Bax Inhibitor-1: between stress and survival

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Abstract

Cellular gatekeepers are essential to maintain order within a cell and anticipate signals of stress to promote survival. BAX inhibitor-1 (BI-1), also named transmembrane BAX inhibitor motif containing-6 (TMBIM6), is a highly conserved endoplasmic reticulum (ER) transmembrane protein. Originally identified as an inhibitor of BAX-induced apoptosis, its pro-survival properties have been expanded to include functions targeted against ER stress, calcium imbalance, reactive oxygen species accumulation and metabolic dysregulation. Nevertheless, the structural biology and biochemical mechanism of action of BI-1 are still under debate. BI-1 has been implicated in several diseases, including chronic liver disease, diabetes, ischemia/reperfusion injury, neurodegeneration and cancer. While most studies have demonstrated a beneficial role for BI-1 in the ubiquitous maintenance of cellular homeostasis, its expression in cancer cells seems most often to contribute to tumorigenesis and metastasis. Here we summarize what is known about BI-1 and encourage future studies on BI-1's contribution to cellular life and death decisions to advocate its potential as a target for drug development and other therapeutic strategies.

Graphical abstract

Disturbances in normal cell function activate evolutionarily conserved stress responses that aim to compensate for damage but can eventually trigger cell death. Bax Inhibitor-1 (BI-1) functions as a molecular rheostat, interacting with a broad range of partners to promote cell survival. Here we review the proposed models and mechanisms of BI-1 and its role in various diseases.
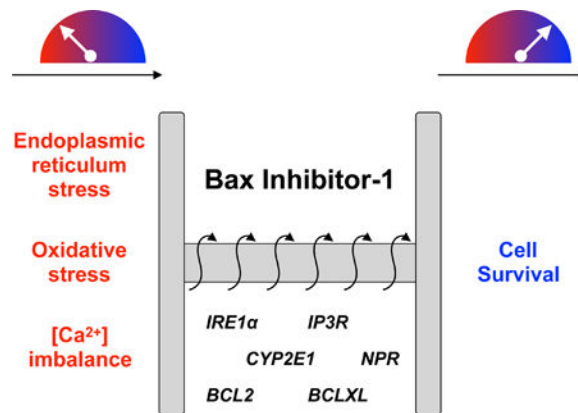
Conflicts of Interest
None to declare.

Keywords

TMBIM6; ER stress; Cell death; Calcium; Disease

Introduction

A large number of regulatory proteins are involved in the signaling pathways that determine cell fate. BAX Inhibitor-1 (BI-1) is the founding member of the transmembrane BAX Inhibitor-1-containing motif (TMBIM) family, which includes ancestral regulators of cell death due to their high degree of genetic and functional conservation[1, 2]. These hydrophobic TMBIM proteins share a UPF0005 consensus motif in the C-terminus that codifies for six or seven transmembrane-spanning regions and is conserved in several species[2]. The cytoprotective function of the TMBIM family was found to regulate evolutionarily conserved mechanisms of stress resistance in animals, plants and yeast[3, 4]. While all six TMBIM family members seem to protect against apoptosis, as discussed *in extenso* [2, 5, 6], this review will concentrate on the function of TMBIM6, the original BI-1, in the animal kingdom.

Initially, a functional screen for BAX suppressors in yeast identified BI-1 to have a protective functional effect over BAX-induced cell death[7]. While it seemed reasonable to name the protein after its observed function, the cytoprotective function of BI-1 was surprisingly not due to a direct physical interaction with BAX. Rather, BI-1 co-immunoprecipitated with the anti-apoptotic BCL2 family proteins BCL2 and BCL_{XL}, but not the pro-apoptotic proteins BAX or BAK due the requirement of a BH4 domain for BI-1 interaction[7]. In mammalian cell lines, *BI-1* overexpression protected against, while its antisense promoted, apoptosis[7].

A recent neuroscience review has provided a brief overview of BI-1-like proteins and focused on the functions of BI-1 to threshold ER stress-induced oxidative damage and inflammation[8]. The present review rather compiles the most recent findings supporting all cytoprotective functions of BI-1 (ER stress and IRE1α signaling, calcium flux, as well as ROS regulation), highlights the implication of BI-1 signaling in disease (i.e. liver,

neurological, immune disorders) and provides a synthesis of the evidence linking BI-1 to cancer.

I.   Structure and distribution of BI-1

BI-1 is a highly hydrophobic protein containing 237 amino acids (~26kDa) located almost exclusively on the ER, as illustrated by immunofluorescence microscopy and fractionation studies[7, 9]. For the most part, BI-1 is characterized to have six fully transmembrane domains, with a cytosolic orientation of both N and C termini, and a C-terminal re-entrant loop[7, 10, 11] (Figure 1). A number of interacting partners bind to increase the anti-apoptotic activity of BI-1, including BCL2 and BCLxL at the N-terminal, and IRE1α, inositol 1,4,5-trisphosphate receptor (IP3R), NADPH-P450 reductase (NPR) and cytochrome P450 species 2E1 (CYP2E1) at the C-terminal[12]. Indeed, deletion of a C-terminal domain of BI-1 abrogates its cytoprotective activity to suppress BAX-induced cell death in yeast[3]. The first BLAST searches revealed that the human and rat genes of BI-1 were essentially identical to the testis enhanced gene transcript due to its presence in the testis[13, 14]. BI-1 is also abundantly expressed in the liver and kidney, and is detected in the heart, skeletal muscle, spleen, brain, placenta, lung and pancreas[7, 9]. Although BI-1-deficient mice are viable and develop normally, they exhibit increased vulnerability to tissue damage when challenged[15].

II.   The various cytoprotective roles of BI-1

BI-1 interacts with a broad range of partners to protect against many facets of cell death (Figure 2). Nonetheless, apoptosis is initially a defense mechanism. Both physiological and pathological stimuli can trigger apoptosis through caspase activation classified into two main pathways: 1) intrinsic apoptosis engaged by intracellular stress conditions (DNA damage or accumulation of unfolded proteins, cytosolic Ca^{2+} or reactive oxygen species); and 2) extrinsic apoptosis that transduces signals from extracellular ligands through death receptors. The activation of either pathway results in mitochondrial outer membrane permeabilization (MOMP) and the release of apoptogenic factors, such as cytochrome c, that activate the caspase cascade of apoptosis. Interestingly, fibroblasts, hepatocytes and neurons isolated from *BI-1*-deficient mice are selectively hypersensitive to apoptosis induced by pharmacological ER stress activators such as thapsigargin and tunicamycin, compared to other intrinsic or extrinsic apoptotic stimuli[15].

