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HSPA5 Gene Encoding Hsp70 Chaperone BiP in the Endoplasmic Reticulum

Jie Wang^a, Jessica Lee^a, David Liem^a, and Peipei Ping^{a,*}

^aDepartments of Physiology, Medicine (Cardiology) and Bioinformatics, NIH BD2K Center of Excellence for Biomedical Computing, University of California Los Angeles, Los Angeles, CA, 90095, USA

Abstract

The *HSPA5* gene (Ensembl ID: ENSG00000044574 (WTSI/EMBL-EBI, 2015)) encodes the binding immunoglobulin protein (BiP), an Hsp70 family chaperone localized in the ER lumen. As a highly conserved molecular chaperone, BiP assists in a wide range of folding processes via its two structural domains, a nucleotide-binding domain (NBD) and substrate-binding domain (SBD). BiP is also an essential component of the translocation machinery for protein import into the ER, a regulator for Ca²⁺ homeostasis in the ER, as well as a facilitator of ER-associated protein degradation (ERAD) via retrograde transportation of aberrant proteins across the ER membrane. When unfolded/misfolded proteins in the ER overwhelm the capacity of protein folding machinery, BiP can initiate the unfolded protein response (UPR), decrease unfolded/misfolded protein load, induce autophagy, and crosstalk with apoptosis machinery to assist in the cell survival decision. Post-translational modifications (PTMs) of BiP have been shown to regulate BiP's activity, turnover, and availability upon different extrinsic or intrinsic stimuli. As a master regulator of ER function, BiP is associated with cancer, cardiovascular disease, neurodegenerative disease, and immunological diseases. BiP has been targeted in cancer therapies and shows promise for application in other relevant diseases.

Keywords

unfolded protein response (UPR); ER-associated protein degradation (ERAD); calcium homeostasis; apoptosis; post-translational modification; drug target

1. Introduction: *HSPA5* encodes the Hsp70 chaperone BiP

In the native cellular environment, polypeptides are constantly at risk of misfolding or aggregating into cytotoxic complexes. Molecular chaperones resolve this cytotoxicity by assisting with protein refolding and preventing protein aggregation (Kampinga & Craig,

*To Whom Correspondence Should be Addressed: Peipei Ping, Ph.D., FAHA, FISHR, UCLA David Geffen School of Medicine, Departments of Physiology, Medicine (Cardiology) and Bioinformatics, Suite 1-609 MRL Building, 675 Charles E. Young Dr. South, Los Angeles, CA 90095-1760, Phone: 310-267-5624, peipeiping@earthlink.net.

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2010). Binding immunoglobulin protein (BiP) is an ER chaperone belonging to the Hsp70 family, which is well known for facilitating a wide range of folding processes. The name of the gene encoding BiP, *HSPA5* (Ensembl ID: ENSG00000044574 (WTSI/EMBL-EBI, 2015)), reflects its status as the fifth member of the heat shock protein family A. The encoded protein was first identified in the mid-1970s under the name 78kDa glucose-regulated protein (GRP78, Uniprot ID: P11021 (EMBL-EBI, 2002)), as its synthesis is induced by glucose starvation (Shiu, Pouyssegur, & Pastan, 1977; Munro & Pelham, 1986). In 1983, Haas and Wabl showed that GRP78 was identical to BiP, which was separately found to bind to immunoglobulin heavy chains in pre-B cells (Haas & Wabl, 1983).

BiP is primarily found in the ER, widely expressed in different tissues, and conserved across eukaryotic species, with various half-life lengths depending on genetic makeup and microenvironment. This protein contains two structural domains, a nucleotide-binding domain (NBD) and substrate-binding domain (SBD). The interplay between these two domains facilitates BiP's ATPase activity and substrate binding ability, allowing BiP to translocate proteins into the ER, fold and hold protein substrates in the ER, initiate the unfolded protein response (UPR), and assist in ER-associated degradation (ERAD). Under various stress conditions, BiP can undergo functional change via post-translational modifications (PTMs), which play important roles in maintaining protein homeostasis and allow the cell to adapt to environmental changes. Since BiP is a master regulator in the ER, changes in its expression, activation, or inhibition have been associated with major diseases, such as cancer, cardiovascular disease, and neurodegenerative disease.

2. The half-life of BiP in different species

BiP is highly conserved in structure and function among eukaryotes and is widely expressed in a variety of human tissues and cell types (Ensembl ID: ENSG00000044574 (WTSI/EMBL-EBI, 2015); Human Protein Atlas: *HSPA5*). Inside the cell, BiP is enriched in the ER, though it may also localize to the mitochondria-associated ER membrane (MAM) and cell surface. The half-life of BiP greatly varies across species and tissue type, ranging from a few hours to 15 days (Figure 1A-B) (Lau et al., 2016). Such a broad range for BiP's half-life can be attributed to the distinct genetic background and epigenetic, transcriptional, and translational regulation. In certain species and cell lines, such as yeast (*Saccharomyces cerevisiae*), tumor cells, and plants, BiP is considered a long-lived protein with a slow turnover rate (Figure 1B) (Gülow, Bienert, & Haas, 2002; Belle et al., 2006; Hammond et al., 2016). As an example, the half-life of yeast BiP is longer than 85% of all proteins in the proteome (Figure 1A) (Belle et al., 2006). Meanwhile, BiP in other species, such as rodent species, is relatively short-lived, with a consistently longer half-life than only 30% of proteins in each proteome (Figure 1A) (Price et al., 2010; Kim et al., 2012; Lau et al., 2016). Even within the same species and tissue type, BiP's half-life can fluctuate from 3.6 to 4.6 days (statistically significant by t-test) in different genetic strains, indicating that genetic variation may also regulate half-life length (Figure 1D). In addition to genetic variation among different species, BiP's half-life changes according to tissue type within the same species and strains (Figure 1C). For example, BiP expressed in the liver of both mouse (*Mus musculus*) and bank vole (*Myodes glareolus*) displays a distinctly shorter half-life than BiP in other tissue types (Figure 1C). The shorter half-life of liver BiP may be attributed to the

