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REVIEW

Bax Inhibitor 1 in apoptosis and disease

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Bax inhibitor 1 (BI-1) was originally discovered as an inhibitor of Bax-induced apoptosis; this review highlights the fundamental importance of BI-1 in a wider context, including in tissue homeostasis and as a regulator of cellular stress. BI-1 has been shown to interact with a broad range of partners to inhibit many facets of apoptosis, such as reactive oxygen species production, cytosolic acidification and calcium levels as well as endoplasmic reticulum stress signalling pathways. BI-1's anti-apoptotic action initially enables the cell to adapt to stress, although if the stress is prolonged or severe the actions of BI-1 may promote apoptosis. This almost universal anti-apoptotic capacity has been shown to be manipulated during infection with enteropathogenic and enterohaemorrhagic *Escherichia coli* inhibiting host cell death through direct interaction between their effector NleH and BI-1. In addition, BI-1 activity is important in a large number of cancers, promoting metastasis by modulating actin dynamics, a process dependent upon the BI-1 C-terminus and BI-1:actin interaction. Manipulation of BI-1 therefore has the potential for significant therapeutic benefit in a wide range of human diseases.
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Introduction

Apoptosis is essential in multicellular organisms for development, tissue homeostasis, wound healing and clearance of pathogens (Elmore, 2007). It is evolutionarily conserved in eukaryotes, including yeasts, nematodes and plants (Madeo *et al*., 2002) (Reviewed in detail in Wang *et al*., 1999; Hengartner, 2000; Circu and Aw, 2010; Wyllie, 2010).

Apoptosis generally occurs through the action of cysteine-dependent aspartate-specific proteases (caspases). A caspase-independent pathway also exists, mediated by apoptosis-inducing factor, but will not be discussed in detail here (for review see Cande *et al*., 2002). Caspases are synthesized as inactive pro-enzymes, which are activated by proteolysis and go on to cleave many cellular proteins thus inducing apoptosis. The process of apoptosis is initiated by either intracellular (intrinsic) or extracellular (extrinsic) signals. The extrinsic pathway is activated by external signals (for example Fas ligand, TRAIL and tumour necrosis factor (TNFα)). This induces the recruitment of intracellular proteins to their respective receptor, formation of the death inducing signalling complex, culminating in the cleavage of caspases-8 and -10. Both caspases activate the effector caspase-3 and cleave the pro-apoptotic protein BID. Cleaved BID is targeted to the mitochondria where it promotes lysis and activation of the caspase-cascade. The intrinsic pathway is activated by intracellular damage/stress/signalling for example, growth factor deprivation, DNA damage or release of endoplasmic reticulum (ER) Ca^{2+}. Such stresses result in mitochondrial lysis releasing pro-apoptotic proteins, one of which is cytochrome *c* which induces the formation of a death signalling complex (apoptosome) cleaving caspase-9 and inducing the caspase cascade.

Apoptosis is a highly regulated process. FLIPs (FADD-like interleukin-1 β-converting enzyme-like protease-inhibitory proteins) inhibit the extrinsic pathway by inhibiting caspase-8 cleavage. In addition, the global regulators IAPs (inhibitors of apoptosis proteins) inhibit the activation of caspases, while BCL-2 family members act at the mitochondria and ER membranes to block mitochondrial permeabilization (Green and Reed, 1998; Kuo *et al*., 1998; Hengartner, 2000; Ferri and Kroemer, 2001; Igney and Krammer, 2002; Elmore, 2007). BCL-2 family members are functionally divided into pro- and anti-apoptotic proteins on the basis of the presence/absence of BCL-2 homology (BH) domains. Anti-apoptotic BCL-2 members, possess four BH domains, and are mainly localized at the mitochondrial outer membrane, although they can also be found in the cytosol and at the ER (Chipuk *et al*., 2010). These anti-apoptotic proteins preserve mitochondrial membrane integrity, in part, by antagonizing the pro-apoptotic BCL-2 members. The pro-apoptotic BCL-2 members consist of 'effectors', which contain BH 1–3 domains (Kvansakul *et al*., 2008; Chipuk *et al*., 2010), and 'BH-3 only' proteins, which contain a single BH3 domain. Two known BCL-2 effectors are Bcl-2-associated x protein (Bax)