A.   Implication of BI-1 in ER stress-induced cell death and the IRE1α pathway

—The ER functions as a platform, central to several stress signaling pathways. In overwhelming conditions that cause unfolded or misfolded proteins to accumulate in the ER lumen, the ER activates the unfolded protein response (UPR) signaling cascade *via* the transmembrane sensors inositol-requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK) and activating transcription factor (ATF6). The UPR first aims to alleviate cellular stress and re-establish proteostasis, but may activate cell death in response to irreversible cell damage.

ER stress-induced apoptosis was shown to require the pro-apoptotic members BAX and BAK of the BCL2 family to initiate mitochondrial dysfunction and cell death[16]. Afterwards, a study reported for the first time that BAX and BAK can colocalize to the

IRE1α sensor on the ER membrane, uncovering a direct link between an essential gateway for cell death and the most conserved pathway of the UPR. BAX and BAK physically form a protein complex with the cytosolic C-terminal domain of IRE1α that appears essential for IRE1α activation and amplified apoptosis[17]. Interestingly, BI-1 binds to this same cytosolic region, directly inhibiting IRE1α signaling and downstream *XBP1* mRNA splicing[18, 19]. The presence of BI-1 drastically reduces the binding of BAX to IRE1α, suggesting that BI-1 and BAX/BAK compete for their binding site on IRE1α[18], with the winner dictating IRE1α activity and ultimately deciding cell fate. However, *BI-1* knockdown in *BAX/BAK* double-knockout cells did not restore the normal levels of *XBP1* mRNA splicing, suggesting that BI-1 operates upstream of BAX and BAK in the control of the IRE1α-XBP1 pathway[18]. An added foe of BI-1 in IRE1α regulation is the bifunctional apoptosis regulator (BAR), an ER-associated RING-type E3 ligase that ubiquitinates BI-1 to tag it for subsequent proteosomal-mediated degradation[20]. The dynamic post-translational regulation of BI-1 by BAR at the ER membrane thus impedes the inhibitory influence of BI-1 on IRE1α signaling. Hence, prolonged activation of IRE1α during ER stress may promote cell death through the kinase-dependent pathway TRAF2-JNK or the RNase-dependent pathways involving XBP1 splicing and RIDD of mRNAs encoding essential cell-survival proteins or miRs[21]. In parallel, CHOP may also direct the subsequent activation of the pro-apoptotic BCL2 family member pathway by the transcriptional induction of BIM[22], inducing cell death[21].

B.   Proposed mechanisms of BI-1-regulated calcium flux—The ER is the primary cellular store for Ca^{2+}. The ER mainly orchestrates Ca^{2+} efflux through IP3R channels that passively release Ca^{2+} from the ER and Ca^{2+} influx through sarcoplasmic/ER calcium ATPase (SERCA) channels that actively pump Ca^{2+} into the ER. Due to its location on the ER membrane, BI-1 is involved in $[Ca^{2+}]$ regulation in the ER lumen. *BI-1*-overexpressing cells have lower resting ER Ca^{2+} levels and higher ER Ca^{2+} leakage[23]. An advantage of lower $[Ca^{2+}]$ in the ER due to *BI-1* overexpression is the controlled discharge of Ca^{2+} from the ER to the cytosol, leading to reduced Ca^{2+} uptake by mitochondria[23], thus protecting the cell from Ca^{2+} overload that triggers apoptosis. To better study its function, potential membrane topologies of BI-1 have been illustrated but remain controversial. While algorithms seem consistent in predicting the first six transmembrane domains of BI-1 with a cytoplasmic N-terminal, they differ in the structural characterization of the last ~20 residues of the protein that argue for or against the presence of a seventh transmembrane domain. As the HA-tagged C-terminal of BI-1 mapped to the cytosol, this region was suggested to bear a membranous re-entrant loop or a short cytosolic helix tail[10]. While still under debate, several mechanisms have been proposed to explain how BI-1 might mediate Ca^{2+} efflux from the ER as a/an: 1) Ca^{2+}/H^+ antiporter; 2) Ca^{2+} leak channel; or 3) IP3R sensitizer (Figure 3). It remains important to note that although these mechanisms have been studied independently, they may not be mutually exclusive.

i.   The first hypothesis -: states that BI-1 functions as a Ca^{2+}/H^+ antiporter, allowing Ca^{2+} release into the cytosol[24, 25] (Figure 3A). To regulate ER Ca^{2+} levels, the lysine-rich C-terminal cytosolic domain (EKDKKKEKK) of BI-1 was suggested to mediate Ca^{2+} channel gating in a pH-dependent manner, as *BI-1*-overexpressing cells showed increased release of

Ca^{2+} in response to acidic conditions[26]. Although most evidence supports a cytoprotective function for BI-1 in fine-tuning the Ca^{2+} leak from the ER, a cell death-promoting phenotype for BI-1 was observed under low pH conditions (5.0 – 6.4), since acidification in *BI-1*-overexpressing cultures dangerously increased Ca^{2+} release from the ER and dose-dependently reduced cell viability[26].

While resting cells have largely monomeric forms of BI-1, cytosolic acidification in stressed cells induces the oligomerization and antiporter activity of BI-1, whose initial function is to exchange ER-stored Ca^{2+} with H^+, reduce cytosolic acidification and promote cell survival[24]. The Ca^{2+}/H^+ antiporter activities of BI-1 may be stimulated by interaction with BH4 domains (present in BCL2 and BCLxL) through enhanced protein oligomerization[25]. Nevertheless, no direct evidence has been shown for the association of BI-1 with proton ions and Bultynck *et al.* commented that such a Ca^{2+}/H^+ antiporter function appears unlikely[27]. They claim the C-terminal of BI-1 does not have the biochemical properties to sense acidic pH changes (< 6.8), as its lysine-rich regions might only be adapted to sense more basic pH changes. They further question whether persistent H^+ gradients even occur in physiological conditions, given the high permeability of ER membranes for protons that renders the exchange of Ca^{2+} with H^+ futile[27]. In that respect, a pH-sensitive Ca^{2+} leak channel function seems more probable.