liver's regenerative ability and high metabolic activity, which are accompanied by increased protein synthesis, compared to other organs (Price et al., 2010; Hammond et al., 2016).

3. Structure: the conserved allosteric cycle

3.1 Allosteric coupling of two domains

The structure of BiP consists of an NBD and an SBD (Figure 2). The NBD binds and hydrolyzes ATP, and the SBD binds polypeptides (Zhuravleva & Gierasch, 2015). The NBD consists of two large globular subdomains (I and II), with each further divided into two small subdomains (A and B). The subdomains are separated by a cleft where the nucleotide, one Mg^{2+} , and two K^{+} ions bind and connect all four domains (IA, IB, IIA, IIB) (Flaherty, DeLuca-Flaherty, & McKay, 1990; Mayer & Bukau, 2005). The SBD is also divided into two subdomains: SBD α and SBD β . SBD β serves as a binding pocket for client proteins or peptides, and SBD α serves as a helical lid to cover the binding pocket (Zhuravleva & Gierasch, 2015; Leu et al., 2014; Liebscher & Roujeinikova, 2009). An interdomain linker connects the NBD and SBD, favoring formation of an NBD–SBD interface (Zhuravleva & Gierasch, 2015).

The activity of BiP is regulated by an allosteric ATPase cycle (Figure 3): when ATP is bound to the NBD, the lid opens and induces a conformational change in the SBD to lower BiP's substrate binding affinity. Upon ATP hydrolysis, ADP is now bound to the NBD, and the lid closes on the bound substrate. This creates high affinity for substrate binding and protects the bound substrate from premature folding or aggregation. Exchange of ADP for ATP then reopens the lid and releases the substrate (Szabo et al., 1994; Schmid et al., 1994; Zuiderweg et al., 2013). This allosteric ATPase cycle is tightly regulated by nucleotide and substrate binding. Substrate binding can stimulate ATP hydrolysis while disruption of nucleotide binding can either interfere with the substrate release or abolish substrate binding (Gaut & Hendershot, 1993; Brot et al., 1994; Wei, Gaut, & Hendershot, 1995).

3.2 Sequence and structural relationship of BiP to other HSP70 family members

As a member of Hsp70 family, BiP's protein sequence, structure, and allosteric cycle are highly conserved among the Hsp70 family proteins. More than half of BiP's sequence is identical to that of major Hsp70 family members, such as HS71A (HSPA1A), HS71B (HSPA1B), GRP75 (HSPA9), HSP7C (HSPA8), HSP72 (HSPA2), HSP76 (HSPA6), and HSP71L (HSPA1L) (Figure 4). The high sequence similarity leads to two conserved functional domains among all Hsp70s: a NBD at the N-terminus and a SBD at the C-terminus. In addition to these shared domains, BiP also has unique features that set its structure apart from the other Hsp70 proteins: an ER signal sequence at the N-terminus and a KDEL retrieval sequence at the C-terminus. These two features enable BiP to translocate into the ER and remain as an ER protein (Figure 2).

4. Function: maintaining ER homeostasis and regulating signal transduction

4.1 Protein folding, holding, and translocation into the ER

As one of the most abundant proteins in the ER, BiP maintains ER homeostasis by folding and holding secretory proteins and monitoring the protein load in the ER lumen (Lièvre et al., 1997). BiP can actively fold their substrates as a foldase or simply hold and restrict a substrate from refolding or aggregating as a holdase. Intact ATPase activity and peptide binding ability are required for BiP to fold proteins, as BiP mutants defective in either activity fail to fold substrate (Simons et al., 1995). BiP's foldase activity is regulated by its cofactors and cochaperones such as J proteins and nucleotide exchange factors (Behnke, Feige, & Hendershot, 2015). The NBD of BiP contains a Mg^{2+} binding site, which stimulates ATPase activity in vitro, and a Ca^{2+} binding site, which inhibits ATPase activity in vitro (Sriram et al., 1997; Wei & Hendershot, 1995). While the foldase activity of BiP is highly dependent on intact ATPase activity, the holdase activity can be enhanced by decreased ATPase activity. A mutant of BiP defective in ATP binding can serve as a better holdase than wild type BiP, likely due to the absence of an ATP-induced conformational change that would normally facilitate substrate release. Mutants with defective ATPase activity can also lead to decreased substrate binding (Wei et al., 1995).

