wang, Huibregtse, & Hayes, 2000). In Ebola and Marburg virus, the interaction of BAG3 and VP40 led to an inhibition of replication due to BAG3 sequestering VP40, therefore limiting its function (Liang et al., 2017). However, a different effect of BAG3 was shown in adenovirus where it interacts with Ad penton base protein, which is a viral capsid constituent responsible for virus internalization. Knockdown of BAG3 impaired the internalization of the virus, suggesting the regulatory function of BAG3 in the viral life cycle in adenoviruses (Gout et al., 2010).

Besides the WW domain, the BAG domain of BAG3 mediates interactions with viral proteins. ORF29p, a latency-associated protein for Varicella-zoster virus (VZV), is bound by BAG3 through its BAG domain. BAG3, Hsp70/Hsc70, and Hsp90 colocalize with ORF29p during lytic replication of VZV. Knockdown of BAG3 results in a dramatic decrease in the replication efficiency of VZV (Kyratsous & Silverstein, 2007). BAG3 expression is therefore crucial for the survival of this specific type of virus. In the case of HIV-1 infection, the opposite is likely the case, as BAG3 has been suggested to suppress the expression of the HIV-1 gene, stunting the spread of this infection (Rosati et al., 2007).

In addition to direct interactions with viral proteins, BAG3 has been shown to have downstream effects and have an indirect role in virus survival. The Herpes Simplex virus (HSV) is an example of a virus that is modulated by BAG3. Specifically, BAG3 colocalized with ICP0, a transcriptional activator, which together resulted in increases in viral protein accumulation in the cell (Kyratsous & Silverstein, 2008). This interaction isn't direct but shows the dependence of these proteins on each other in order to maintain efficient viral production. Similar functions of BAG3 were found in studies in SARS-CoV. Studies have suggested that SARS-CoV relies heavily on the expression of BAG3 in the host cell in order to replicate, as knocking down BAG3 led to dramatic decreases in viral production (L. Zhang, Zhang, Zhang, Lin, & Ge, 2010). BAG3 can be regulated by other proteins as well, as demonstrated in Epstein-Barr virus (EBV). In this study, the viral oncoprotein, EBNA3A, was able to induce BAG3 and it's chaperone proteins, one of them being Hsp70 (Young, Anderton, Paschos, White, & Allday, 2008). The mechanism involves interacting with the Hsp70 complex, therefore having an indirect effect on BAG3. By doing so, it had been suggested that the EBNA3A protein would maintain stability and prevalence when upregulating these molecular chaperones and co-chaperones (Young et al., 2008). Besides the interaction with virus proteins, BAG3 was also found to interact with host proteins to regulate the integration process of virus infiltration. Overall, BAG3 can lead to either negative or positive effects on the virus life cycle. Variability between viruses accounts for these vast differences, as well as the complexity of BAG3 and its interactors.

Conclusions and future directions:

BAG3, as a co-chaperone, plays multiple roles in supporting the diverse functions of the different cell types, including cancer cells, myocytes, and neurons. BAG3 exhibits these various functions by interacting with specific proteins through its different domains. Identifying the interactors of each particular domain of BAG3 is key to understanding the various functions of BAG3. A variety of transgenic mouse and zebrafish models have been used to study the functions of BAG3 using site mutations in different domains. The association of point mutations in BAG3 and the phenotype (cardiomyopathy and neuropathy) have been extremely helpful in the molecular dissection of BAG3's function. Large-scale whole genomic sequencing in the different inherited diseases may help us to better understand BAG3.

Although the functions of BAG3 are diverse, we can still identify two significant threads; cell survival and proteostasis. These two threads show a certain degree of specificity considering the different cell types. For example, the function of BAG3 in regulating cell

survival primarily occurs in cancer cells, while maintaining proteostasis is more likely associated with neurodegeneration and cardiomyopathy. Silencing of BAG3 is reported to inhibit the chemotherapy resistance of cancer cells, while overexpression of BAG3 promotes the degradation of misfolded proteins in neurodegeneration. However, these two threads are also intertwined with each other. For example, BAG3 regulates the proteostasis of pro-apoptotic proteins, which affect cell survival. Overall, it is clear that further studies are to define BAG3 interactors and the signaling pathways that it regulates. In the future, understanding these BAG3 interactions will allow the development of therapeutic strategies for cancer, myopathies and neurodegenerative diseases.

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Abbreviations:

AAV	Adeno-associated virus
AD	Alzheimer’s Disease
Ago2	Argonaute 2
ALS	Amyotrophic lateral sclerosis
BAG1	Bcl-2-associated athanogene 1
BAG3	Bcl-2-associated athanogene 3
Bcl-2	B-cell lymphoma-2
Bcl-xL	B-cell lymphoma-extra large
CAIR-1	CAI stressed-1
CapZβ1	F-actin-capping protein subunit beta1
CDE	Constitutive decay degradation element
CHIP	C-terminus Hsp70 Interacting Protein
CHMP2B	Charged Multivesicular Body Protein 2B
CMT	Charcot-Marie-Tooth disease
Cx43	Connexin 43
CXCR4	C-X-C Motif Chemokine Receptor 4
EBNA3A	Epstein–Barr virus nuclear antigen 3A
EBV	Epstein-Barr virus
EMT	Epithelial-mesenchymal transition

FAK	F focal adhesion kinase
GWAS	Genome-wide association study
HD	Huntington's disease
HK2	hexokinase 2
Hsc70	Heat shock cognate 71 kDa protein
Hsp70	70 kDa heat shock protein
HspB	Heat shock protein B
HuR	Human antigen R
IKK-γ	cytokine-responsive IκB kinase -gamma
IL	Interleukin
IPV domain	Ile-Pro-Val domain
Lamp1	Lysosomal-associated membrane protein 1
LC3B	Microtubule-associated proteins 1A/1B light chain 3B
MAPK	Mitogen-activated protein kinase
Mcl-1	Myeloid-cell leukemia 1
MFM	myofibrillar myopathy
mTOR	Mechanistic target of rapamycin
NBR1	Neighbor of BRCA1 gene 1
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
PD	Parkinson's disease
PDACs	Pancreatic ductal adenocarcinomas
PLCγ1	Phospholipase C, gamma 1
PXXP	proline-rich region
RAPGEF2	Rap guanine nucleotide exchange factor 2
ROBO receptors	Roundabout receptors
SARS-CoV	Severe acute respiratory syndrome-associated coronavirus
Skp2	S-phase kinase-associated protein 2
SNPs	Single nucleotide polymorphisms

SOD1	superoxide dismutase 1
SQSTM	Sequestosome-1
Src	Proto-oncogene tyrosine-protein kinase Src
STAT3	Signal transducer and activator of transcription 3
VZV	Varicella-zoster virus
WW domain	tryptophan-tryptophan domain

References

Adriaenssens E, Tedesco B, Mediani L, et al. (2020). BAG3 Pro209 mutants associated with myopathy and neuropathy relocate chaperones of the CASA-complex to aggresomes. Sci Rep, 10(1), 8755. 10.1038/s41598-020-65664-z [PubMed: 32472079]

Ammirante M, De Laurenzi V, Graziano V, Turco MC, & Rosati A (2011). BAG3 is required for IKKalpha nuclear translocation and emergence of castration resistant prostate cancer. Cell Death Dis, 2, e139. 10.1038/cddis.2011.23 [PubMed: 21451574]

Ammirante M, Rosati A, Arra C, et al. (2010). IKK{gamma} protein is a target of BAG3 regulatory activity in human tumor growth. Proc Natl Acad Sci U S A, 107(16), 7497–7502. 10.1073/pnas.0907696107 [PubMed: 20368414]

An MX, Li S, Yao HB, et al. (2017). BAG3 directly stabilizes Hexokinase 2 mRNA and promotes aerobic glycolysis in pancreatic cancer cells. J Cell Biol, 216(12), 4091–4105. 10.1083/jcb.201701064 [PubMed: 29114069]

Andersen AG, Fornander F, Schroder HD, et al. (2018). BAG3 myopathy is not always associated with cardiomyopathy. Neuromuscul Disord, 28(9), 798–801. 10.1016/j.nmd.2018.06.019 [PubMed: 30061062]

Antonietti P, Linder B, Hehlgans S, et al. (2017). Interference with the HSF1/HSP70/BAG3 Pathway Primes Glioma Cells to Matrix Detachment and BH3 Mimetic-Induced Apoptosis. Mol Cancer Ther, 16(1), 156–168. 10.1158/1535-7163.MCT-16-0262 [PubMed: 27777286]

Aragam KG, Chaffin M, Levinson RT, et al. (2018). Phenotypic Refinement of Heart Failure in a National Biobank Facilitates Genetic Discovery. Circulation. 10.1161/CIRCULATIONAHA.118.035774

Arimura T, Ishikawa T, Nunoda S, Kawai S, & Kimura A (2011). Dilated cardiomyopathy-associated BAG3 mutations impair Z-disc assembly and enhance sensitivity to apoptosis in cardiomyocytes. Hum Mutat, 32(12), 1481–1491. 10.1002/humu.21603 [PubMed: 21898660]