ii. **The second hypothesis -:** states that the presumed C-terminal ER-membrane dipping domain of BI-1 located after its six transmembrane domains forms a Ca^{2+}-channel pore responsible for a Ca^{2+} leak from the ER to the cytosol[11] (Figure 3B). To clarify the effect of pH on BI-1 function, Kiviluoto *et al.* optimized pH assays specifically in intact ER of permeabilized cells, as opposed to using artificial microscomal preparations or proteoliposomes. While it was corroborated that the Ca^{2+} leak property of BI-1 was pH-dependent, the Ca^{2+} flux peaks at a physiological pH and decreases under increasingly acidic conditions[28]. Specifically, BI-1 is less efficient to provoke Ca^{2+}-release at pH values < 6.8, with BI-1-mediated Ca^{2+} leakage completely blocked at pH 6.0[28]. The examination of the crystal structure of the bacterial homolog of BI-1 from *Bacillus subtilis* (BsYetJ) led to its characterization as a *bona fide* functional bacterial BI-1 homolog of human BI-1 (but with seven transmembrane domains and a luminal N-terminal) with Ca^{2+} leak activity dependent on pH-driven conformational changes[29]. This group recently established, with functional and computational data, that the modulation of BsYetJ activity was ion-dependent, with the protonation of its aspartate residues (Asp171–195 dyad) controlling pore opening and pH-dependent Ca^{2+} binding[5, 30]. Thus, BI-1-regulated Ca^{2+} flux is rather pH dependent in bell-shaped fashion: at pH 6, critical aspartate residues forming the Ca^{2+} pore in BI-1 become protonated, resulting in an open structure but loss of negative charges critical for Ca^{2+} binding; pH 7 allows for formation of a negatively charged pore for Ca^{2+} passage; and at pH 8, BI-1 is closed due to deprotonation of aspartate residues and formation of hydrogen bonds that lock the structure[5, 30]. A simplified illustration of BI-1's pH-dependent pore function features an neutral pore impearmeable to Ca^{2+} at pH 6, an open electronegative pore permeable to Ca^{2+} at pH 7, and a closed pore at pH 8[27] (Figure 3C).

iii. The third hypothesis -: states that BI-1 can sensitize IP3R independently of its Ca^{2+} channel activity, contributing to reduce steady-state Ca^{2+} concentration in the ER[31, 32] (Figure 3D). Purified and ectopically-expressed BI-1 from transfected HeLa cells co-immunoprecipitated with IP3R, further identified to bind at the C-terminal of BI-1[31]. This suggests that BI-1 and IP3R can directly influence each other's channel activity by forming protein complexes. By increasing controlled ER Ca^{2+} leakage into the cytosol, BI-1 sensitization of IP3R reduces otherwise-destined Ca^{2+} transfer from the ER into the mitochondria at MAMs[32]. By regulating steady-state levels of ER Ca^{2+} via IP3R, BI-1 influences mitochondrial bioenergetics and stimulates autophagy to promote cellular recovery when faced with metabolic stress[32]. Similar to *BI-1*-overexpressing cells, *BAX/BAK* double-knockout cells have reduced steady-state ER Ca^{2+} levels due to increased ER Ca^{2+} leakage driven by IP3R[33]. On the contrary, BCL2 appears to prevent IP3R-mediated Ca^{2+} leakage through the inhibitory binding of its BH4 domain to the regulatory and coupling domain of IP3R[34, 35]. Knowing that BI-1 interacts directly with BCL2 as well as IP3R adds yet another degree of complexity to understanding cellular $[Ca^{2+}]$ regulation and anti-apoptotic function (as extensively reviewed in [36, 37]). While small leakage of Ca^{2+} is necessary to avoid overload in the ER, too much ER Ca^{2+} depletion disturbs protein production and the Ca^{2+}-dependent activities of many intraluminal chaperones, suggesting that many actors are certainly at play to fine-tune $[Ca^{2+}]$ in fundamental organelles and the cell itself. In certain circumstances, BI-1 may even play both the role of calcium Ca^{2+} leak channel itself as well as IP3R sensitizer. Whether BI-1 is able to use both properties at once or chooses to alternate between either mechanism has yet to be determined.

C. Potential for BI-1 to limit ROS-induced cell death—If produced in excess, reactive oxygen species (ROS), a natural by-product of metabolic pathways, leads to oxidative stress that can damage cellular architecture and initiate apoptosis. Due to its protein folding function, the ER is closely associated with ROS production. The microsomal monooxygenase (MMO) system of the ER, also called microsomal electron transfer chain, leads to the release of ROS such as O^-_2 and H_2O_2[38]. BI-1 may protect against ROS accumulation by interacting with the MMO members NPR and CYP2E1 in conditions of ER stress[39–41]. *BI-1* overexpression limited the production of H_2O_2 in a dose-dependent and exponential decaying manner, seemingly due to the direct interaction of BI-1's C-terminal with NPR and CYP2E1 to block electron transfer and ROS production[39]. Likewise, *BI-1* overexpression in ER stress-exposed cells led to reduced CYP2E1 and membrane lipid peroxidation. Enhanced lysosomal activity in *BI-1*-overexpressing cells appears linked to CYP2E1 degradation[41] and BI-1 was reported to maintain high lysosomal activity under ER stress, leading to lysosomal acidification and increased autophagy[42]. Forced expression of *BI-1* in HEK293 cells and MEFs suppressed mitochondria-mediated ROS production and associated apoptosis due to activated ERK signaling[43]. Another possible way for BI-1 to limit ROS production is by stimulating the antioxidant response. Cells overexpressing *BI-1* showed increased levels of the redox-sensitive transcription factor NRF2 and its antioxidant enzyme target heme oxygenase-1 (HO-1), expressed to counteract ROS accumulation[44]. Conversely, siRNA-mediated knockdown of *BI-1* led to reduced HO-1 levels and increased sensitivity to cell death[44]. However, *BI-1*-deficient MEFs were

later shown to exhibit no differences in HO-1 transcription levels[18]. Therefore, more work must be done to clarify this connection.

III. Implication of BI-1 in disease

Increasing reports investigate the physiopathological role of BI-1 in various contexts, from metabolic diseases affecting the liver[19, 45, 46] or kidneys[47] to lung disease[48], infection [49], neurological disorders[50, 51] and cancer. Most of the literature associates the anti-apoptotic function of BI-1 with cancer development (Table 1). Nevertheless, the expression of BI-1 has yet to be shown to correlate with cancer prognosis. Although seemingly ubiquitous, the endogenous expression of BI-1 may differ among tissues or cell types and at different stages of development, leading to various cell-type and time-specific roles. While non-exhaustive, protein profiling by two-dimensional electrophoresis and mass spectrometry from liver, brain, heart, lung and kidney tissue samples identified altered differential protein expressions of GRP75, peroxiredoxin 6, fumarylacetoacetate hydrolase, selenium-binding protein 2, phosphatidylethanolamine-binding protein 1 and ferritin light chain 1 in tissues from $BI\text{-}1^{-/-}$ compared to $BI\text{-}1^{+/+}$ mice, leaving clues about BI-1 function in those organs[52]. Despite this interest, few researchers have addressed the implication of BI-1 in disease.