BiP is also required to import polypeptides into the ER lumen or ER membrane in an ATPase-dependent manner. Mutations in the NBD of BiP that abolish BiP's ATPase activity block the translocation of a number of proteins (immunoglobulin, invertase, carboxypeptidase Y, α factor, BiP) into the ER lumen (Vogel, Misra, & Rose, 1990; Nguyen, Law, & Williams, 1991; Brodsky & Schekman, 1993). The role of BiP in ER translocation has been contested in two controversial models: the ratchet model suggests that BiP can prevent backsliding of the translocation substrate through the translocon, and the power stroke model states that BiP undergoes a conformational change that pulls the translocation substrate through the translocon (Matlack et al., 1999; Liebermeister, Rapoport, & Heinrich, 2001; Elston, 2002). In addition to directing substrates through the translocon, BiP can gate ER translocation in a nucleotide-dependent manner. The ADP-bound conformation of BiP seals the translocon, blocking protein translocation and Ca^{2+} leakage, while the ATP-bound conformation of BiP reopens the translocon pore, facilitating import of precursor polypeptides into the ER (Schäuble et al., 2012; Alder et al., 2005).

4.2 ER-associated protein degradation (ERAD)

ERAD maintains ER protein load by eliminating misfolded/unfolded proteins in the ER. As a chaperone protein, BiP facilitates degradation through recognizing substrates, enhancing substrate solubility, and releasing substrates upon retro-translocation into the cytosol.

BiP recognizes the hydrophobic regions of protein substrates in the ER lumen and targets them for degradation. The transmembrane segment of a canonical mammalian ERAD substrate, T cell receptor α (TCR α), was recently shown to mislocalize to the ER lumen. Once the hydrophobic transmembrane segments become luminal, BiP directly binds to them and targets them for degradation (Feige & Hendershot, 2013). BiP is also required to

solubilize proteins prior to their degradation. Reduced protein solubility by BiP mutants leads to impaired degradation (Stolz & Wolf, 2010; Plemper et al., 1997; Simons et al., 1995). Equally important is BiP's ability to release substrates, as failure to do so would block the degradation of ERAD substrates. Dissociation of BiP from an unassembled immunoglobulin light chain correlates with the degradation kinetics of the substrate (Knittler, Dirks, & HAAs, 1995). To facilitate efficient substrate degradation, BiP can complex with its cochaperones, ER-localized DnaJ 4 (ERdj4) and ERdj5, as well as with other ERAD components, specifically degradation in ER protein 1 (Derlin-1), ER degradation enhancer, mannosidase alpha-like 1 (EDE1), and valosin-containing protein (VCP) (Lai et al., 2012; Dong et al., 2008; Ushioda et al., 2008).

4.3 Unfolded protein response (UPR) and UPR-induced apoptosis

An imbalance between a load of unfolded/misfolded proteins in the ER and the capacity of protein folding machinery leads to UPR (Ron & Walter, 2007). UPR is initiated by three classes of ER stress transducers—inositol-requiring enzyme 1 (IRE1), protein kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6)—and executed in three stages: (1) lowering protein synthesis and translocation into the ER, (2) upregulating UPR target genes to enhance protein folding capacity in the ER, and (3) inducing apoptosis mainly via IRE1, PERK, and ATF6 signaling when UPR fails to reestablish homeostasis (Ron & Walter, 2007; Hetz, 2012; Minamino, Komuro, & Kitakaze, 2010; Hetz, Chevet, & Oakes, 2015).

BiP is both an essential regulator of the UPR and a UPR target gene product. The UPR initiation model has been well studied and suggests that the binding of BiP to the ER luminal sensor domains of IRE1 and PERK regulates UPR activation, whereas binding of BiP to the Golgi-localization signal of ATF6 regulates ATF6 Golgi transportation and activation (Bertolotti et al., 2000; Shen et al., 2002). During ER stress, BiP dissociates from the three transducers, effectively activating their respective UPR pathways (Korennykh & Walter, 2012). As a UPR target gene product, BiP is upregulated when UPR transcription factors associate with the UPR element in BiP's DNA promoter region (Yoshida et al., 2001).

Though UPR is initiated to protect the cell, prolonged UPR can lead to cell death (Hetz et al., 2015). For instance, the PERK-induced phosphorylation of eukaryotic initiation factor 2 α (eIF2 α) can activate ATF4, which in turn induces CCAAT-enhancer-binding protein homologous protein (CHOP), a transcription factor promoting apoptosis (Harding and Ron, 2002). An alternative pathway through ATF6 can likewise activate apoptosis through CHOP (Minamino et al., 2010). Nonetheless, as a master regulator of UPR, BiP also assists with cell survival decisions following prolonged UPR activation. BiP has been shown to bind to BCL2-interacting killer (BIK), an apoptotic BH3-only protein located primarily in the ER. Overexpression of BiP inhibits the pro-apoptotic activity of BIK and BAX while knockdown of BiP sensitizes the apoptotic response in estrogen-dependent MCF-7/BUS breast cancer cells subjected to estrogen starvation (Fu, Li, & Lee, 2007). BiP can also complex with apoptotic machinery such as caspase-7 and 12 to promote cell survival (Rao et

al., 2002). Specifically, BiP binds to caspase-7 via the NBD and blocks caspase-7 activation and etoposide-induced apoptosis (Reddy et al., 2003).