Arndt V, Dick N, Tawo R, et al. (2010). Chaperone-assisted selective autophagy is essential for muscle maintenance. Curr Biol, 20(2), 143–148. 10.1016/j.cub.2009.11.022 [PubMed: 20060297]

Booms A, Pierce S, & Coetzee GA (2020). Parkinson's Disease Genetic Risk Evaluation in Microglia Highlights Autophagy and Lysosomal Genes. BioRxiv. 10.1101/2020.08.17.254276

Brockmann C, Leitner D, Labudde D, et al. (2004). The solution structure of the SODD BAG domain reveals additional electrostatic interactions in the HSP70 complexes of SODD subfamily BAG domains. FEBS Lett, 558(1–3), 101–106. 10.1016/S0014-5793(03)01490-X [PubMed: 14759524]

Cao YL, Yang YP, Mao CJ, et al. (2017). A role of BAG3 in regulating SNCA/alpha-synuclein clearance via selective macroautophagy. Neurobiol Aging, 60, 104–115. 10.1016/j.neurobiolaging.2017.08.023 [PubMed: 28941726]

Carra S, Seguin SJ, Lambert H, & Landry J (2008a). HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. J Biol Chem, 283(3), 1437–1444. 10.1074/jbc.M706304200 [PubMed: 18006506]

Carra S, Seguin SJ, Lambert H, & Landry J (2008b). HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. J Biol Chem, 283(3), 1437–1444. 10.1074/jbc.M706304200 [PubMed: 18006506]

Carra S, Seguin SJ, & Landry J (2008). HspB8 and Bag3: a new chaperone complex targeting misfolded proteins to macroautophagy. Autophagy, 4(2), 237–239. 10.4161/auto.5407 [PubMed: 18094623]

Chen HY, Liu P, Sun M, et al. (2010). [Bag3 gene expression in chronic lymphocytic leukemia and its association with patients' prognosis]. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 18(4), 838–842. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20723284 [PubMed: 20723284]

Chen Y, Yang LN, Cheng L, et al. (2013). Bcl2-associated athanogene 3 interactome analysis reveals a new role in modulating proteasome activity. Mol Cell Proteomics, 12(10), 2804–2819. 10.1074/mcp.M112.025882 [PubMed: 23824909]

Chiappetta G, Ammirante M, Basile A, et al. (2007). The antiapoptotic protein BAG3 is expressed in thyroid carcinomas and modulates apoptosis mediated by tumor necrosis factor-related apoptosis-inducing ligand. J Clin Endocrinol Metab, 92(3), 1159–1163. 10.1210/jc.2006-1712 [PubMed: 17164298]

Chiappetta G, Basile A, Barbieri A, et al. (2014). The anti-apoptotic BAG3 protein is expressed in lung carcinomas and regulates small cell lung carcinoma (SCLC) tumor growth. Oncotarget, 5(16), 6846–6853. 10.18632/oncotarget.2261 [PubMed: 25149536]

Choi JS, Lee JH, Kim HY, Chun MH, Chung JW, & Lee MY (2006). Developmental expression of Bis protein in the cerebral cortex and hippocampus of rats. Brain Res, 1092(1), 69–78. 10.1016/j.brainres.2006.02.137 [PubMed: 16690035]

Choi JS, Lee JH, Shin YJ, et al. (2009). Transient expression of Bis protein in midline radial glia in developing rat brainstem and spinal cord. Cell Tissue Res, 337(1), 27–36. 10.1007/s00441-009-0794-x [PubMed: 19415333]

Citro R, d'Avenia M, De Marco M, et al. (2013). Polymorphisms of the antiapoptotic protein bag3 may play a role in the pathogenesis of tako-tsubo cardiomyopathy. Int J Cardiol, 168(2), 1663–1665. 10.1016/j.ijcard.2013.03.050 [PubMed: 23582692]

Colvin TA, Gabai VL, Gong J, et al. (2014). Hsp70-Bag3 interactions regulate cancer-related signaling networks. Cancer Res, 74(17), 4731–4740. 10.1158/0008-5472.CAN-14-0747 [PubMed: 24994713]

Crippa V, Sau D, Rusmini P, et al. (2010). The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). Hum Mol Genet, 19(17), 3440–3456. 10.1093/hmg/ddq257 [PubMed: 20570967]

d'Avenia M, Citro R, De Marco M, et al. (2015). A novel miR-371a-5p-mediated pathway, leading to BAG3 upregulation in cardiomyocytes in response to epinephrine, is lost in Takotsubo cardiomyopathy. Cell Death Dis, 6, e1948. 10.1038/cddis.2015.280 [PubMed: 26512958]

D'Avila F, Meregalli M, Lupoli S, et al. (2016). Exome sequencing identifies variants in two genes encoding the LIM-proteins NRAP and FHL1 in an Italian patient with BAG3 myofibrillar myopathy. J Muscle Res Cell Motil, 37(3), 101–115. 10.1007/s10974-016-9451-7 [PubMed: 27443559]

Das CK, Linder B, Bonn F, et al. (2018). BAG3 Overexpression and Cytoprotective Autophagy Mediate Apoptosis Resistance in Chemoresistant Breast Cancer Cells. Neoplasia, 20(3), 263–279. 10.1016/j.neo.2018.01.001 [PubMed: 29462756]

de Denus S, Mottet F, Korol S, et al. (2020). A genetic association study of heart failure: more evidence for the role of BAG3 in idiopathic dilated cardiomyopathy. ESC Heart Fail. 10.1002/ehf2.12934

De Marco M, Basile A, Iorio V, et al. (2018). Role of BAG3 in cancer progression: A therapeutic opportunity. Semin Cell Dev Biol, 78, 85–92. 10.1016/j.semcdb.2017.08.049 [PubMed: 28864347]

Dominguez F, Cuenca S, Bilinska Z, et al. (2018). Dilated Cardiomyopathy Due to BLC2-Associated Athanogene 3 (BAG3) Mutations. J Am Coll Cardiol, 72(20), 2471–2481. 10.1016/j.jacc.2018.08.2181 [PubMed: 30442290]

Doong H, Price J, Kim YS, et al. (2000). CAIR-1/BAG-3 forms an EGF-regulated ternary complex with phospholipase C-gamma and Hsp70/Hsc70. Oncogene, 19(38), 4385–4395. 10.1038/sj.onc.1203797 [PubMed: 10980614]

Du ZX, Meng X, Zhang HY, Guan Y, & Wang HQ (2008). Caspase-dependent cleavage of BAG3 in proteasome inhibitors-induced apoptosis in thyroid cancer cells. Biochem Biophys Res Commun, 369(3), 894–898. 10.1016/j.bbrc.2008.02.112 [PubMed: 18325327]

Esposito V, Baldi C, Zeppa P, et al. (2017). BAG3 Protein Is Over-Expressed in Endometrioid Endometrial Adenocarcinomas. J Cell Physiol, 232(2), 309–311. 10.1002/jcp.25489 [PubMed: 27414463]

Esslinger U, Garnier S, Korniat A, et al. (2017). Exome-wide association study reveals novel susceptibility genes to sporadic dilated cardiomyopathy. PLoS One, 12(3), e0172995. 10.1371/journal.pone.0172995 [PubMed: 28296976]

Falco A, Rosati A, Festa M, et al. (2013). BAG3 is a novel serum biomarker for pancreatic adenocarcinomas. Am J Gastroenterol, 108(7), 1178–1180. 10.1038/ajg.2013.128 [PubMed: 23821002]

Fang X, Bogomolovas J, Wu T, et al. (2017). Loss-of-function mutations in co-chaperone BAG3 destabilize small HSPs and cause cardiomyopathy. J Clin Invest, 127(8), 3189–3200. 10.1172/JCI94310 [PubMed: 28737513]

Feldman AM, Gordon J, Wang J, et al. (2016). BAG3 regulates contractility and Ca(2+) homeostasis in adult mouse ventricular myocytes. J Mol Cell Cardiol, 92, 10–20. 10.1016/j.yjmcc.2016.01.015 [PubMed: 26796036]

Festa M, Del Valle L, Khalili K, et al. (2011). BAG3 protein is overexpressed in human glioblastoma and is a potential target for therapy. Am J Pathol, 178(6), 2504–2512. 10.1016/j.ajpath.2011.02.002 [PubMed: 21561597]

Franaszczyk M, Bilinska ZT, Sobieszczanska-Malek M, et al. (2014). The BAG3 gene variants in Polish patients with dilated cardiomyopathy: four novel mutations and a genotype-phenotype correlation. J Transl Med, 12, 192. 10.1186/1479-5876-12-192 [PubMed: 25008357]

Fu H, Possenti A, Freer R, et al. (2019). A tau homeostasis signature is linked with the cellular and regional vulnerability of excitatory neurons to tau pathology. Nat Neurosci, 22(1), 47–56. 10.1038/s41593-018-0298-7 [PubMed: 30559469]