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and Bcl-2 antagonist killer 1 (Bak). These effectors act by forming homo-oligomeric pores in the mitochondrial outer membrane leading to disruption and release of pro-apoptotic proteins, which in turn promote the release of ER Ca^{2+}. The second group of pro-apoptotic proteins, the BH3-only proteins, are further subdivided on the basis of their protein interaction profile into those that interact solely with BCL-2 anti-apoptotic proteins (for example, HRK and Noxa) or additionally with effector BCL-2 members (for example, BID and BIM) (Chipuk *et al*., 2010).

This review highlights the importance of a fourth regulator of apoptosis, bax inhibitor 1 (BI-1). BI-1 enables the cell to adapt to a broad range of stresses, which is attributable to its numerous interacting partners. This review will discuss the many roles of BI-1 and highlight how BI-1 has been manipulated to inhibit apoptosis during bacterial infection and tumourigenesis.

Regulation of apoptosis by Bax inhibitor-1

Bax inhibitor 1 (also known as testis-enhanced gene transcript) was discovered during a functional assay screen for suppressors of Bax-induced cell death (Xu and Reed, 1998). Bax expression is lethal in yeast; using a human HepG2 complementary DNA expression library a small protein of 237 amino acids (26.538 kDa) was identified, which conferred resistance to Bax and was therefore named BI-1 (Xu and Reed, 1998). The human *bi-1* gene is found on the 12q12–q13 chromosome. BLAST searches revealed highly conserved, uncharacterised homologues of the human BI-1 in mice (92% identity, 95% similarity), *Caenorhabditis elegans* (21% identity, 23% similarity) and *Arabidopsis thaliana* (29% identity, 45% similarity) (Figure 1a) (Xu and Reed, 1998).

Northern blot analysis showed that BI-1 is widely expressed in humans and found in organs inclusive of the heart, lungs, liver, stomach, colon, kidney, brain, placenta, skeletal muscle and pancreas (Xu and Reed, 1998; Grzmil *et al*., 2006). BI-1 expression levels in these organs vary and fluctuate during development (Grzmil *et al*., 2006; Tanaka *et al*., 2006). During pre- and early-natal lung development BI-1 expression peaked during times of high apoptosis (Jean *et al*., 1999), indicating a possible link between cell-death and BI-1 expression. How BI-1 expression is regulated in tissues remains unknown, however, upstream of the *bi-1* gene are two alternative promoters (P1 and P2), which are independent of TATA elements. It is thought the proximal P1 promoter is regulated by cell-type specific factors, while the distal P2 promoter is constitutive in all cell types (Jean *et al*., 1999). Moreover, 15 p53 tumour suppressor sites are present in the BI-1 promoter region. Although no relationship between p53 expression and BI-1 expression was observed in adenocarcinomas (Tanaka *et al*., 2006), the recognised importance of p53 in inhibiting cancer leads to speculation that BI-1 may be deregulated in cancers with p53 mutations.

BI-1 is a transmembrane protein bearing putative nuclear targeting sequences, but overall lacking any functional motifs. The transmembrane regions are deemed to be mostly α-helical, with both experimental and bio-informatic evidence indicating BI-1 is a six transmembrane protein exposing its N- and C-termini into the cytosol (Figure 1b) (Xu and Reed, 1998).

Transfection and fractionation studies showed that BI-1 is localized to both the ER and nuclear envelope with only a small proportion associated with mitochondrial membranes (Figure 1c) (Xu and Reed, 1998). Cross-linking and co-immunoprecipitation revealed that BI-1 interacts with Bcl-2 and Bcl-xL by their BH4 domains, but not with Bax or Bak (Xu and Reed, 1998). Therefore, regulation of Bax by BI-1 is not through direct protein:protein interactions.