4.4 Cell-surface BiP as a signaling regulator in apoptosis and cell survival

In addition to ER, BiP has been shown to be expressed on the cell surface under pathological conditions and has emerged as an important regulator of cell signaling and viability (Pfaffenbach & Lee, 2011; Ni, Zhang, & Lee, 2011). Cell-surface BiP majorly exists as a peripheral protein on the plasma membrane via interactions with other cell-surface proteins, including glycosylphosphatidylinositol (GPI)-anchored proteins (Tsai et al., 2015). The cell-surface translocation of BiP is dependent on BiP's SBD and is actively enhanced by ER stress (Tsai et al., 2015; Arap et al., 2004; Zhang et al., 2010). Saturation of the KDEL receptor, which functions as part of the ER retrieval machinery, increases cell-surface localization of BiP. Alternatively, BiP with a KDEL deletion exhibits higher surface localization compared to full-length BiP (Zhang et al., 2010).

Cell-surface BiP promotes cell survival and apoptosis via interactions with a wide range of proteins. BiP can interact with and activate $\alpha 2$ -macroglobulin and cripto, which, in turn, activate the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signaling cascades to promote cancer cell proliferation (Misra, Deedwania, & Pizzo, 2006; Shani et al., 2008; Kelber et al., 2009). Furthermore, BiP can bind to plasminogen kringle 5 and coxsackie A9 virus as a signaling receptor (Gonzalez-Gronow et al., 2007; Triantafilou et al., 2002). Plasminogen kringle 5 induces apoptosis by competitive binding of BiP with caspase-7 (Davidson et al., 2005). Upon coxsackie A9 virus entry, BiP binds to histocompatibility complex class I (MHC-I) to form a receptor complex, followed by MHC-I-mediated internalization (Triantafilou et al., 2002).

An 82-kDa tumor-specific variant of cell-surface BiP with an O-linked carbohydrate moiety has been found specific to malignant cells (Rauschert et al., 2008). This cell-surface epitope can serve as an excellent target for antibodies to neutralize the protective effects on tumor cells, disable mechanisms of drug resistance, and directly kill cancer cells by inducing apoptosis (Rauschert et al., 2008; Díaz-Zaragoza et al., 2015).

4.5 Calcium regulation

Under physiological conditions, the Ca^{2+} concentration in the ER ranges from 100 to 800 μM , which is several orders of magnitude higher than that in the cytosol ($\sim 100 \text{ nM}$) (Samtleben et al., 2013). As one of the most abundant proteins in the ER, BiP serves as a high-capacity Ca^{2+} binding protein to store Ca^{2+} in the ER. BiP can store around 25% of the ER Ca^{2+} , with a stoichiometry of 1–2 moles of Ca^{2+} per mole of BiP (Lièvremonet et al., 1997). In addition, BiP is involved in regulating Ca^{2+} release from the ER. The absence of available BiP leads to increased Ca^{2+} leakage from ER to cytosol through the Sec61 complex (Schäuble et al., 2012). BiP has been reported to form a complex at the MAM with the sigma-1 ER receptor (Sig-1R), which prevents protein aggregation, and inositol 1,4,5-trisphosphate receptor type 3 (IP3R3), which transmits apoptotic Ca^{2+} signals to the mitochondria. Ca^{2+} depletion at the MAM dissociates BiP from Sig-1R and enhances

Sig-1R's chaperone activity to stabilize IP3R3, prolonging Ca^{2+} signaling from the ER to mitochondria (Hayashi & Su, 2007).

5. Regulation of BiP by post-translational modifications (PTMs)

PTMs of BiP have been shown to regulate BiP's activity, turnover, and availability upon different extrinsic or intrinsic stimuli. So far, BiP has been shown to be sulfenylated, glutathionylated, ADP-ribosylated, phosphorylated, AMPylated, and citrullinated. However, interactions between different modifications within one BiP protein remain unknown.

5.1 Oxidation

Redox fluctuations within the ER can be detrimental to protein stability and homeostasis. BiP has been identified as a redox signaling protein, which can sense and respond to oxidative stress through reversible oxidation of its cysteine residue (Wang et al., 2014; Wang & Sevier, 2016). This N-terminal cysteine is highly conserved among eukaryotes, and the majority of BiP in mammalian cells possess a second conserved cysteine residue.

In mammalian cells, the conserved N-terminal cysteine of BiP in the NBD has been identified as a glutathionylated protein during diamide-induced oxidative stress using high-throughput proteomic approach (Lind et al., 2002). This conserved cysteine in the yeast BiP can be both glutathionylated (-SG) and sulfenylated (-OH) upon ER-induced oxidative stress (Wang et al., 2014; Wang & Sevier, 2016). Both cysteine oxidation forms can abolish the ATPase activity of BiP and enhance BiP's ability to prevent protein aggregation. During oxidative stress, when proteins experience increased the risk of misfolding and aggregation, sulfenylation and glutathionylation of BiP's cysteine function to turn BiP from a foldase into a holdase, a shift that is beneficial for the ER (Wang et al., 2014; Wang & Sevier, 2016). It is generally accepted that the reversibility of oxidation is essential for regulating a redox signaling molecule. Upon removal of oxidative stress, the constitutive modification that abolished BiP's folding ability is no longer beneficial. Highly dynamic reversal of BiP's oxidation has been observed when oxidants were removed, restoring BiP's function as a foldase. Meanwhile, mutants that mimic constitutively oxidized BiP inhibited cell proliferation upon stress removal and even under non-stress conditions, emphasizing the importance of reversible oxidation for BiP as a redox signaling molecule (Wang & Sevier, 2016).