Fu J, Ma M, Song J, Pang M, Li G, & Zhang J (2020). BAG3 p.Pro209Ser mutation identified in a Chinese family with Charcot-Marie-Tooth disease. J Neurol, 267(4), 1080–1085. 10.1007/s00415-019-09680-8 [PubMed: 31853710]

Fuchs M, Poirier DJ, Seguin SJ, et al. (2009). Identification of the key structural motifs involved in HspB8/HspB6-Bag3 interaction. Biochem J, 425(1), 245–255. 10.1042/BJ20090907 [PubMed: 19845507]

Furusawa Y, Yunoki T, Hirano T, et al. (2018). Identification of genes and genetic networks associated with BAG3dependent cell proliferation and cell survival in human cervical cancer HeLa cells. Mol Med Rep, 18(4), 4138–4146. 10.3892/mmr.2018.9383 [PubMed: 30106105]

Gamerdinger M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, & Behl C (2009). Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. EMBO J, 28(7), 889–901. 10.1038/emboj.2009.29 [PubMed: 19229298]

Gamerdinger M, Kaya AM, Wolfrum U, Clement AM, & Behl C (2011). BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins. EMBO Rep, 12(2), 149–156. 10.1038/embor.2010.203 [PubMed: 21252941]

Garnier S, Hengstenberg C, Lamblin N, et al. (2015). Involvement of BAG3 and HSPB7 loci in various etiologies of systolic heart failure: Results of a European collaboration assembling more than 2000 patients. Int J Cardiol, 189, 105–107. 10.1016/j.ijcard.2015.04.003 [PubMed: 25889438]

Gentilella A, & Khalili K (2011). BAG3 expression in glioblastoma cells promotes accumulation of ubiquitinated clients in an Hsp70-dependent manner. J Biol Chem, 286(11), 9205–9215. 10.1074/jbc.M110.175836 [PubMed: 21233200]

Gentilella A, Passiatore G, Deshmane S, Turco MC, & Khalili K (2008). Activation of BAG3 by Egr-1 in response to FGF-2 in neuroblastoma cells. Oncogene, 27(37), 5011–5018. 10.1038/onc.2008.142 [PubMed: 18469860]

Ghasemi Tahrir F, Gupta M, Myers V, et al. (2019). Role of Bcl2-associated Athanogene 3 in Turnover of Gap Junction Protein, Connexin 43, in Neonatal Cardiomyocytes. Sci Rep, 9(1), 7658. 10.1038/s41598-019-44139-w [PubMed: 31114002]

Gout E, Gutkowska M, Takayama S, Reed JC, & Chroboczek J (2010). Co-chaperone BAG3 and adenovirus penton base protein partnership. J Cell Biochem, 111(3), 699–708. 10.1002/jcb.22756 [PubMed: 20607728]

Grigore AD, Jolly MK, Jia D, Farach-Carson MC, & Levine H (2016). Tumor Budding: The Name is EMT. Partial EMT. J Clin Med, 5(5). 10.3390/jcm5050051

Guilbert SM, Lambert H, Rodrigue MA, Fuchs M, Landry J, & Lavoie JN (2018). HSPB8 and BAG3 cooperate to promote spatial sequestration of ubiquitinated proteins and coordinate the cellular adaptive response to proteasome insufficiency. FASEB J, 32(7), 3518–3535. 10.1096/fj.201700558RR [PubMed: 29405094]

Habata S, Iwasaki M, Sugio A, et al. (2015). BAG3 increases the invasiveness of uterine corpus carcinoma cells by suppressing miR-29b and enhancing MMP2 expression. Oncol Rep, 33(5), 2613–2621. 10.3892/or.2015.3831 [PubMed: 25738313]

Habata S, Iwasaki M, Sugio A, et al. (2016). BAG3-mediated Mcl-1 stabilization contributes to drug resistance via interaction with USP9X in ovarian cancer. Int J Oncol, 49(1), 402–410. 10.3892/ijo.2016.3494 [PubMed: 27120977]

Harty RN, Brown ME, Wang G, Huibregtse J, & Hayes FP (2000). A PPxY motif within the VP40 protein of Ebola virus interacts physically and functionally with a ubiquitin ligase: implications for filovirus budding. Proc Natl Acad Sci U S A, 97(25), 13871–13876. 10.1073/pnas.250277297 [PubMed: 11095724]

Heberle H, Meirelles GV, da Silva FR, Telles GP, & Minghim R (2015). InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. BMC Bioinformatics, 16, 169. 10.1186/s12859-015-0611-3 [PubMed: 25994840]

Henderson MX, Trojanowski JQ, & Lee VM (2019). alpha-Synuclein pathology in Parkinson's disease and related alpha-synucleinopathies. Neurosci Lett, 709, 134316. 10.1016/j.neulet.2019.134316 [PubMed: 31170426]

Hiebel C, Sturner E, Hoffmeister M, et al. (2020). BAG3 Proteomic Signature under Proteostasis Stress. Cells, 9(11). 10.3390/cells9112416

Hipp MS, Park SH, & Hartl FU (2014). Proteostasis impairment in protein-misfolding and -aggregation diseases. Trends Cell Biol, 24(9), 506–514. 10.1016/j.tcb.2014.05.003 [PubMed: 24946960]

Hishiya A, Kitazawa T, & Takayama S (2010). BAG3 and Hsc70 interact with actin capping protein CapZ to maintain myofibrillar integrity under mechanical stress. Circ Res, 107(10), 1220–1231. 10.1161/CIRCRESAHA.110.225649 [PubMed: 20884878]

Homma S, Iwasaki M, Shelton GD, Engvall E, Reed JC, & Takayama S (2006). BAG3 deficiency results in fulminant myopathy and early lethality. Am J Pathol, 169(3), 761–773. 10.2353/ajpath.2006.060250 [PubMed: 16936253]

Im CN, Yun HH, Song B, et al. (2016). BIS-mediated STAT3 stabilization regulates glioblastoma stem cell-like phenotypes. Oncotarget, 7(23), 35056–35070. 10.18632/oncotarget.9039 [PubMed: 27145367]

Inomata Y, Nagasaka S, Miyate K, et al. (2018). Bcl-2-associated athanogene 3 (BAG3) is an enhancer of small heat shock protein turnover via activation of autophagy in the heart. Biochem Biophys Res Commun, 496(4), 1141–1147. 10.1016/j.bbrc.2018.01.158 [PubMed: 29409895]

Iwasaki M, Homma S, Hishiya A, Dolezal SJ, Reed JC, & Takayama S (2007). BAG3 regulates motility and adhesion of epithelial cancer cells. Cancer Res, 67(21), 10252–10259. 10.1158/0008-5472.CAN-07-0618 [PubMed: 17974966]

Iwasaki M, Tanaka R, Hishiya A, Homma S, Reed JC, & Takayama S (2010). BAG3 directly associates with guanine nucleotide exchange factor of Rap1, PDZGEF2, and regulates cell adhesion. Biochem Biophys Res Commun, 400(3), 413–418. 10.1016/j.bbrc.2010.08.092 [PubMed: 20800573]

Janin A, N'Guyen K, Habib G, et al. (2017). Truncating mutations on myofibrillar myopathies causing genes as prevalent molecular explanations on patients with dilated cardiomyopathy. Clin Genet, 92(6), 616–623. 10.1111/cge.13043 [PubMed: 28436997]

Ji C, Tang M, Zeidler C, Hohfeld J, & Johnson GV (2019). BAG3 and SYNPO (synaptopodin) facilitate phospho-MAPT/Tau degradation via autophagy in neuronal processes. Autophagy, 15(7), 1199–1213. 10.1080/15548627.2019.1580096 [PubMed: 30744518]

Judge LM, Perez-Bermejo JA, Truong A, et al. (2017). A BAG3 chaperone complex maintains cardiomyocyte function during proteotoxic stress. JCI Insight, 2(14). 10.1172/jci.insight.94623

Karpel-Massler G, Shu C, Chau L, et al. (2015). Combined inhibition of Bcl-2/Bcl-xL and Usp9X/Bag3 overcomes apoptotic resistance in glioblastoma in vitro and in vivo. Oncotarget, 6(16), 14507–14521. 10.18632/oncotarget.3993 [PubMed: 26008975]

Kathage B, Gehlert S, Ulbricht A, et al. (2017). The cochaperone BAG3 coordinates protein synthesis and autophagy under mechanical strain through spatial regulation of mTORC1. Biochim Biophys Acta Mol Cell Res, 1864(1), 62–75. 10.1016/j.bbamcr.2016.10.007 [PubMed: 27756573]

Klimek C, Kathage B, Wordehoff J, & Hohfeld J (2017). BAG3-mediated proteostasis at a glance. J Cell Sci, 130(17), 2781–2788. 10.1242/jcs.203679 [PubMed: 28808089]

Kogel D, Linder B, Brunschweiger A, Chines S, & Behl C (2020). At the Crossroads of Apoptosis and Autophagy: Multiple Roles of the Co-Chaperone BAG3 in Stress and Therapy Resistance of Cancer. Cells, 9(3). 10.3390/cells9030574