Several lines of evidence suggest that BI-1 modulates the intrinsic, but not the Fas- and TNFα-induced extrinsic apoptotic pathways. Overexpression of BI-1 in interleukin-3-dependent pro-B lymphocytes (FL5.12) significantly inhibited apoptosis induced by the topoisomerase inhibitor, etoposide and global kinase inhibitor, staurosporine, both initiators of the intrinsic pathway (Xu and Reed, 1998). Whilst human diploid fibroblasts (GM701) overexpressing BI-1 grew during growth factor deprivation and inhibited reactive oxygen species (ROS) accumulation (Lee *et al*., 2007). BI-1 antisense transfected into human embryonic kidney 293 cells induced apoptosis, independent of cell stress inducers (Xu and Reed, 1998).

BI-1 anti-apoptotic activity is caspase-independent, as treating BI-1 overexpressing cells with tunicamycin, an N-linked glycosylation inhibitor, in the presence of the global caspase inhibitor zVAD-fmk, did not result in translocation of Bax to the mitochondria (Chae *et al*., 2004). In contrast, BI-1 overexpressing cells treated with staurosporine in the presence of zVAD-fmk, showed that Bax was translocated to the mitochondria (Chae *et al*., 2004). Taken together, these results suggest that the anti-apoptotic activity of BI-1 is more specific to ER stress inducing agents.

BI-1 modulation of ER stress-induced apoptosis

The modulation of ER-stress-induced apoptosis by BI-1 is linked to ER stress signalling, which initially enables the cell to adapt to stress, but can result in apoptosis, (reviewed in detail Xu *et al*., 2005). The ER is associated with the synthesis, initial post-translational modification, folding and maturation of proteins and is a major regulator of intracellular Ca^{2+} homeostasis (Shiraishi *et al*., 2006). Improper protein maturation, unfavourable environmental conditions, protein redox and glycosylation status alterations or nutrient and ATP deprivation, disrupt ER function inducing ER stress and the unfolded protein response (UPR). The UPR is a signalling cascade enabling the cell to adapt to different stresses. If unresolved or prolonged, this will result in ER Ca^{2+} release, promoting apoptosis and pro-apoptotic