In addition to sulfenylation and glutathionylation, the N-terminal cysteine can form an intramolecular disulfide bond with C-terminal cysteine by activated (oxidized) glutathione peroxidase 7 (GPx7) (Wei et al., 2012). In vitro data has demonstrated that the disulfide bond formation enhances binding of BiP to denatured luciferase, suggesting that oxidation of BiP enhances its holdase function (Wei et al., 2012).

5.2 ADP-ribosylation and phosphorylation

Different forms of BiP have been detected in separate pools, allowing for regulation of BiP's availability and activity: (1) by forming complexes with other proteins, (2) as a free unmodified monomer, or (3) as an oligomer (Freiden, Gaut, & Hendershot, 1992). ADP-ribosylation and phosphorylation of BiP hinder BiP's substrate binding and keeps BiP in a

pool of inactive BiP oligomers (Freiden et al., 1992). As the unfolded protein load declines, BiP is reversibly ADP-ribosylated or phosphorylated at the SBD. The phosphorylation occurs at residue Thr520 in *Chlamydomonas* and yeast BiP while the ADP-ribosylation occurs at residues Arg470 and Arg492 of hamster BiP (Díaz-Troya et al., 2011; Chambers et al., 2012). These modifications obstruct the SBD and inactivate BiP's chaperone capability. Although the modifications have been mapped for only a subset of BiP orthologs, all of the modification sites are widely conserved among the eukaryotic BiPs. In cells with higher levels of BiP, both BiP phosphorylation and ADP-ribosylation are reduced either due to the accumulation of misfolded/unfolded proteins or due to glucose starvation (Díaz-Troya et al., 2011; Chambers et al., 2012). Both phosphorylation and ADP-ribosylation are restricted to the unbound BiP pool, specifically, the oligomerized form, while the bound BiP pool is unmodified (Hendershot, Ting, & Lee, 1988; Freiden et al., 1992; Chambers et al., 2012).

5.3 AMPylation

When the protein load in the ER is low, BiP has been shown to be AMPylated in the NBD. HYPE, a protein with a single Fic domain that catalyzes AMP modification, facilitates AMPylation of BiP at Ser365 and Thr366 near the nucleotide-binding site, potentially influencing nucleotide binding during ER stress (Ham et al., 2014; Sanyal et al., 2015). Similar to ADP-ribosylation, AMPylation of BiP is reversible, and the level of AMPylated BiP decreases when the unfolded protein load is increased upon UPR activation (Sanyal et al., 2015). However, at the molecular level, whether AMPylation activates or inactivates BiP's ATPase activity is still a matter for debate: Ham et al. studied BiP AMPylation using a recombinant NBD and observed decreased ATPase activity upon AMPylation while Sanyal et al. used a full-length BiP and observed increased ATPase activity (Ham et al., 2014; Sanyal et al., 2015). As of yet, it is not clear how enhanced ATPase activity helps cells to adapt to a decreased protein load.

Other PTMs exist in addition to those mentioned above, but these are still undergoing investigation. For example, citrullination on BiP is known to contribute to the inflammatory response in rheumatoid arthritis patients (Shoda et al., 2011); however, the detailed molecular mechanism and disease pathology has yet to be elucidated.

6. Clinical Significance

Considering its role in managing ER homeostasis, BiP has been implicated in a broad spectrum of disease pathologies. BiP's clinical relevance in six major disease classifications is discussed below and summarized in Figure 5.

6.1 Cancer

BiP is a prominent chaperone protein in the UPR of normal cells; however, this role has led to the resilience of cancer cells against therapeutic efforts. BiP is overexpressed in cancers of a diverse range of tissue and cell types, including such major cancers as breast cancer, prostate cancer, lung cancer, and liver cancer. Moreover, BiP overexpression is associated with enhanced proliferation, metastasis, and drug and chemotherapy resistance of primary cancer cells, as well as with overall poor clinical outcome (Ni & Lee, 2007; Uckun et al.,

2011; Chen et al., 2014). BiP promotes cancer cell growth and survival through different molecular mechanisms. Glucose and oxygen deprivation trigger UPR signaling and upregulates BiP expression to address the aggregation of misfolded proteins in the ER (Fu et al., 2007). BiP also localizes to the cell surface, where it acts as a receptor for activating anti-apoptotic signaling pathways (Misra et al., 2006). Meanwhile, BiP in the ER lumen may control Ca^{2+} leakage to the cytosol, thus removing the apoptotic stimulus (Lièvremonet et al., 1997). As a result, treatments that inhibit BiP activity may augment the effectiveness of chemotherapy and anticancer drugs. To exemplify, retaspimycin hydrochloride, a small molecule Hsp90 inhibitor that dissociates BiP-Hsp90 complexes, increased the sensitivity of mantle cell lymphoma cells to the anticancer proteasome inhibitor bortezomib (Uckun et al., 2011). Moreover, a doxorubicin conjugate of Pep42, PEP-DOX, has been demonstrated to induce apoptosis in previously chemotherapy-resistant B-lineage acute lymphoblastic leukemia cells by targeting their surface-expressed BiP molecules (Uckun et al., 2011). More impressively, the anticancer drug OSU-03012, derived from celecoxib, kills tumor cells directly by downregulating BiP and interfering with PERK signaling in the ER stress response (Booth et al., 2015a). Additional BiP inhibitors include nucleoside analogs; natural inhibitors such as epigallocatechin gallate, a flavonoid found in green tea (*Camellia sinensis*); and bacterial proteins such as AB5 subtilase toxin (SubAB). These three agents all promote cell death by targeting the NBD of BiP, thereby inhibiting its anti-apoptotic capabilities (Martin et al., 2013).