Konersman CG, Bordini BJ, Scharer G, et al. (2015). BAG3 myofibrillar myopathy presenting with cardiomyopathy. Neuromuscul Disord, 25(5), 418–422. 10.1016/j.nmd.2015.01.009 [PubMed: 25728519]

Kong DH, Li S, Du ZX, et al. (2016). BAG3 elevation inhibits cell proliferation via direct interaction with G6PD in hepatocellular carcinomas. Oncotarget, 7(1), 700–711. 10.18632/oncotarget.6396 [PubMed: 26621836]

Kostera-Pruszczyk A, Suszek M, Ploski R, et al. (2015). BAG3-related myopathy, polyneuropathy and cardiomyopathy with long QT syndrome. J Muscle Res Cell Motil, 36(6), 423–432. 10.1007/s10974-015-9431-3 [PubMed: 26545904]

Kruger L, & Mandelkow EM (2016). Tau neurotoxicity and rescue in animal models of human Tauopathies. Curr Opin Neurobiol, 36, 52–58. 10.1016/j.conb.2015.09.004 [PubMed: 26431808]

Kyratsous CA, & Silverstein SJ (2007). BAG3, a host cochaperone, facilitates varicella-zoster virus replication. J Virol, 81(14), 7491–7503. 10.1128/JVI.00442-07 [PubMed: 17475647]

Kyratsous CA, & Silverstein SJ (2008). The co-chaperone BAG3 regulates Herpes Simplex Virus replication. Proc Natl Acad Sci U S A, 105(52), 20912–20917. 10.1073/pnas.0810656105 [PubMed: 19088197]

Lee HC, Cherk SW, Chan SK, et al. (2012). BAG3-related myofibrillar myopathy in a Chinese family. Clin Genet, 81(4), 394–398. 10.1111/j.1399-0004.2011.01659.x [PubMed: 21361913]

Lee JH, Takahashi T, Yasuhara N, Inazawa J, Kamada S, & Tsujimoto Y (1999). Bis, a Bcl-2-binding protein that synergizes with Bcl-2 in preventing cell death. Oncogene, 18(46), 6183–6190. 10.1038/sj.onc.1203043 [PubMed: 10597216]

Lei Z, Brizzee C, & Johnson GV (2015). BAG3 facilitates the clearance of endogenous tau in primary neurons. Neurobiol Aging, 36(1), 241–248. 10.1016/j.neurobiolaging.2014.08.012 [PubMed: 25212465]

Li C, An MX, Jiang JY, et al. (2019). BAG3 Suppresses Loading of Ago2 to IL6 mRNA in Pancreatic Ductal Adenocarcinoma. Front Oncol, 9, 225. 10.3389/fonc.2019.00225 [PubMed: 31001483]

Li C, Jiang JY, Wang JM, et al. (2018). BAG3 regulates stability of IL-8 mRNA via interplay between HuR and miR-4312 in PDACs. Cell Death Dis, 9(9), 863. 10.1038/s41419-018-0874-5 [PubMed: 30154469]

Li N, Chen M, Cao Y, et al. (2018). Bcl-2-associated athanogene 3(BAG3) is associated with tumor cell proliferation, migration, invasion and chemoresistance in colorectal cancer. BMC Cancer, 18(1), 793. 10.1186/s12885-018-4657-2 [PubMed: 30081850]

Li N, Du ZX, Zong ZH, et al. (2013). PKCdelta-mediated phosphorylation of BAG3 at Ser187 site induces epithelial-mesenchymal transition and enhances invasiveness in thyroid cancer FRO cells. Oncogene, 32(38), 4539–4548. 10.1038/onc.2012.466 [PubMed: 23108398]

Liang J, Sagum CA, Bedford MT, et al. (2017). Chaperone-Mediated Autophagy Protein BAG3 Negatively Regulates Ebola and Marburg VP40-Mediated Egress. PLoS Pathog, 13(1), e1006132. 10.1371/journal.ppat.1006132 [PubMed: 28076420]

Lin H, Tang M, Ji C, et al. (2021). BAG3 regulation of Rab35 mediates the ESCRT/endolysosome pathway and tau clearance. BioRxiv. 10.1101/2021.01.25.428055

Lin YH, Li J, Swanson ER, & Russell B (2013). CapZ and actin capping dynamics increase in myocytes after a bout of exercise and abates in hours after stimulation ends. J Appl Physiol (1985), 114(11), 1603–1609. 10.1152/japplphysiol.01283.2012 [PubMed: 23493359]

Liu BQ, Zhang S, Li S, et al. (2017). BAG3 promotes stem cell-like phenotype in breast cancer by upregulation of CXCR4 via interaction with its transcript. Cell Death Dis, 8(7), e2933. 10.1038/cddis.2017.324 [PubMed: 28703799]

Luders J, Demand J, & Hohfeld J (2000). The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. J Biol Chem, 275(7), 4613–4617. 10.1074/jbc.275.7.4613 [PubMed: 10671488]

Luo YB, Peng Y, Lu Y, et al. (2020). Expanding the Clinico-Genetic Spectrum of Myofibrillar Myopathy: Experience From a Chinese Neuromuscular Center. Front Neurol, 11, 1014. 10.3389/fneur.2020.01014 [PubMed: 33041974]

Mani J, Antonietti P, Rakel S, et al. (2016). Knockdown of BAG3 sensitizes bladder cancer cells to treatment with the BH3 mimetic ABT-737. World J Urol, 34(2), 197–205. 10.1007/s00345-015-1616-2 [PubMed: 26100943]

McDermott-Roe C, Lv W, Maximova T, et al. (2019). Investigation of a dilated cardiomyopathy-associated variant in BAG3 using genome-edited iPSC-derived cardiomyocytes. JCI Insight, 4(22). 10.1172/jci.insight.128799

Meister-Broekema M, Freilich R, Jagadeesan C, et al. (2018). Myopathy associated BAG3 mutations lead to protein aggregation by stalling Hsp70 networks. Nat Commun, 9(1), 5342. 10.1038/s41467-018-07718-5 [PubMed: 30559338]

Meng X, Kong DH, Li N, et al. (2014). Knockdown of BAG3 induces epithelial-mesenchymal transition in thyroid cancer cells through ZEB1 activation. Cell Death Dis, 5, e1092. 10.1038/cddis.2014.32 [PubMed: 24577090]

Miszalski-Jamka K, Jefferies JL, Mazur W, et al. (2017). Novel Genetic Triggers and Genotype-Phenotype Correlations in Patients With Left Ventricular Noncompaction. Circ Cardiovasc Genet, 10(4). 10.1161/CIRCGENETICS.117.001763

Myers VD, Gerhard GS, McNamara DM, et al. (2018). Association of Variants in BAG3 With Cardiomyopathy Outcomes in African American Individuals. JAMA Cardiol, 3(10), 929–938. 10.1001/jamacardio.2018.2541 [PubMed: 30140897]

Ng D, Johnston JJ, Teer JK, et al. (2013). Interpreting secondary cardiac disease variants in an exome cohort. Circ Cardiovasc Genet, 6(4), 337–346. 10.1161/CIRCGENETICS.113.000039 [PubMed: 23861362]

Nickel W, & Rabouille C (2009). Mechanisms of regulated unconventional protein secretion. Nat Rev Mol Cell Biol, 10(2), 148–155. 10.1038/nrm2617 [PubMed: 19122676]

Norland K, Sveinbjornsson G, Thorolfsdottir RB, et al. (2019). Sequence variants with large effects on cardiac electrophysiology and disease. Nat Commun, 10(1), 4803. 10.1038/s41467-019-12682-9 [PubMed: 31641117]

Norton N, Li D, Rieder MJ, et al. (2011). Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. Am J Hum Genet, 88(3), 273–282. 10.1016/j.ajhg.2011.01.016 [PubMed: 21353195]

Noury JB, Maisonobe T, Richard P, Delague V, Malfatti E, & Stojkovic T (2018). Rigid spine syndrome associated with sensory-motor axonal neuropathy resembling Charcot-Marie-Tooth disease is characteristic of Bcl-2-associated athanogene-3 gene mutations even without cardiac involvement. Muscle Nerve, 57(2), 330–334. 10.1002/mus.25631 [PubMed: 28224639]

Odgerel Z, Sarkozy A, Lee HS, et al. (2010). Inheritance patterns and phenotypic features of myofibrillar myopathy associated with a BAG3 mutation. Neuromuscul Disord, 20(7), 438–442. 10.1016/j.nmd.2010.05.004 [PubMed: 20605452]

Ogen-Shtern N, Ben David T, & Lederkremer GZ (2016). Protein aggregation and ER stress. Brain Res, 1648(Pt B), 658–666. 10.1016/j.brainres.2016.03.044 [PubMed: 27037184]