A.   Hepatic disorders—The fact that BI-1 is prominently expressed in the liver has incited scientists to investigate the contribution of BI-1 in hepatic disorders. Studies characterize BI-1 as a hepatoprotective factor in metabolic disorders associated with obesity, ischemia-reperfusion injury and liver regeneration.

i.   Insulin resistance and Non-Alcoholic Fatty Liver Disease: BI-1 was identified as a critical regulator of ER stress responses in the development of obesity-associated insulin resistance[19]. *BI-1* mRNA expression was downregulated by at least 30% in livers from *ob/ob*, *db/db* and 12-week high-fat diet (HFD)-fed mice compared to lean controls. Adenoviral transfer to restore hepatic BI-1 expression in these mice severely blunted the IRE1α-dependent splicing of XBP1 mRNA and improved insulin sensitivity and glucose tolerance due to dramatically reduced expression of G6Pase and PEPCK responsible for gluconeogenesis[19]. Hepatic BI-1 was further demonstrated to protect against insulin resistance by limiting ER stress and CYP2E1-dependent ROS accumulation in livers of mice fed a HFD for 8 weeks[40]. In *BI-1*-deficient hepatocytes, the knockdown of *CYP2E1* significantly reduced fatty acid-induced ROS production and improved insulin signaling as represented by increased phosphorylated AKT, FOXO-1 and GSK3 levels[40].

Hepatic insulin resistance is strongly associated with hepatic lipid accumulation in a pathology called Non-Alcoholic Fatty Liver Disease, in which patients also exhibit increased expression of ER stress markers[53]. During chronic HFD feeding, BI-1 was proposed to mediate fatty acid synthesis and ApoB lipoprotein secretion associated with the ER oxidative folding environment in the liver[54]. Livers of *BI-1*-deficient mice fed a HFD revealed excessive post-translational oxidation of protein disulfide isomerase and ER stress associated with lipid accumulation[54]. Our results on liver disease progression highlight the important role of BI-1 in limiting IRE1α activity from driving the transition from steatosis

(fatty liver) to Non-Alcoholic Steatohepatitis characterized by inflammation, hepatocyte apoptosis and often fibrosis. In livers of patients with Non-Alcoholic Fatty Liver Disease, we found *BI-1* mRNA downregulation to be accompanied by greater IRE1α RNase signaling[46]. Our current data also reveal that BI-1 confers protection against NLRP3 inflammasome activation and caspase-1 or caspase-11-dependent pyroptosis[46].

ii.   Liver injury inflicted by ischemia-reperfusion or CCl₄ injection: Hypoxia and ischemia-reperfusion insults activate ER stress-associated cell death. Livers from *BI-1*-deficient mice subjected to ischemia-reperfusion presented greater hepatocellular injury and higher expression of CHOP, spliced XBP1, cleaved ATF6 and phosphorylated JNK compared to control mice[55]. Interestingly after ischemia-reperfusion, livers of wild-type mice contained a significant 2-fold upregulation in *BI-1* mRNA levels, possibly in an effort to limit tissue injury[55]. Similarly, BI-1 protects against microvascular injury and mitochondrial dysfunction due to cardiac ischemia-reperfusion injury[56] and inflammation, ER and oxidative stress in the context of neonatal hypoxic-ischemic injury[57]. The cytoprotective role of BI-1 was also shown to have a detoxifying effect in mice with CCl₄-induced liver injury and ROS accumulation[58]. The transplantation of mesenchymal stem cells as a therapeutic intervention promoted an antioxidant response and rescued *BI-1* mRNA levels during the recovery from CCl₄-induced liver injury[58].

iii.   Liver regeneration: In response to massive hepatocellular injury, the liver demonstrates significant regenerative capacity to restore liver mass, thus providing a context for studying *in vivo* mechanisms of cell growth regulation. Regenerating hepatocytes from *BI-1*-deficient mice subjected to a two-third liver resection entered the cell cycle faster than control mice[45]. This was demonstrated by increased incorporation of BrdUrd, accelerated expression of cyclin family proteins (cyclin D1, cyclin D3, Cdc2, Cdk2 and Cdk4) with hyperphosphorylation of retinoblastoma protein and faster degradation of p27 in regenerating *BI-1*-deficient hepatocytes[45]. Faster regeneration in livers from *BI-1*-deficient mice also correlated with increased dephosphorylation and nuclear translocation of NFAT1, a Ca^{2+}-dependent event linked to calcineurin activation that promotes cytokine gene expression and facilitates hepatocyte entry into the cell cycle[45]. This suggests that the effects of *BI-1* deficiency on ER Ca^{2+} homeostasis may account for the accelerated proliferation of hepatocytes.

B.   Immune system regulation and infection—Lymphocytic BI-1 was suggested to play a key role in the adaptive immune response by regulating intracellular Ca^{2+} homeostasis. Spleens of *BI-1*-deficient mice presented significant alterations in B-cell maturation due increased ER and cytosolic Ca^{2+} levels that activated NFkB signaling[59]. Purified B cells from *BI-1*-deficienct mice were further associated with attenuated function and increased spontaneaous cell death[59]. Recently, BI-1 was suggested to protect against influenza virus by inhibiting ROS-mediated cell death[49].

C.   Neurological disorders—BI-1 has been reported to possess a neuroprotective role, even at the earliest of developmental stages to promote neurogenesis. Mouse embryonic stem cells with ectopic *BI-1* expression were protected from apoptosis, through suppression

of p38 activation, and primed for neuronal differentiation, mediated by the MEK/ERK pathway, towards a neuroectodermal lineage[60]. In mature neurons *in vitro*, BI-1 limited neuronal cell death induced by oxygen-glucose-deprivation or pharmacological ER stress-induced toxicity with thapsigargin[61]. In forms of acute brain injury inflicted by middle cerebral artery occlusion, *BI-1* deficiency in mice increased sensitivity to stroke[15], while enforced neuronal *BI-1* expression reduced CHOP expression and protected against stroke injury[50]. In response to traumatic brain injury delivered by cortical impact, neuronal *BI-1* overexpression protected against impaired motor function, lesion volume and percentage of CHOP-positive and TUNEL-positive cells[50].

BI-1 was also found to promote resilience in animal models of depression. Mice with enforced neuronal expression of *BI-1* exhibited spontaneous recovery from learned helplessness and were protected from developing a depressive-like phenotype induced by serotonin or catecholamine depletion[51]. A possible explanation for improved behavior was altered Ca^{2+} dynamics in primary cortical neuron cultures from *BI-1* transgenic mice. Interestingly, neurons with enhanced *BI-1* expression had lower basal cytosolic Ca^{2+} levels and a limited increase in intracellular $[Ca^{2+}]$ from thapsigargin-induced SERCA inhibition than neurons from wild-type mice[51]. The contribution of *BI-1* in Ca^{2+}-related psychiatric conditions was further investigated in *BI-1$^{-/-}$* mice shown to be more vulnerable to chronic mild stress-induced depressive-like behavior than wild-type mice[62]. After 6 weeks of exposure to a variety of unpredictable environmental, social and physical stressors, the brains of *BI-1*-deficient mice exhibited ROS accumulation and slight ER stress with a slight but significant decrease in the levels of the monoamines serotonin, norepinephrine and dopamine compared to wild-type mice[62]. This was proposed to be mediated by *BI-1*-dependent Ca^{2+} regulation and associated monoamine oxidase-A activity responsible for regulating serotonin, norepinephrine and dopamine levels[62].