6.2 Cardiovascular disease

While ER stress is initially protective, prolonged periods of ER stress can be harmful, especially to the heart, resulting in a wide array of pathological conditions, including cardiac hypertrophy, alcoholic cardiomyopathy, and ischemia-reperfusion (Minamino et al., 2010; Li & Ren, 2008; Doroudgar et al., 2009). ER stress-induced cardiac dysfunction has been associated with significant upregulation of BiP and attributed to the resulting dysregulation of intracellular Ca^{2+} (Roe & Ren, 2013). BiP can also suppress the development of atherosclerosis through alleviating homocysteine-induced ER stress, preventing apoptosis of vascular endothelial cells, inhibiting the activation of genes responsible for cholesterol/triglyceride biosynthesis, and suppressing tissue factor procoagulant activity, all of which can contribute to the buildup of atherosclerotic plaques (Ni & Lee, 2007).

Additionally, ER chaperones, including BiP, were markedly induced in patients with dilated cardiomyopathy and heart failure, indicating that ER-initiated apoptosis may contribute to cardiac disease development (Okada et al., 2004). As a result, some anticancer drugs, such as proteasome inhibitors, have been associated with heart failure complications. Induction of BiP was found to rescue cardiomyocytes from proteasome inhibitor-induced apoptosis and, therefore, may mitigate the cardiac repercussions of anticancer therapies (Fu et al., 2008).

6.3 Neurodegenerative disease

ER stress has been illustrated in numerous studies to participate in the pathogenesis of neurodegenerative disease (Ni & Lee, 2007; Okada et al., 2004; Kudo et al., 2008). BiP plays an important role in cerebellar ataxia-related diseases, including Huntington's disease and cerebral infarction. In the Huntington's disease model, BiP has been shown to bind to

huntingtin aggregates and limit huntingtin aggregation at high concentrations (Muchowski, Schaffar, & Sittler..., 2000). As a result, cells expressing both mutant huntingtin and decreased BiP availability show hypersensitivity to ER stress (Lajoie & Snapp, 2011). In Marinesco-Sjögren syndrome, which is characterized by cerebellar ataxia, BiP prevents neuronal cell death induced by ER stress by correcting misfolded proteins; this role has been demonstrated in woozy mice and patients with Marinesco-Sjögren syndrome (Zhao et al., 2005; Anttonen et al., 2005). In cells with spinocerebellar ataxia type 17 causative mutations, BiP is expressed at decreased levels (Lee et al., 2009). Moreover, a chemical inducer of BiP, named BIX, has demonstrably reduced cerebral infarction in cerebral ischemic mice (Kudo et al., 2008).

In Parkinson's disease, elevated expression of BiP and other UPR proteins have been reported in many cellular and animal models of Parkinson's disease (Baek et al., 2016). Interestingly, the SH-SY5Y cell model exhibited lower BiP mRNA levels when treated with the neurotoxin 1-methyl-4-phenylpyridinium (MPP+); however, treating the same cell model with 6-hydroxydopamine, another Parkinson's disease inducer, raised BiP mRNA levels. BiP's neuroprotective properties have led it to play an influential role in therapeutic treatments. In the MPTP mouse model of Parkinson's disease, 2-deoxy-D-glucose treatment has been suggested to reduce neural injury through BiP-induced neuroprotective pathways. Similarly, overexpression of BiP was found to attenuate α -synuclein cytotoxicity in rat and SH-SY5Y cell models by reprogramming ER stress signaling pathways to avert apoptotic cell death (Gorbatyuk & Gorbatyuk, 2013).

In contrast to the aforementioned disease types, enhanced BiP chaperone function, among other chaperones, has been strongly implicated in Alzheimer's disease (Booth, Roberts, & Dent, 2015b; Ni & Lee, 2007). Since BiP and other chaperones facilitate protein expression in neurons and, by extension, their tau hyperphosphorylation (García-Barroso et al., 2013), OSU-03012 and other BiP inhibitors may prevent or delay the onset and progression of the disease (Booth et al., 2015b). However, neurons already laden with β -amyloid protein, a major component of extracellular senile plaques in Alzheimer's disease, may be rescued by BiP's chaperone activity (Zhao et al., 2005; Anttonen et al., 2005). Thus, BiP inhibitors may be more effective when applied in the earliest stages of Alzheimer's disease and withheld in the late stages (Booth et al., 2015b).

6.4 Autoimmune disease

While well established in the UPR and related pathways, BiP is increasingly recognized for its immunomodulatory and anti-inflammatory role in the immune response, particularly in rheumatoid arthritis. BiP was initially thought to be an autoantigen of rheumatoid arthritis, but recent evidence indicates that BiP may actually be a responder to, not instigator of, the inflammatory process (Corrigall et al., 2001; Panayi & Corrigall, 2014; Brownlie et al., 2006). In many of these studies, the presence of BiP autoantibodies could be attributed to the secretion of BiP into the extracellular environment in response to hypoxia and glucose starvation caused by the influx of inflammatory cells (Panayi & Corrigall, 2014). This explanation may also apply to inflammatory diseases of other tissues and organ systems, such as in rheumatic heart disease (Fae et al., 2008).