Overton HA, Ihara T, & Bishop DH (1987). Identification of the N and NSS proteins coded by the ambisense S RNA of Punta Toro phlebovirus using monospecific antisera raised to baculovirus

expressed N and NSS proteins. Virology, 157(2), 338–350. 10.1016/0042-6822(87)90276-5 [PubMed: 2950650]

Pappas CT, Bhattacharya N, Cooper JA, & Gregorio CC (2008). Nebulin interacts with CapZ and regulates thin filament architecture within the Z-disc. Mol Biol Cell, 19(5), 1837–1847. 10.1091/mbc.E07-07-0690 [PubMed: 18272787]

Pasillas MP, Shields S, Reilly R, et al. (2015). Proteomic analysis reveals a role for Bcl2-associated athanogene 3 and major vault protein in resistance to apoptosis in senescent cells by regulating ERK1/2 activation. Mol Cell Proteomics, 14(1), 1–14. 10.1074/mcp.M114.037697 [PubMed: 24997994]

Paulson HL, Shakkottai VG, Clark HB, & Orr HT (2017). Polyglutamine spinocerebellar ataxias - from genes to potential treatments. Nat Rev Neurosci, 18(10), 613–626. 10.1038/nrn.2017.92 [PubMed: 28855740]

Qiu S, Sun L, Jin Y, An Q, Weng C, & Zheng J (2017). Silencing of BAG3 promotes the sensitivity of ovarian cancer cells to cisplatin via inhibition of autophagy. Oncol Rep, 38(1), 309–316. 10.3892/or.2017.5706 [PubMed: 28628188]

Qiu S, Sun L, Zhang Y, & Han S (2019). Downregulation of BAG3 attenuates cisplatin resistance by inhibiting autophagy in human epithelial ovarian cancer cells. Oncol Lett, 18(2), 1969–1978. 10.3892/ol.2019.10494 [PubMed: 31423267]

Quintana MT, Parry TL, He J, et al. (2016). Cardiomyocyte-Specific Human Bcl2-Associated Anthanogene 3 P209L Expression Induces Mitochondrial Fragmentation, Bcl2-Associated Anthanogene 3 Haploinsufficiency, and Activates p38 Signaling. Am J Pathol, 186(8), 1989–2007. 10.1016/j.ajpath.2016.03.017 [PubMed: 27321750]

Rafiq MA, Chaudhry A, Care M, Spears DA, Morel CF, & Hamilton RM (2017). Whole exome sequencing identified 1 base pair novel deletion in BCL2-associated athanogene 3 (BAG3) gene associated with severe dilated cardiomyopathy (DCM) requiring heart transplant in multiple family members. Am J Med Genet A, 173(3), 699–705. 10.1002/ajmg.a.38087 [PubMed: 28211974]

Rauch JN, Tse E, Freilich R, et al. (2017). BAG3 Is a Modular, Scaffolding Protein that physically Links Heat Shock Protein 70 (Hsp70) to the Small Heat Shock Proteins. J Mol Biol, 429(1), 128–141. 10.1016/j.jmb.2016.11.013 [PubMed: 27884606]

Raudvere U, Kolberg L, Kuzmin I, et al. (2019). g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res, 47(W1), W191–W198. 10.1093/nar/gkz369 [PubMed: 31066453]

Romano MF, Festa M, Pagliuca G, et al. (2003). BAG3 protein controls B-chronic lymphocytic leukaemia cell apoptosis. Cell Death Differ, 10(3), 383–385. 10.1038/sj.cdd.4401167 [PubMed: 12700638]

Rosati A, Basile A, D'Auria R, et al. (2015). BAG3 promotes pancreatic ductal adenocarcinoma growth by activating stromal macrophages. Nat Commun, 6, 8695. 10.1038/ncomms9695 [PubMed: 26522614]

Rosati A, Bersani S, Tavano F, et al. (2012). Expression of the antiapoptotic protein BAG3 is a feature of pancreatic adenocarcinoma and its overexpression is associated with poorer survival. Am J Pathol, 181(5), 1524–1529. 10.1016/j.ajpath.2012.07.016 [PubMed: 22944597]

Rosati A, Leone A, Del Valle L, Amini S, Khalili K, & Turco MC (2007). Evidence for BAG3 modulation of HIV-1 gene transcription. J Cell Physiol, 210(3), 676–683. 10.1002/jcp.20865 [PubMed: 17187345]

Rubinsztein DC, Leggo J, Coles R, et al. (1996). Phenotypic characterization of individuals with 30–40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36–39 repeats. Am J Hum Genet, 59(1), 16–22. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8659522 [PubMed: 8659522]

Ruparelia AA, McKaige EA, Williams C, et al. (2020). Metformin rescues muscle function in BAG3 myofibrillar myopathy models. Autophagy, 1–17. 10.1080/15548627.2020.1833500

Ruparelia AA, Oorschot V, Vaz R, Ramm G, & Bryson-Richardson RJ (2014). Zebrafish models of BAG3 myofibrillar myopathy suggest a toxic gain of function leading to BAG3 insufficiency. Acta Neuropathol, 128(6), 821–833. 10.1007/s00401-014-1344-5 [PubMed: 25273835]

Saffitz JE, Laing JG, & Yamada KA (2000). Connexin expression and turnover : implications for cardiac excitability. Circ Res, 86(7), 723–728. 10.1161/01.res.86.7.723 [PubMed: 10764404]

Sahlin E, Green A, Gustavsson P, et al. (2019). Identification of putative pathogenic single nucleotide variants (SNVs) in genes associated with heart disease in 290 cases of stillbirth. PLoS One, 14(1), e0210017. 10.1371/journal.pone.0210017 [PubMed: 30615648]

Santoro A, Nicolin V, Florenzano F, Rosati A, Capunzo M, & Nori SL (2017). BAG3 is involved in neuronal differentiation and migration. Cell Tissue Res, 368(2), 249–258. 10.1007/s00441-017-2570-7 [PubMed: 28144784]

Sarparanta J, Jonson PH, Golzio C, et al. (2012). Mutations affecting the cytoplasmic functions of the co-chaperone DNAJB6 cause limb-girdle muscular dystrophy. Nat Genet, 44(4), 450–455, S451–452. 10.1038/ng.1103 [PubMed: 22366786]

Schanzer A, Rupp S, Graf S, et al. (2018). Dysregulated autophagy in restrictive cardiomyopathy due to Pro209Leu mutation in BAG3. Mol Genet Metab, 123(3), 388–399. 10.1016/j.ymgme.2018.01.001 [PubMed: 29338979]

Selcen D, Muntoni F, Burton BK, et al. (2009). Mutation in BAG3 causes severe dominant childhood muscular dystrophy. Ann Neurol, 65(1), 83–89. 10.1002/ana.21553 [PubMed: 19085932]

Semmler AL, Sacconi S, Bach JE, et al. (2014). Unusual multisystemic involvement and a novel BAG3 mutation revealed by NGS screening in a large cohort of myofibrillar myopathies. Orphanet J Rare Dis, 9, 121. 10.1186/s13023-014-0121-9 [PubMed: 25208129]

Shamas-Din A, Kale J, Leber B, & Andrews DW (2013). Mechanisms of action of Bcl-2 family proteins. Cold Spring Harb Perspect Biol, 5(4), a008714. 10.1101/cshperspect.a008714 [PubMed: 23545417]

Shi H, Chen W, Dong Y, Lu X, Zhang W, & Wang L (2018). BAG3 promotes chondrosarcoma progression by upregulating the expression of beta-catenin. Mol Med Rep, 17(4), 5754–5763. 10.3892/mmr.2018.8611 [PubMed: 29484408]

Shy M, Rebelo AP, Feely SM, et al. (2018). Mutations in BAG3 cause adult-onset Charcot-Marie-Tooth disease. J Neurol Neurosurg Psychiatry, 89(3), 313–315. 10.1136/jnnp-2017-315929 [PubMed: 28754666]

Sondermann H, Scheufler C, Schneider C, Hohfeld J, Hartl FU, & Moarefi I (2001). Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. Science, 291(5508), 1553–1557. 10.1126/science.1057268 [PubMed: 11222862]

Sturner E, & Behl C (2017). The Role of the Multifunctional BAG3 Protein in Cellular Protein Quality Control and in Disease. Front Mol Neurosci, 10, 177. 10.3389/fnmol.2017.00177 [PubMed: 28680391]

Sugio A, Iwasaki M, Habata S, et al. (2014). BAG3 upregulates Mcl-1 through downregulation of miR-29b to induce anticancer drug resistance in ovarian cancer. Gynecol Oncol, 134(3), 615–623. 10.1016/j.ygyno.2014.06.024 [PubMed: 24992675]

Takayama S, Xie Z, & Reed JC (1999). An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. J Biol Chem, 274(2), 781–786. 10.1074/jbc.274.2.781 [PubMed: 9873016]

Tang M, Ji C, Pallo S, Rahman I, & Johnson GVW (2018). Nrf2 mediates the expression of BAG3 and autophagy cargo adaptor proteins and tau clearance in an age-dependent manner. Neurobiol Aging, 63, 128–139. 10.1016/j.neurobiolaging.2017.12.001 [PubMed: 29304346]