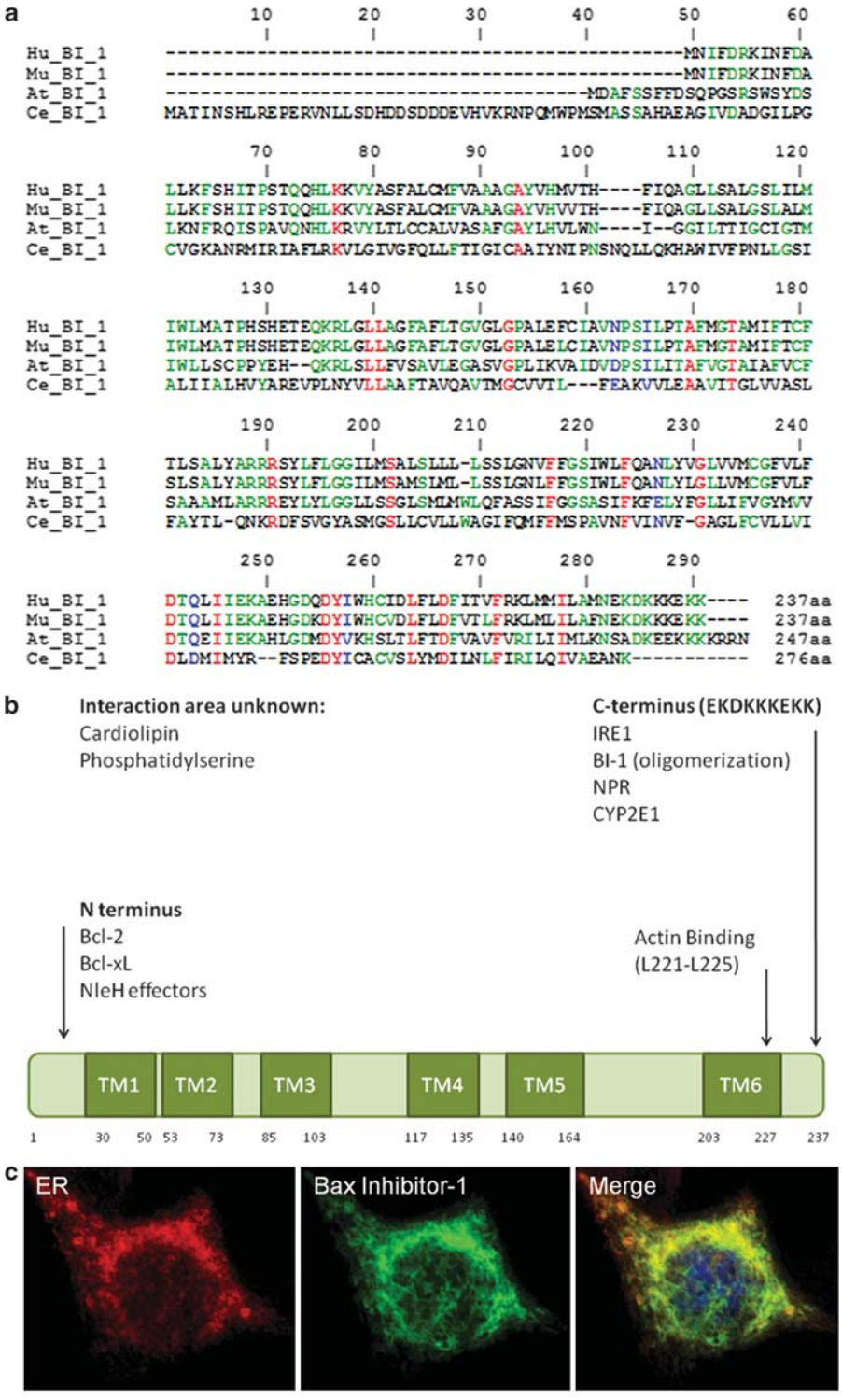

Figure 1 (**a**) Amino acid sequence alignment of the evolutionarily conserved BI-1 of human (Hu_BI_1), *Mus musculus* (Mu_BI_1), *Arabidopsis thaliana* (At_BI_1) and *Caenorhabiditis elegans* (Ce_BI_1). (**b**) Human BI-1 is an ER resident protein consisting of six predicted transmembrane domains. The anti-apoptotic activity of BI-1 is largely mediated by binding various partner proteins. (**c**) Immunofluorescent staining showing co-localization of BI-1 with the ER marker calnexin.

ER signalling for example, JNK phosphorylation resulting in induction of CHOP, a pro-apoptotic transcription factor (for a review of Ca^{2+} role in apoptosis see Rizzuto *et al*., 2003).

ER stress signalling is mediated by three transmembrane proteins ATF6 (activating transcription factor 6), PERK (PKR-like ER kinase) and IRE1 (inositol-requiring-1), which are kept inactive through interaction