Results have seldom been shown for BI-1 in the context of neurodegenerative diseases. Alzheimer's disease is characterized by hundreds of mutations in presenilin, the catalytic subunit of γ-secretase responsible for the generation of β-amyloid peptides from the amyloid precursor protein[63], and BI-1 was recently reported to be a binding protein of free presenilin 1, independent of intact γ-secretase[63]. While this finding is a first step towards opening investigations on BI-1 implication in neurodegenerative disease, it's a wonder the cytoprotective role for BI-1 has not been explored with greater therapeutic interest in such ER stress-associated diseases characterized by the accumulation of misfolded proteins, protein aggregates and neuronal cell death.

D.   Cancer development and progression—Cancer results from an imbalance between apoptosis and cell proliferation, fueled by the formation of a microenvironment with hypoxic, nutrient-deprived and acidic conditions. As BI-1 is characterized as an anti-apoptotic protein, it is not surprising that BI-1 is found to be upregulated in numerous different tumor samples (as reviewed in [2, 6, 64, 65]. Besides the frequent upregulation of BI-1 expression, BI-1 downregulation was observed human patients with stomach, colon, kidney, lung and rectal cancers[66]. With the expression of BI-1 in cancer cells depending on tumor type, the relevance of BI-1 expression in clinical outcomes remains to be stated clearly for therapeutic intervention.

i.   Liver cancer: As mentioned above, the noteworthy ability of the liver to regenerate rapidly may predispose this organ to carcinogenesis. As regenerating hepatocytes in *BI*-1-deficient mice duplicate and proliferate faster[45], one would assume BI-1 to protect against hepatocellular carcinoma development. The examination of liver biopsies from patients with hepatocellular carcinoma confirmed significantly downregulated *BI-1* mRNA expression in tumoral and surrounding cirrhotic tissues[67].

ii.   Breast cancer: BI-1 expression was observed in several human breast cancer cell lines, with highest levels of expression in estrogen-independent MDA-MB-453 compared to HCC70, T-47D, MCF-7, ZR-75–1 and MDA-MB-231 breast carcinoma cells (listed from highest to lowest expression)[66]. A cancer profiling array from different human tumors and corresponding normal tissues from individual female patients revealed upregulated BI-1 expression in breast cancer, but also in ovarian and cervical cancer[66]. While the expression of BI-1 in breast cancer was initially observed to be hormone independent, an estrogen-induced Krüppel-like zinc finger transcription factor (Klf10) directly suppressed BI-1 transcription, leading to increased cytosolic $[Ca^{2+}]$ concentration and apoptosis in breast cancer cells[68]. Treatment with estradiol-17β revealed a dose-dependent decrease in BI-1 protein levels in estrogen-dependent MCF-7 breast cancer cells, and the knockdown of *Klf10* reversed the effects of estradiol-17β-induced BI-1 downregulation[68]. *Klf10* overexpression in MCF-7 cells led to lower BI-1 and higher apoptotic marker protein expression[68], contradicting the previous report of unaffected apoptosis in MCF-7 cells after *BI-1* knockdown[66].

iii.   Prostate and testicular cancer: Upregulated gene expression of BI-1 was singled out of a cDNA array analysis comparing a set of capsule-invasive prostate tumor and matched normal prostate tissue and confirmed by quantitative RT-PCR in epithelial tissue samples from prostate cancer patients[69]. Downregulation of BI-1 in human PC-3, LNCaP, and DU-145 prostate carcinoma cells lowered cell viability attributable to increased apoptosis and possibly necrosis, visible by higher active caspase-3-positive staining and *in situ* end labeling that recognizes DNA strand breaks[69]. Additionally, a study explored the protective function of BI-1 in the testis against cisplatin, a gold-standard chemotherapy drug for testicular cancer, which can cause reproductive toxicity[70]. Cisplatin-treated BI-1-deficient mice presented greater testicular damage and reduced testosterone-synthesis-associated proteins compared to control mice[70]. BI-1 protection was attributed to the regulation of ROS metabolism by the antioxidant AKT-Nrf2-HO-1 axis and improved ER folding capacitance[70]. While lentivirus-mediated testicular expression of BI-1 ultimately rescued BI-1-deficient mice from testicular injury and reduced testosterone production, experimentation using models of testicular cancer will be needed to further characterize these toxicity mechanisms associated with anticancer therapy.

iv.   Nasopharyngeal carcinoma: BI-1 expression was explored in CNE-2Z and CNE-1 human nasopharyngeal carcinoma cell lines[71]. The downregulation of BI-1 in these cells led to apoptosis triggered by a decrease in the anti-apoptotic/pro-apoptotic ratios of BCL-XL/BAX and BCL2/BAX and subsequent increase in active caspase-3[71]. Subsequently, BI-1 downregulation as a target for human gene therapy was tested by lentivirus-mediated

RNA interference[72]. Administration of lenti-shBI-1 in a nasopharyngeal carcinoma xenograft model established by inoculating nude mice with was CNE-1 cells led to significantly inhibited tumor growth[72], demonstrating the therapeutic value of BI-1 inhibition in nasopharyngeal carcinoma treatment.

v. Lung cancer: The characterization of BI-1 expression in lung cancer remains ambiguous. One study found that BI-1 was positively expressed in all pulmonary adenocarcinomas with a bronchioloalveolar carcinoma (17 of 32 tumors examined), while absolutely no expression was detected in the other histological subtypes classified as papillary, acinar and/or solid tumors[73]. Clinically, bronchioloalveolar carcinoma (now referred to as "lepidic predominant adenocarcinoma", a subtype of non-small cell lung cancers) is characterized by well-differentiated but noninvasive tumors and a favorable prognosis. Hence, although positive BI-1 expression was present in this type of lung cancer, it is not associated with adenocarcinomas having more aggressive behaviors. More recently, BI-1 expression was detected in primary lesions and metastasis lymph nodes of clinical non-small cell lung cancer samples, although no information on the histological cancer subtype of the samples was provided[74]. Using human lung adenocarcinoma cell lines, BI-1 was less expressed in the highly metastatic Anip973 than in its parental line AGZY83-a associated with low metastatic capacity[74]. Thus, the differential expression of BI-1 in cells with varying metastatic potential and classified into separate subtypes of lung adenocarcinoma requires further investigation.