Extracellular BiP facilitates anti-inflammatory signaling by inducing interleukin (IL)-10 and other anti-inflammatory cytokines, downregulating tumor necrosis factor alpha (TNF- α) and IL-6, and shifting the T-helper type 1 (Th1)/Th2 balance towards the Th2 profile (Corrigall et al., 2001; Shields, Panayi, & Corrigall, 2011). Additionally, BiP regulates the differentiation of peripheral blood monocytes into dendritic cells and reduces their expression of human leukocyte antigen-antigen D related (HLA-DR) and cluster of differentiation 86 (CD86), which are critical to their antigen presentation function (Corrigall et al., 2001; Shields et al., 2011). By interacting with the mature dendritic cells, BiP regulates the development of T cells, which have been implicated in the disease progression of rheumatoid arthritis (Brownlie et al., 2006; Corrigall, Vittecoq, & Panayi, 2009). As further evidence of BiP's protective role, recombinant human (rhu) BiP administered intravenously or subcutaneously into mice with collagen-induced arthritis, the equivalent of human rheumatoid arthritis in animal models, not only prevented onset of disease but also caused full remission after onset (Panayi & Corrigall, 2014; Brownlie et al., 2006; Corrigall et al., 2009). These effects persist even after ceasing administration of rhuBiP. RhuBiP is currently undergoing a double-blind, placebo-controlled Phase I/IIa clinical trial and, if proven effective, will provide an appealing alternative to long-term drug therapy (Panayi & Corrigall, 2014).

6.5 Metabolic disease

ER stress is also involved in the pathogenesis of diabetes mellitus (Ni & Lee, 2007; Okada et al., 2004). In eIF2 α -mutant mice fed a high-fat diet, BiP sequesters proinsulin within the ER, thus interfering with its maturation into insulin, increasing its susceptibility to degradation by ERAD, and effectively reducing insulin levels. Furthermore, BiP heterozygosity is proposed to protect against high fat diet-induced obesity, type 2 diabetes, and pancreatitis by upregulating protective ER stress pathways. BiP is also necessary for adipogenesis and glucose homeostasis in adipose tissues (Scheuner et al., 2005).

6.6 Infectious disease

In addition to human and other higher eukaryotes, BiP and its evolutionarily conserved orthologs play essential roles in the biology and life cycles of viruses, bacteria, protozoa, and yeast (Booth et al., 2015b). Prokaryotic BiP orthologs were found to interact with key proteins such as RecA, which is vital to bacterial DNA replication. As a result, these bacterial Hsp70 chaperones represent a promising set of targets for antibiotic development, particularly in regards to combatting antibiotic-resistant strains (Booth et al., 2015b). Notably, the anticancer drug OSU-03012 re-sensitized "superbug" strains of *Neisseria gonorrhoeae* to several standard-of-care antibiotics (Booth et al., 2015a).

Meanwhile, a virulent strain of Shiga toxigenic *Escherichia coli* undermines the cell survival of its host organism by producing SubAB, which inhibits BiP by cleaving it at the region linking the NBD and the SBD. Although this strain may threaten human health, SubAB itself may become an effective tool to specifically eliminate BiP expressed in human cancers (Ni & Lee, 2007).

In contrast, viruses capitalize on the expression of BiP in their eukaryotic hosts to ensure successful replication and release. Some viruses infect cells through cell-surface BiP, and most stimulate expression of additional host BiP to chaperone the profusion of viral proteins produced. Moreover, some viral proteins avert the ER stress death response by suppressing the PERK-eIF2 α signaling response, thus ensuring the host cell's survival over the duration of its replication. Viruses that evade detection through this strategy may be vulnerable to drugs like OSU-03012 that counteract viral modulation with increased PERK signaling (Rathore, Ng, & Vasudevan, 2013; Booth et al., 2015b; Booth et al., 2015a).

7. Conclusions

BiP is an ER chaperone protein belonging to the Hsp70 family, and its high conservation across species stands testament to its vital role in the biology of each organism. As a molecular chaperone, BiP contributes to protein folding, import into the ER, protein removal through ERAD, and regulation of Ca²⁺ homeostasis in the ER. In addition, BiP activates the UPR pathway, promoting autophagy and inhibiting apoptosis. These functions are commonly induced by ER stress, as evidenced by BiP's association with cancer, cardiovascular disease, neurodegenerative disease, metabolic disease, autoimmune disease, and infectious disease. To better understand BiP's contribution to cell survival in response to ER stress and other disease conditions, the interplay between BiP's roles in UPR, autophagy, and apoptosis require further investigation. Current studies on drugs targeting BiP have yielded promising results, particularly in enhancing specific cancer treatments and potential concomitant heart failure, and can be expanded to identify new chemical inhibitors and inducers of BiP to treat other relevant diseases. Though PTMs of BiP have been characterized, their effect on BiP in a pathological context remains unclear. Thus, further research into the area can refine future clinical applications involving BiP.