Tapia-Rojas C, Cabezas-Opazo F, Deaton CA, Vergara EH, Johnson GVW, & Quintanilla RA (2019). It's all about tau. Prog Neurobiol, 175, 54–76. 10.1016/j.pneurobio.2018.12.005 [PubMed: 30605723]

Tarnovskaya S, Kiselev A, Kostareva A, & Frishman D (2017). Structural consequences of mutations associated with idiopathic restrictive cardiomyopathy. Amino Acids, 49(11), 1815–1829. 10.1007/s00726-017-2480-8 [PubMed: 28831623]

Toro R, Perez-Serra A, Campuzano O, et al. (2016). Familial Dilated Cardiomyopathy Caused by a Novel Frameshift in the BAG3 Gene. PLoS One, 11(7), e0158730. 10.1371/journal.pone.0158730 [PubMed: 27391596]

Valdez BC, Murray D, Ramdas L, et al. (2008). Altered gene expression in busulfan-resistant human myeloid leukemia. Leuk Res, 32(11), 1684–1697. 10.1016/j.leukres.2008.01.016 [PubMed: 18339423]

van der Kolk NM, Arts P, van Uden IW, et al. (2016). Progressive multifocal leukoencephalopathy in an immunocompetent patient. Ann Clin Transl Neurol, 3(3), 226–232. 10.1002/acn3.279 [PubMed: 27042682]

Villard E, Perret C, Gary F, et al. (2011). A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy. Eur Heart J, 32(9), 1065–1076. 10.1093/eurheartj/ehr105 [PubMed: 21459883]

Wang CY, Guo ST, Croft A, et al. (2018). BAG3-dependent expression of Mcl-1 confers resistance of mutant KRAS colon cancer cells to the HSP90 inhibitor AUY922. Mol Carcinog, 57(2), 284–294. 10.1002/mc.22755 [PubMed: 29068469]

Wise DR, & Thompson CB (2010). Glutamine addiction: a new therapeutic target in cancer. Trends Biochem Sci, 35(8), 427–433. 10.1016/j.tibs.2010.05.003 [PubMed: 20570523]

Xiang W, Yang CY, & Bai L (2018). MCL-1 inhibition in cancer treatment. Onco Targets Ther, 11, 7301–7314. 10.2147/OTT.S146228 [PubMed: 30425521]

Xiang Y, Stine ZE, Xia J, et al. (2015). Targeted inhibition of tumor-specific glutaminase diminishes cell-autonomous tumorigenesis. J Clin Invest, 125(6), 2293–2306. 10.1172/JCI75836 [PubMed: 25915584]

Xiao H, Cheng S, Tong R, et al. (2014). BAG3 regulates epithelial-mesenchymal transition and angiogenesis in human hepatocellular carcinoma. Lab Invest, 94(3), 252–261. 10.1038/labinvest.2013.151 [PubMed: 24365746]

Xu Z, Graham K, Foote M, et al. (2013). 14-3-3 protein targets misfolded chaperone-associated proteins to aggresomes. J Cell Sci, 126(Pt 18), 4173–4186. 10.1242/jcs.126102 [PubMed: 23843611]

Yan J, Liu C, Jiang JY, et al. (2017). BAG3 promotes proliferation of ovarian cancer cells via post-transcriptional regulation of Skp2 expression. Biochim Biophys Acta Mol Cell Res, 1864(10), 1668–1678. 10.1016/j.bbamcr.2017.06.004 [PubMed: 28624440]

Yang X, Tian Z, Gou WF, et al. (2013). Bag-3 expression is involved in pathogenesis and progression of colorectal carcinomas. Histol Histopathol, 28(9), 1147–1156. 10.14670/HH-28.1147 [PubMed: 23553442]

Yeo CD, Park GS, Kang N, et al. (2015). Bis Expression in Patients with Surgically Resected Lung Cancer and its Clinical Significance. Ann Surg Oncol, 22 Suppl 3, S1365–1370. 10.1245/s10434-015-4576-2 [PubMed: 25968616]

Young P, Anderton E, Paschos K, White R, & Allday MJ (2008). Epstein-Barr virus nuclear antigen (EBNA) 3A induces the expression of and interacts with a subset of chaperones and co-chaperones. J Gen Virol, 89(Pt 4), 866–877. 10.1099/vir.0.83414-0 [PubMed: 18343826]

Yuan Y, Jiang JY, Wang JM, et al. (2019). BAG3-positive pancreatic stellate cells promote migration and invasion of pancreatic ductal adenocarcinoma. J Cell Mol Med, 23(8), 5006–5016. 10.1111/jcmm.14352 [PubMed: 31119886]

Zhang L, Zhang ZP, Zhang XE, Lin FS, & Ge F (2010). Quantitative proteomics analysis reveals BAG3 as a potential target to suppress severe acute respiratory syndrome coronavirus replication. J Virol, 84(12), 6050–6059. 10.1128/JVI.00213-10 [PubMed: 20392858]

Zhang Y, & Weinberg RA (2018). Epithelial-to-mesenchymal transition in cancer: complexity and opportunities. Front Med, 12(4), 361–373. 10.1007/s11684-018-0656-6 [PubMed: 30043221]

Zhao S, Wang JM, Yan J, et al. (2019). BAG3 promotes autophagy and glutaminolysis via stabilizing glutaminase. Cell Death Dis, 10(4), 284. 10.1038/s41419-019-1504-6 [PubMed: 30910998]

Zhou J, Chow HM, Liu Y, et al. (2020). Cyclin-Dependent Kinase 5-Dependent BAG3 Degradation Modulates Synaptic Protein Turnover. Biol Psychiatry, 87(8), 756–769. 10.1016/j.biopsych.2019.11.013 [PubMed: 31955914]

Zhu H, Wu W, Fu Y, et al. (2014). Overexpressed BAG3 is a potential therapeutic target in chronic lymphocytic leukemia. Ann Hematol, 93(3), 425–435. 10.1007/s00277-013-1883-1 [PubMed: 23978946]

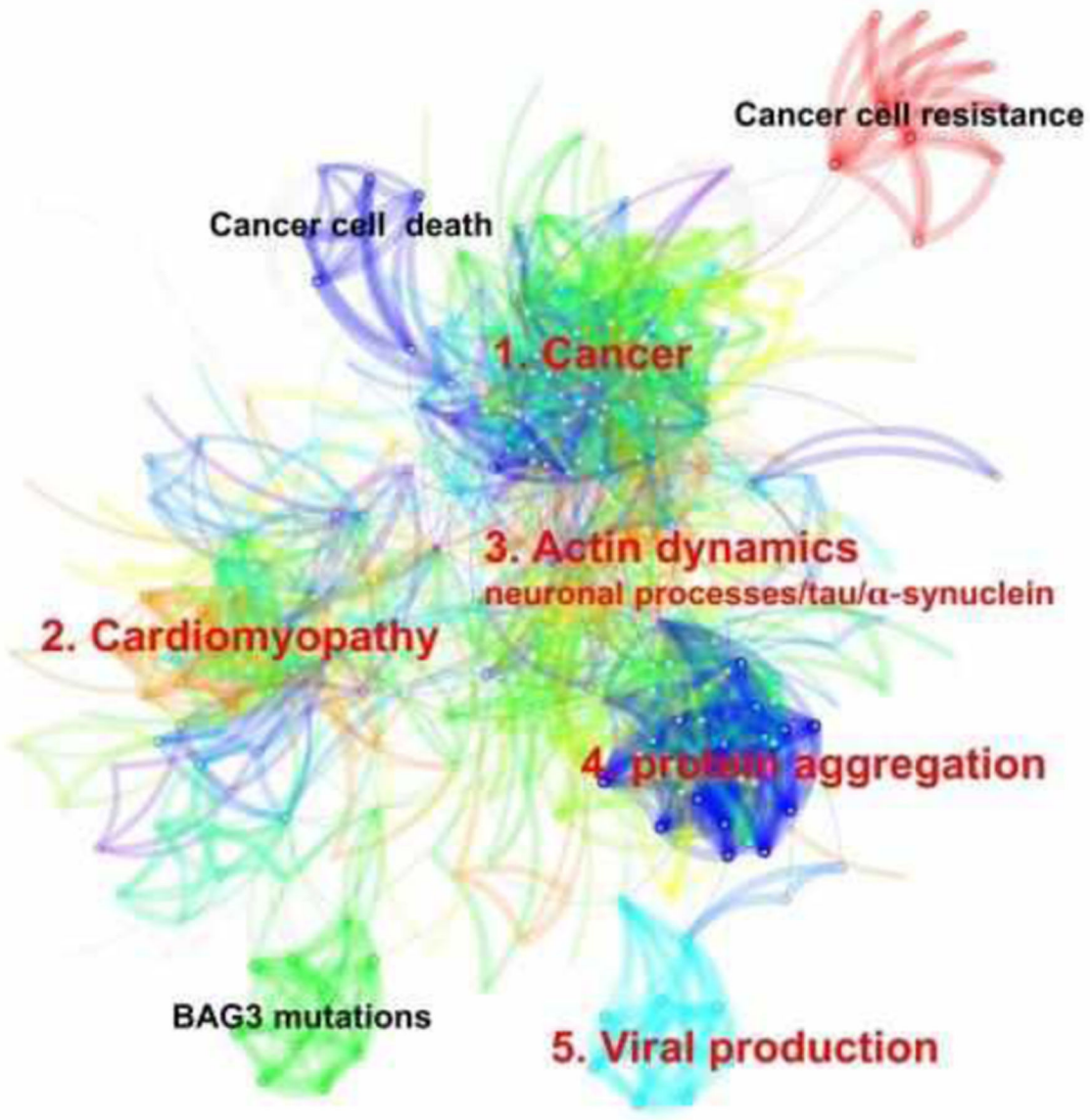

Figure 1. Cluster analysis of research papers about BAG3 in the past decade.