vi. Metastasis: During metastasis, cancer cells may break away from one of the primary cancers aforementioned, travel through the blood or lymph system and form new tumors in other parts of the body. BI-1 may mediate actin polymerization through its dynamic Ca^{2+} regulation and actin binding site, promoting cell adhesion[75]. In a vicious cycle, actin polymerization can enhance ER Ca^{2+} release[76], while high $[Ca^{2+}]$ can drive actin polymerization in a mechanism recently named calcium-mediated actin reset (CaAR)[77]. Varying Ca^{2+} levels under cellular stress is hence relevant in conditions ranging from wound healing to cancer progression as it may be responsible for the reorganization of the actin cytoskeleton, cell shape and movement. BI-1 was further found to promote metastasis by altering glucose metabolism through increased glycolysis and activating the Na^+/H^+ exchanger (NHE)[78]. Glycolytic metabolism imposes an increased acid load on tumor cells. The pH of *BI-1* overexpressing cells was lower as cells excreted more H^+ and increased NHE activity to compensate, leading to enhanced cell invasiveness and mobility as well as acid-sensitive matrix metalloproteinase-2/9 activity[78]. The viability of *BI-1* overexpressing cells was found to be promoted by the intracellular pH regulation through NHE[78]. Furthermore, the interconnection between BI-1, NHE and cancer metastasis was joined by the monoamine carboxylate transporter (MCT) system in the regulation of acidic extracellular pH[79]. Ultimately, blocking NHE or MCT activity inhibited BI-1-induced cellular acidity and cancer metastasis, leading to cell death[79]. Targeting ion exchangers or transporters may provide a therapeutic strategy aimed at limiting BI-1 activity and cancer metastasis.

Understanding the regulatory mechanisms of the BI-1 family in the tumor microenvironment will most surely help to develop and optimize cancer therapy. BI-1 was previously shown to be a pharmacological target of the chemotherapeutic drugs doxorubicin and daunorubicin that lowered cell viability in ER-stressed cells by inhibiting the Ca^{2+}/H^+ antiporter-like activity of BI-1 recreated in proteolisosomes *in vitro*[80]. An immunotherapeutic approach was also suggested for acute myeloid leukemia, since the HLA-A24-binding peptide derived from BI-1 was identified as a novel immunogenic tumor-associated antigen capable of generating leukemia-specific cytotoxic T lymphocytes[81]. BI-1 may thus be a potential tool for cancer vaccine development. Although the functions of BI-1 in cancer cells should be further investigated, these studies collectively suggest that BI-1 activities are associated with cancer progression and propagation. Importantly, regardless of the type tumor analyzed, it remains unknown how a BI-1 signature would impact a patient's outcome. In addition, no correlations have been reported between the level of BI-1 expression and the clinical response of cancer patients to chemotherapeutic drugs. Future work must be done to define BI-1 as a potentially relevant cancer therapeutic target, and to design specific BI-1-targeted novel strategies.

Conclusion

BI-1 is the best-characterized member of the TMBIM family and a highly conserved protein. Multiple hypotheses have emerged on the true function of BI-1 as a transmembrane protein but most agree that it acts as a guardian of ER homeostasis, notably bridging UPR functions and $[Ca^{2+}]$ regulation. As an anti-apoptotic protein, BI-1 controls ER Ca^{2+} as well as ROS production, both potent inducers of cell death if released in excess. That being said, the fact that the structural biochemistry of BI-1 remains to be confirmed may challenge the conclusions made on its alternative functions. The highly hydrophobic nature of BI-1 also makes it difficult to use appropriate antibodies. Mechanistic studies must still be conducted to answer fundamental questions that will pave the way for therapeutic manipulation.

This review has contributed to combine recent progress made to understand the role of BI-1 in cell death and disease. Nevertheless, many questions remain: *"What are the other molecular triggers on the fine line between ER stress-induced adaptation to apoptosis and how do they cooperate with BI-1? Besides IRE1α, can BI-1 directly affect the two other transmembrane sensors of the UPR and their downstream signaling? How can BI-1 lower $[Ca^{2+}]$ in the ER as well in the cytosol if it functions as an ER Ca^{2+} leak channel? When does an anti-apoptotic protein like BI-1 protect cancer cells at the expense of normal cells, and is BI-1 able to recognize one from the other? How does BI-1 expression correlate with the various phases of cancer?* The relatively ubiquitous expression of BI-1 has made it an interesting player in multiple diseases. Future studies and a consolidation of BI-1 function in cellular life and death situations will undeniably lead to novel therapeutic strategies.

Acknowledgements

Abbreviations

ATF6	activating transcription factor
BI-1	BAX Inhibitor-1
BAR	bifunctional apoptosis regulator
CYP2E1	cytochrome P450 species 2E1
HO-1	heme oxygenase-1
HFD	high-fat diet
IP3R	inositol 1,4,5-trisphosphate receptor
IRE1	inositol-requiring enzyme 1
Klf10	Krüppel-like zinc finger transcription factor
MOMP	mitochondrial outer membrane permeabilization
MCT	monoamine carboxylate transporter
NHE	Na^+/H^+ exchanger
NPR	NADPH-P450 reductase
PERK	PKR-like ER kinase
ROS	reactive oxygen species
SERCA	sarcoplasmic/ER calcium ATPase
TMBIM	transmembrane BAX Inhibitor-1-containing motif
UPR	unfolded protein response

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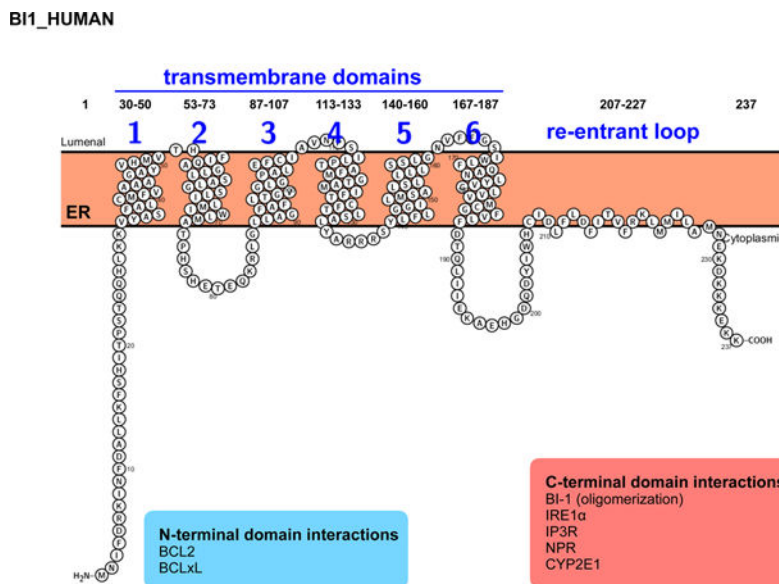
Figure 1: Structure of BAX Inhibitor-1 (BI-1)