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Abbreviations

aa	amino acid
ATF6	activating transcription factor 6
BIK	BCL2-interacting killer
BiP	binding immunoglobulin protein
CD86	cluster of differentiation 86
CHOP	CCAAT-enhancer-binding protein homologous protein
Derlin-1	degradation in ER protein 1

EDEM1	ER degradation enhancer, mannosidase alpha-like 1
eIF2α	eukaryotic initiation factor 2 alpha
ERAD	ER-associated degradation
ERdj4/5	ER-localized DnaJ 4/5
GPI	glycosylphosphatidylinositol
GPx7	glutathione peroxidase 7
Grp78	78kDa glucose-regulated protein
HLA-DR	human leukocyte antigen D-related
IL	interleukin
IP3R3	inositol 1,4,5-trisphosphate receptor type 3
IRE1	inositol-requiring enzyme 1
KDEL	lys-asn-glu-leu
MAM	mitochondria-associated ER membrane
MAPK	mitogen-activated protein kinase
MHC-I	histocompatibility complex class I
MPP+	1-methyl-4-phenylpyridinium
NBD	nucleotide-binding domain
PERK	protein kinase-like ER kinase
PI3K	phosphoinositide 3-kinase
PTM	post-translational modification
rhuBiP	recombinant human BiP
SBD	substrate-binding domain
Sec61	secretory 61
Sig-1R	sigma-1 ER receptor
SubAB	AB5 subtilase toxin
TCRα	T cell receptor α
Th1/2	T-helper type 1/2
TNF-α	tumor necrosis factor alpha
UPR	unfolded protein response

VCP valosin-containing protein

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Highlights

- The structure of BiP is highly conserved while its turnover varies by species.
- BiP majorly functions in maintaining ER protein homeostasis.
- Post-translational modifications of BiP can regulate its localization and function.
- BiP's roles in various disease types make it a prime therapeutic target.

Figure 1. Half-life of BiP in different species and tissue types

BiP's half-life is compared across three species (yeast, mouse, and bank vole), five tissue types (heart, liver, brain, kidney, and skeletal muscle), and six mouse strains (A/J, BALB/cJ, C57BL/6J, CE/J, DBA/2J, and FVB/NJ) (Lau et al., 2016). (A) This set of box- and-whisker plots illustrates how BiP's half-life may fluctuate greatly depending on species and tissue type. While BiP's half-life remains consistently longer than only 30% proteins in the proteomes of both rodent species, the half-life of yeast BiP is longer than 85% of proteins in the yeast proteome. (B) BiP's half-life can vary in different species, from a few hours in yeast up to 15 days in bank vole. In addition to yeast, mouse, and bank vole, the half-life of

BiP in maize, bean, and human tumor cells are shown. (C) BiP's half-life also differs between tissue types within the same species. Notably, liver BiP has a shorter half-life compared to other tissue types in both mouse and bank vole. (D) BiP's half-life can deviate significantly (*) in different strains of mouse cardiac tissue, such as between A/J and BALB/cJ.

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Figure 2. Protein structure of BiP

The upper panel represents the primary structure of BiP, including the nucleotide binding domain (NBD) and substrate binding domain (SBD). In addition to the NBD and SBD, BiP has an N-terminal signal sequence, which is an 18-amino acid long signal peptide for ER targeting and translocation, and a C-terminal ER retrieval sequence, lys-asp-glu-leu (KDEL), which prevents BiP from being secreted from the ER. The lower panel shows the structures of the NBD (PDB: 3IUC) and SBD (PDB: 5e85) of human BiP. The magenta stick represents the ADP that binds to the NBD of BiP.

Figure 3. Allosteric ATPase cycle of BiP

When the nucleotide binding domain (NBD, blue) binds to ATP, the substrate-binding domain (SBD, green) of BiP has low binding affinity to the substrate (purple). When the NBD is bound to ADP, the SBD has high substrate binding affinity. The allosteric ATPase cycle is known to allow BiP to act as a foldase and transport proteins into the ER.

Figure 4. Conservation of Primary Structure Within Hsp70 Family Members

(A) The primary structure of BiP (HSPA5) is compared with that of the other seven members of the Hsp70 family (HSPA1A, HSPA1B, HSPA9, HSPA8, HSPA2, HSPA6, and HSPA1L) using NCBI COBALT sequence alignment tool (https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi). Numbers at the ends of each row indicate the position of the residue, with a maximum of 80 residues per row. Red regions indicate identical amino acid (aa) matches; blue regions indicate less conserved aa matches; and black regions indicate non-conserved aa. (B) For each Hsp70 member (column 1), the number of identical matches with BiP over total length (column 2), as well as the resulting percentage (column 3) are summarized. The high degree of sequence similarity across all eight Hsp70 family members accounts for two shared functional domains: the nucleotide-binding domain (NBD) and substrate-binding domain (SBD).

Figure 5. Clinical significance of BiP

BiP is associated with six major disease classifications: cardiovascular disease, especially heart failure; neurodegenerative disease, including Alzheimer’s and prion disease; autoimmune disease, particularly rheumatoid arthritis; metabolic disease, including diabetes mellitus and obesity; infectious disease, which includes prokaryotic pathogens and eukaryotic parasites employing BiP homologs; and cancer and viral disease, both which rely the host’s BiP expression. The biological mechanisms underlying BiP’s involvement in each disease group—unfolded protein response (UPR), apoptosis, ER- associated degradation (ERAD), Ca²⁺ homeostasis, and other signaling pathways—are illustrated. Treatments for each disease group can be classified as BiP inhibitors, which compromise the survival of infected and cancerous cells, and BiP inducers, which counteract excessive protein misfolding and cell death.