BAG3 is used as the key word to screen for research papers since 2011 in the Web of Science data base. The reviews or comments are filtered out, and the remaining citations were exported to Citespace for cluster analysis based on reference. Five major clusters including cancer, cardiomyopathy, actin dynamics, protein aggregation and viral production and three minor cluster such as cancer cell resistance, cancer cell death, BAG3 mutations are labeled in the diagram.

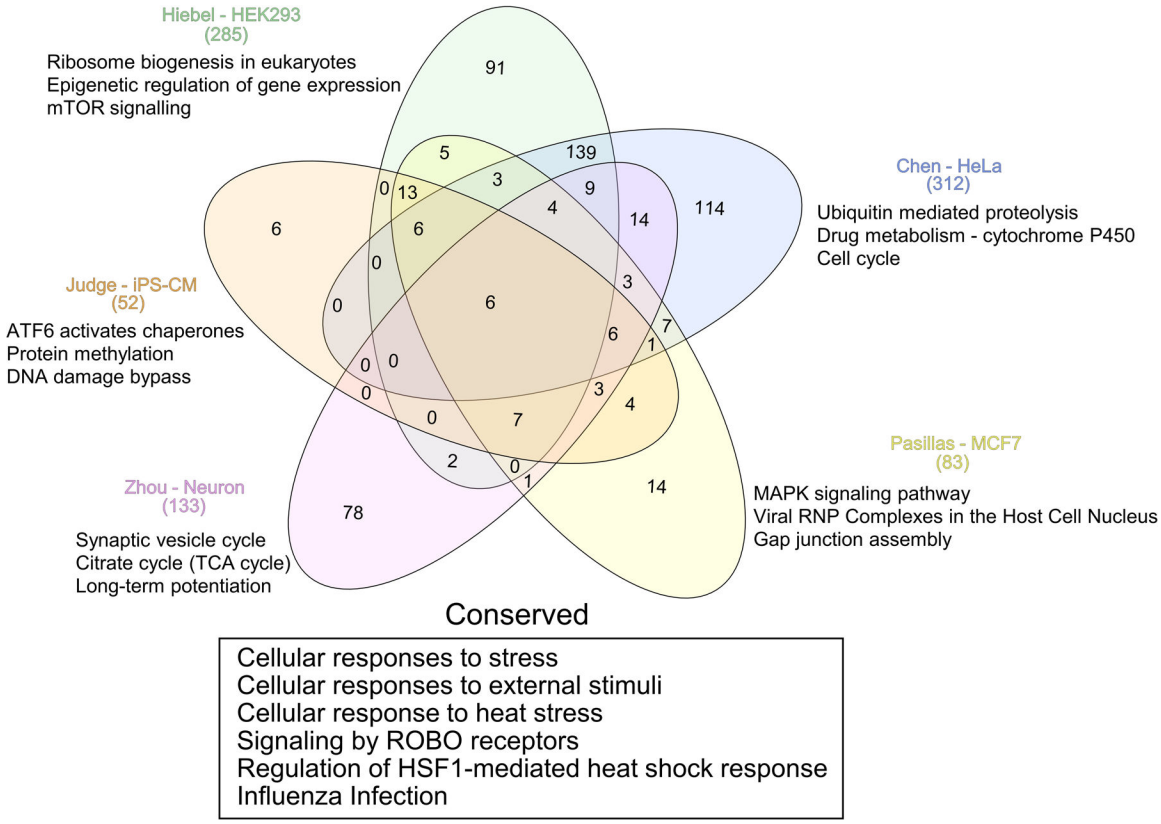

Figure 2. Pathway meta-analysis of BAG3 associated proteins.

Five separate IP-MS studies assessing protein co-immunoprecipitation with BAG3 were compared and analyzed through gProfiler to determine the statistically significant pathways over-represented by BAG3 interacting proteins across cell types. An FDR p value cutoff of 0.05 was used to determine statistically significant pathway over-representation. KEGG and Reactome annotations were considered. A Venn diagram was produced using InteractiVenn (Heberle, Meirelles, da Silva, Telles, & Minghim, 2015).

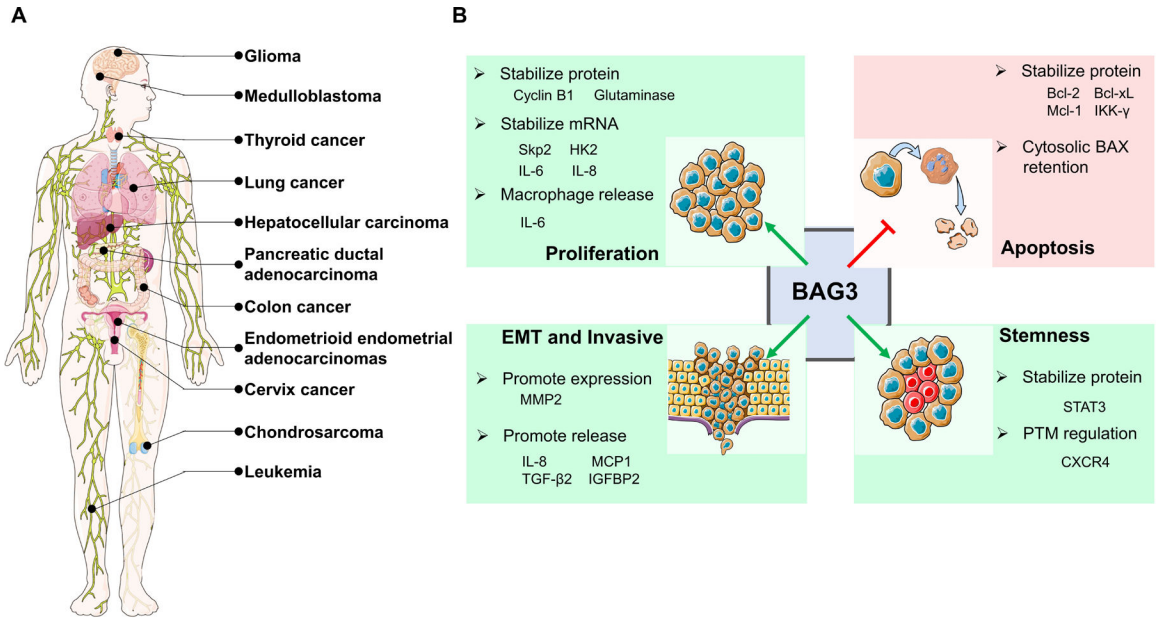

Figure 3. Functions of BAG3 in Cancer.

(A) An illustration showing the cancers related to increased BAG3 expression. (B) A diagram showing that BAG3 promotes proliferation, EMT, invasiveness, and stemness while inhibiting the apoptosis of cancer cells. The underlining molecular mechanism are listed in the box.

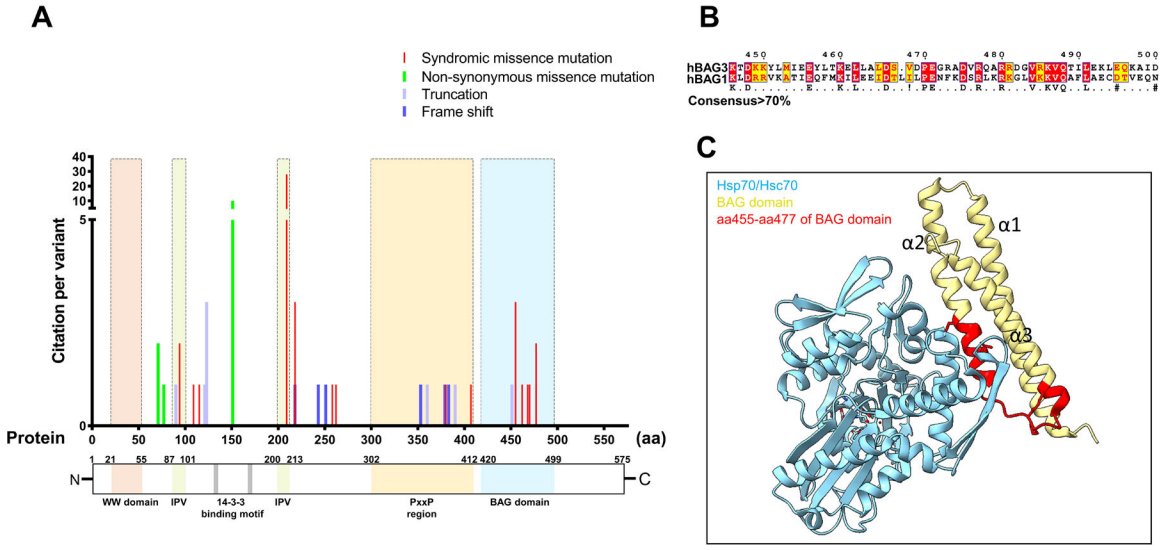

Figure 4. The majority of syndromic mutations in BAG3 are located in the BAG domain.