BAX Inhibitor-1 (BI-1) is an endoplasmic reticulum (ER)-resident protein with six fully transmembrane domains and a C-terminal re-entrant loop. Listed are select interacting partners that bind either to the N- or C-terminal domain of BI-1 (BCL2 apoptosis regulator (BCL2); BCL2 like 1 (BCLxL); inositol-requiring enzyme 1 alpha (IRE1α); inositol 1,4,5-trisphosphate receptor (IP3R); NADPH-P450 reductase (NPR); cytochrome P450 species 2E1 (CYP2E1). The membrane topology model was generated using Protter [82] with the BI1_Human protein sequence from the UniProt protein knowledgebase.
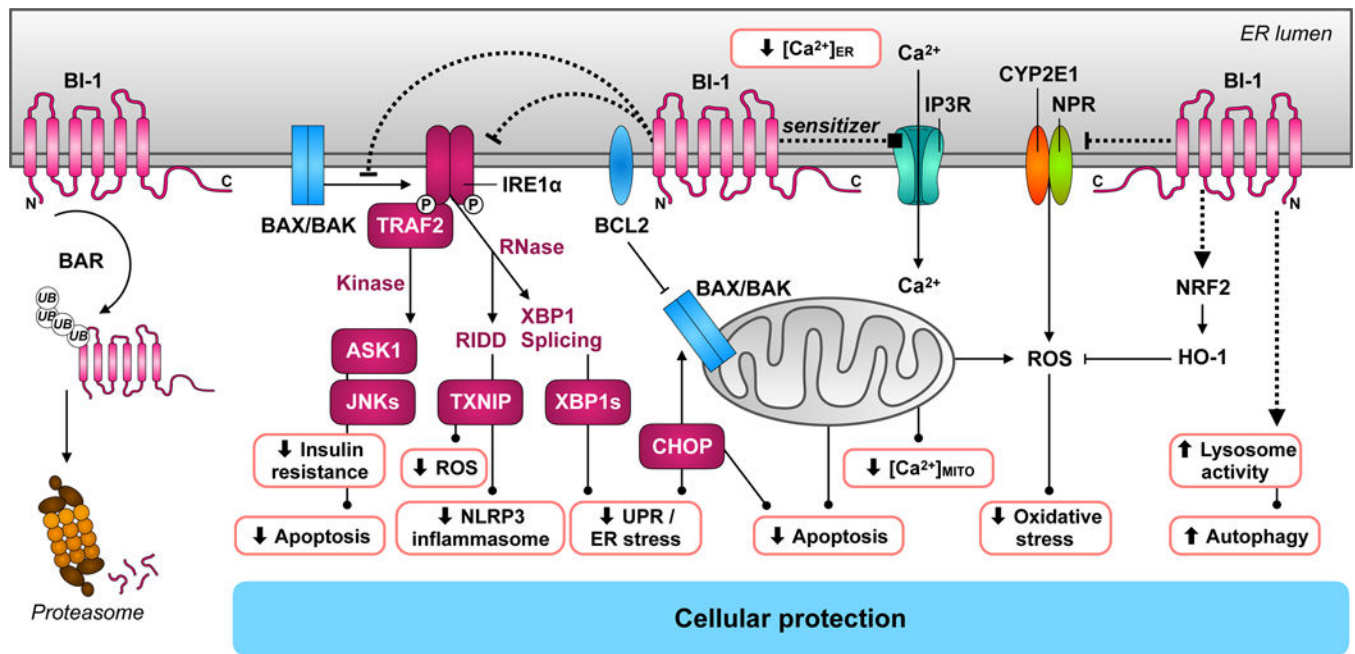
Figure 2: Overview of the cytoprotective functions of BI-1

BI-1 confers cellular protection as an anti-apoptotic protein by limiting multiple stress-inducing pathways surrounding the ER and mitochondria. Nevertheless, BI-1 can be ubiquitinated by the ER-associated RING-type E3 ligase bifunctional apoptosis regulator (BAR) for degradation by the proteasome, thus reducing BI-1 levels in the cell. BI-1 inhibits the activities of the ER stress sensor IRE1α directly and by blocking BAX/BAK binding. BI-1 can increase its anti-apoptotic function through the association with BCL2, which can inhibit BAX/BAK translocation to the mitochondria. BI-1 can regulate Ca^{2+} balance in the ER and mitochondria, notably by sensitizing IP3R function into controlled Ca^{2+} release into the cytosol. BI-1 can impact mitochondrial bioenergetics mediated by reduced $[Ca^{2+}]$, reduced reactive oxygen species (ROS) release and oxidative stress. BI-1 can interact with the microsomal monooxygenase system, composed of NADPH-P450 reductase (NPR) and cytochrome P450 member CYP2E1, to limit the harmful conversion of endogenous or exogenous compounds to ROS, which promote cytosolic acidification and oxidative stress. BI-1 can promote an antioxidant response via NRF2 and heme oxygenase-1 (HO-1) that counteracts ROS. Finally, BI-1 expression can reduce pH in lysosomes, consequently increasing lysosome activity and autophagy. (apoptosis signal regulating kinase 1 (ASK1); BCL2 associated X, apoptosis regulator/BCL2 antagonist/killer 1 (BAX/BAK); BCL2 apoptosis regulator (BCL2); DNA damage inducible transcript 3 (CHOP); inositol 1,4,5-trisphosphate receptor (IP3R); JUN N-terminal kinase (JNK); NLR family pyrin domain containing 3 (NLRP3); TNF receptor associated factor 2 (TRAF2); thioredoxin interacting protein (TXNIP); unfolded protein response (UPR); X-box binding protein 1 (XBP1)).
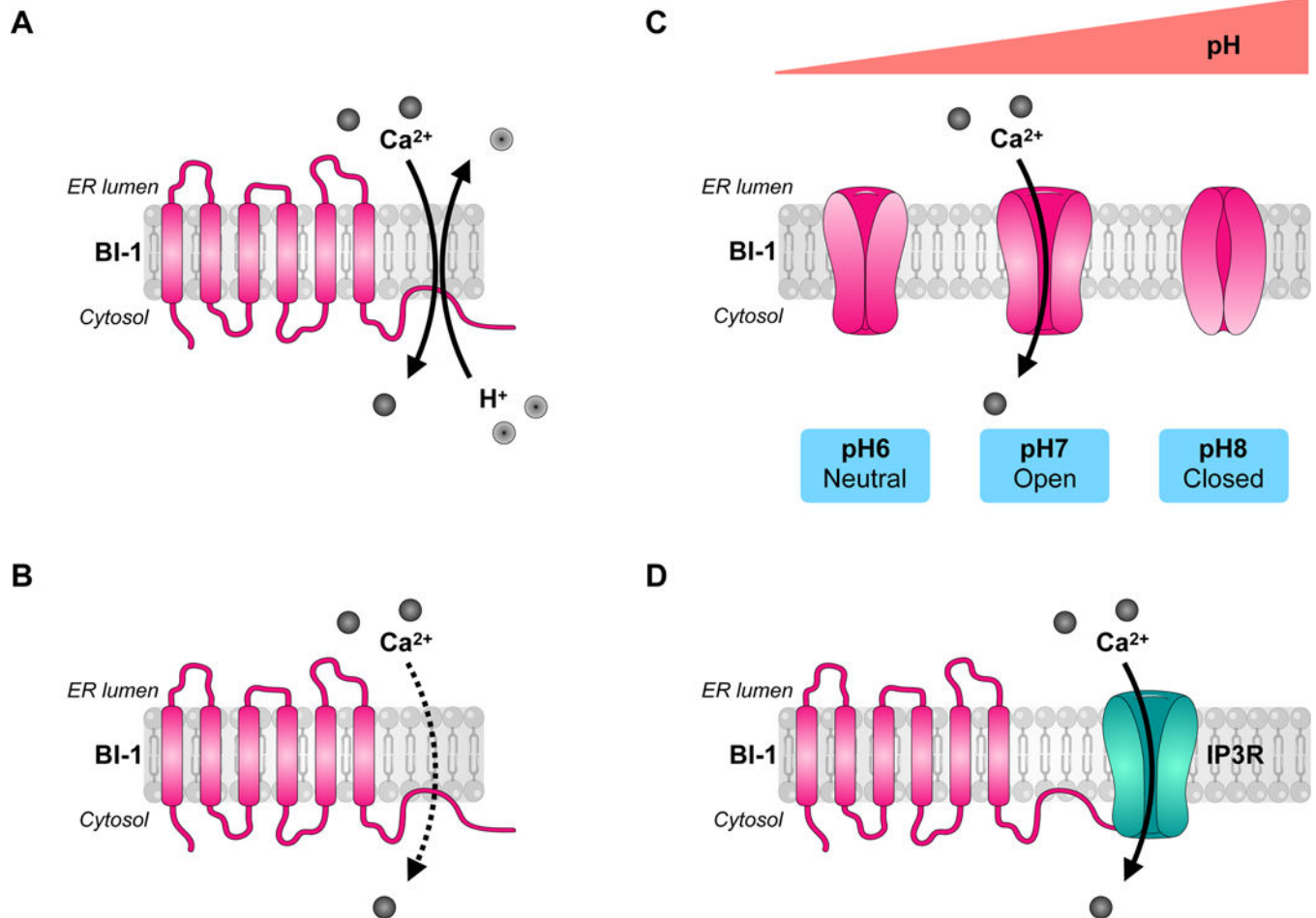
Figure 3: Proposed models of BI-1 Ca²⁺ regulation
The C-terminal of BI-1 may have Ca^{2+} channel or sensitizing properties and be regulated by changes in intracellular pH. **A)** BI-1 could function as a Ca^{2+}/H^+ antiporter. **B)** BI-1 could passively release Ca^{2+} into the cytosol as a Ca^{2+} leak channel. **C)** A simplified view of the status of the Ca^{2+}-permeable pore of BI-1 in terms of intracellular pH, endorsing the model in **B)**. **D)** BI-1 could function as an inositol 1,4,5-trisphosphate receptor (IP3R) sensitizer, directing ER Ca^{2+} flux control.

Table 1:

Overview of BI-1 expression in diseases referenced.

Disease	BI-1 levels	Observations	Reference
Hepatic disorders			
Insulin resistance	*down*	BI-1 overexpression improves hepatic insulin signaling.	[19] [40]
Non-Alcoholic Fatty Liver Disease	*down*	BI-1 KO mice are vulnerable to acute ER stress- and HFD-induced liver injury.	[54] [46]
Hypoxia/ischemia-reperfusion	*up*	After IR, BI-1 KO mice exhibit ER stress and cell death while BI-1 is upregulated in recovering WT mice.	[55]
CCl4	*down*	BI-1 levels increase after mesenchymal stem cell transplantation therapy.	[58]
Partial hepatectomy	*down*	BI-1 KO mice have accelerated liver regeneration.	[45]
Immune system regulation and infection			
Autoimmune encephalomyelitis	*?*	BI-1 KO mice display later onset of clinical symptoms with attenuated T- and B-cell function.	[59]
Influenza	*?*	BI-1 overexpression suppresses virus-induced cell death.	[49]
Neurological disorders			
Stroke	*?*	BI-1 KO mice are vulnerable to cerebral artery occlusion injury. BI-1 transgenic mice have reduced brain injury and better recovery.	[15] [50]
Depression	*?*	BI-1 transgenic mice are protected against learned helplessness and anhedonia. BI-1 mice are vulnerable to stress-induced depression-like behavior.	[51] [62]
Alzheimer's disease	*?*	BI-1 is a stable presenilin 1 interacting partner.	[63]
Other disorders			
Cardiac ischemia-reperfusion	*down*	BI1 transgenic mice are protected from microvascular injury and mitochondrial dysfunction.	[56]
Neonatal hypoxic-ischemic injury	*up*	BI-1 overexpression increases the production of antioxidant enzymes and attenuates inflammation after injury.	[57]
Nephrotoxicity	*down*	BI-1 KO mice are vulnerable to cyclosporine A-induced kidney injury.	[47]
Pulmonary fibrosis	*?*	BI-1 KO mice exhibit high sensitivity and low survival in response to bleomycin-induced lung injury.	[48]
Cancer			
Liver cancer	*down*	Human BI-1 expression is downregulated as liver damage progresses.	[67]
Breast cancer	*up*	BI-1 is upregulated in human breast cancer, though to varying degrees in common breast cancer cell lines. High-dose estrogen can induce Klf10 to suppress BI-1 transcription and induce apoptosis.	[66] [68]
Prostate cancer	*up*	BI-1 downregulation induces prostate carcinoma cell death.	[69]
Testicular cancer	*up*	BI-1 may serve a protective role against cisplatin-associated reproductive toxicity.	[70]
Nasopharyngeal carcinoma	*up*	BI-1 downregulation induces cell death, while BI-1 and Bcl-2 family interactions may contribute to apoptosis-resistance. BI-1 downregulation inhibits tumor growth and increases apoptosis.	[71] [72]
Lung cancer	*(up)*	BI-1 is upregulated in a subtype of non-small cell lung cancers. BI-1 levels vary according to metastatic potential, and patients with BI-1- positive adenocarcinoma have relatively favorable prognoses.	[73] [74]