(A) A diagram that maps the reported variants of BAG3 on the protein sequence. BAG3 variants are color coded and indicate syndromic missense mutations, non-syndromic missense mutations, truncations and frame shifts. (B) Structure-based sequence alignment of BAG domain from BAG3 and BAG1. The sequence ticker of the protein is based on the original sequence of BAG3. (C) The crystal structure of the BAG domain of human BAG1 (yellow) associating with the ATPase domain of bovine Hsp70 (blue, PDB: 1HX1).

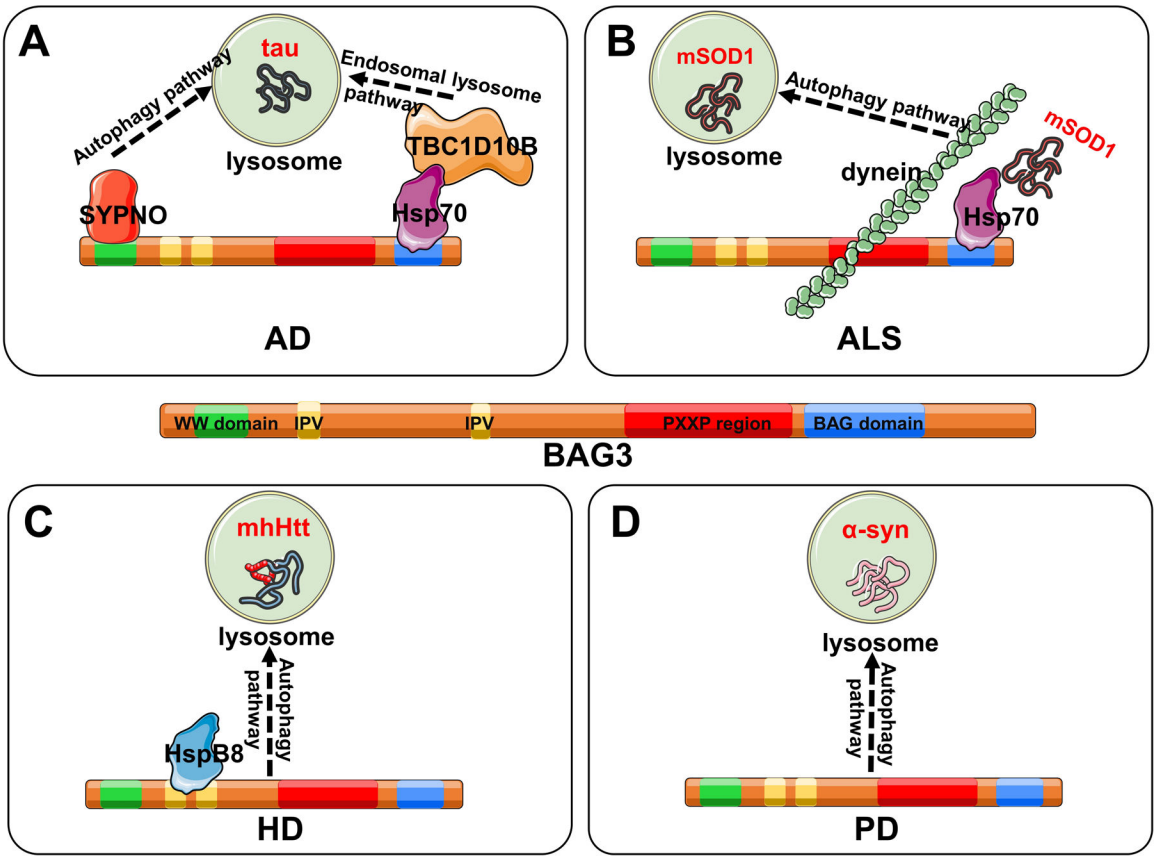

Figure 5. BAG3 in neurodegenerative disorders.

Diagrams illustrate the molecular mechanism of BAG3 in regulating neurodegenerative disorders including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and Parkinson's disease (PD).

Table 1.

A list of reported BAG3 variants and the related disease

Site	Citation per variant	Mutation	Citation	Disease
71	1	R71W	(Norton et al., 2011)	DCM
94	2	I94F	(Miszalski-Jamka et al., 2017; Norton et al., 2011)	DCM
109	1	H109R	(Norton et al., 2011)	DCM
115	1	P115S	(Norton et al., 2011)	DCM
209	23	P209L	(Adriaenssens et al., 2020; Andersen et al., 2018; Arimura, Ishikawa, Nunoda, Kawai, & Kimura, 2011; D'Avila et al., 2016; Dominguez et al., 2018; Guilbert et al., 2018; Inomata et al., 2018; Konersman et al., 2015; Kostera-Pruszczyk et al., 2015; Lee et al., 2012; Luo et al., 2020; Meister-Broekema et al., 2018; Norton et al., 2011; Noury et al., 2018; Odgerel et al., 2010; Quintana et al., 2016; Ruparelia et al., 2020; Ruparelia, Oorschot, Vaz, Ramm, & Bryson-Richardson, 2014; Sarparanta et al., 2012; Schanzer et al., 2018; Selcen et al., 2009; Tarnovskaya, Kiselev, Kostareva, & Frishman, 2017)	MFM /axonal neuropathy/ myopathy/CMT
209	2	P209Q	(Meister-Broekema et al., 2018; Semmler et al., 2014)	MFM/ axonal sensorimotor polyneuropathy
209	3	P209S	(Adriaenssens et al., 2020; Fu et al., 2020; Shy et al., 2018)	CMT
218	3	R218W	(Arimura et al., 2011; Ng et al., 2013; Sahlin et al., 2019)	DCM
258*	1	R258W	(Lee et al., 2012)	MFM
262	1	A262T	(Norton et al., 2011)	DCM
380	1	P380S	(Myers et al., 2018)	DCM
407	1	P407L	(Villard et al., 2011)	DCM
455	3	E455K	(Adriaenssens et al., 2020; Fang et al., 2017; Franaszczyk et al., 2014)	DCM
462	1	I462P	(Arimura et al., 2011)	DCM
468	1	V468M	(Villard et al., 2011)	DCM
470	1	P470S	(Meister-Broekema et al., 2018)	MFM
477	2	R477H	(McDermott-Roe et al., 2019; Norton et al., 2011)	DCM
90	1	R90X	(Norton et al., 2011)	DCM
121	1	A121X	(Norton et al., 2011)	DCM
123	3	R123X	(Janin et al., 2017; Norton et al., 2011; Toro et al., 2016)	DCM
360	1	M306X	(Rafiq et al., 2017)	DCM
390	1	R309X	(Villard et al., 2011)	DCM
451	1	T451X	(Franaszczyk et al., 2014)	DCM
218	1	R218Gfs	(Norton et al., 2011)	DCM
243	1	H243Tfs	(Toro et al., 2016)	DCM
251	1	Q251Rfs	(Norton et al., 2011)	DCM
353	1	Q353Rfs	(Franaszczyk et al., 2014)	DCM
379	1	G379Afs	(Franaszczyk et al., 2014)	DCM
383	1	S385Qfs	(Villard et al., 2011)	DCM
71	2	R71Q	(Citro et al., 2013; Villard et al., 2011)	NO
77	1	P77L	(van der Kolk et al., 2016)	NO
151	10	C151R	(Aragam et al., 2018; Citro et al., 2013; d'Avenia et al., 2015; de Denus et al., 2020; Esslinger et al., 2017; Garnier et al., 2015; Myers et al., 2018; Ng et al., 2013; Norland et al., 2019; Villard et al., 2011)	NO

Site	Citation per variant	Mutation	Citation	Disease
258 †	**1**	**R258W**	(Arimura et al., 2011)	**NO**

† It should be noted that R258W mutation was found in a CMT case (Lee et al., 2012), but is also found in 1 DCM case and 5 control case in another report in which it was considered as a non-disease-associated polymorphism (Arimura et al., 2011). BAG3 variants are coded with colors that indicate syndromic missense mutation(red), non-syndromic missense mutation(green), truncation (purple) and frame shift(blue). DCM, dilated cardiomyopathy; MFM, myofibrillar myopathy; CMT, Charcot-Marie-Tooth disease.