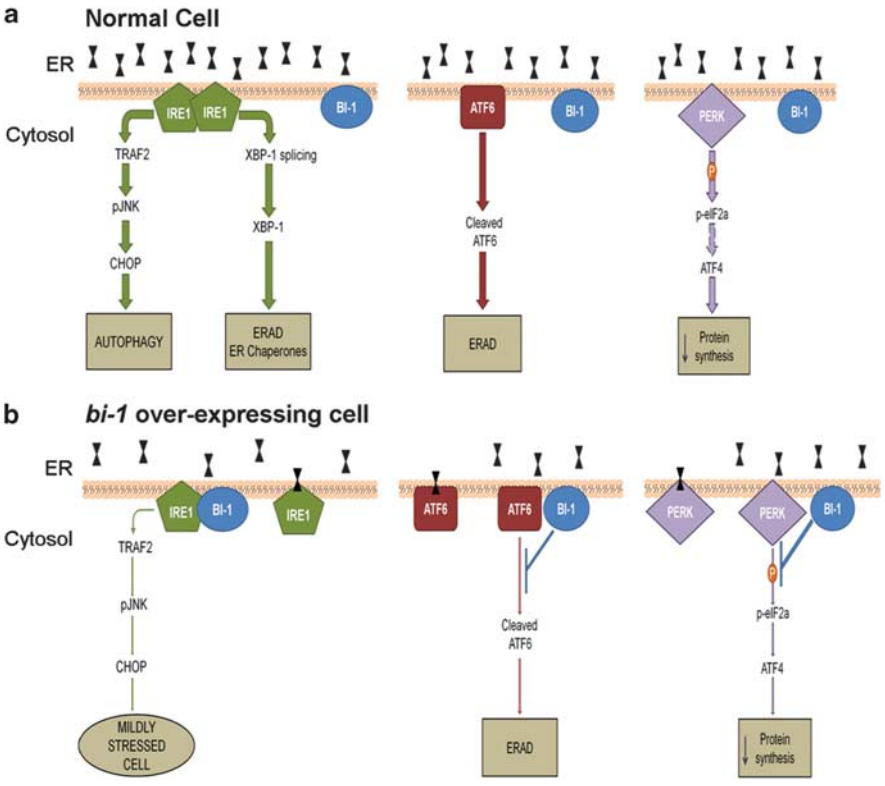

Figure 2 The unfolded protein response (UPR) is a signalling cascade enabling cellular adaptation to stress or promotion of apoptotic pathways. BI-1 regulates the UPR signalling by modulating the ER proteins ATF6, PERK and IRE1. During stress, normal cells upregulate the UPR resulting in adaptation. However, under prolonged or severe stress, the UPR results in apoptosis. Stressed cells overexpressing *bi-1* exhibit a significantly lower UPR resulting in adaptation and cellular survival. In response to stress, cells induce expression of Grp78 (), however, the magnitude of Grp78 expression is reduced by 50% in cells overexpressing BI-1 (Table 1).

with the luminal ER protein Grp78 (Figure 2a) (Imaizumi *et al*., 2001; Shiraishi *et al*., 2006). Initially, adaptation to ER stress induces cleavage of ATF6 releasing its cytosolic fraction, which translocates to the nucleus inducing the expression of ER chaperones and the ER associated degradation machinery (ERAD) genes. In addition to promoting ER chaperones in response to stress, PERK is activated and phosphorylates the eukaryotic initiation factor 2-α (eIF2α). Phosphorylated eIF2α goes on to decrease protein synthesis, by releasing inhibition of ATF4, a transcription factor that reduces the protein load on the ER, further reducing ER stress (Kohno, 2010). IRE1 is a dual enzyme, composed of a kinase domain and RNase domain and has both an anti- and pro-apoptotic role. ER stress induces the oligomerisation of IRE1 and its autophosphorylation thereby activating its RNase activity. The RNase activity promotes the splicing of XBP-1 inducing the formation of the active XBP-1 transcription factor and increasing the expression of ER chaperones and ERAD genes. Moreover, following prolonged or severe stress the cytoplasmic part of IRE1 also interacts with TRAF-2 inducing, to varying degrees, nuclear factor-κB activation, phosphorylation of JNK and p38 MAPK by ASK1 (Urano *et al*., 2010), induction of CHOP, autophagy and ultimately apoptosis (Kim *et al*., 2006; Ogata *et al*., 2006). The UPR response is dependent upon the intensity and duration of the stress, with the cell initially adapting to overcome the stress, before committing to apoptosis.

Interestingly, cells overexpressing BI-1 challenged with ER stress inducers, such as tunicamycin (N-linked glycosylation inhibitor), brefaldin A (an inhibitor of ER to Golgi transport), or during oxygen-glucose deprivation and ischaemic reperfusion injuries, did not exhibit characteristic biochemical and morphological apoptotic changes for example, changes in ER/Golgi morphology, caspase activation, Bax oligomerization, production of apoptotic bodies and membrane blebbing (Chae *et al*., 2004). Inversely, BI-1 knockout cells exhibited hypersensitivity to the same ER stresses, indicating BI-1 aids to inhibit ER stress signalling pathways (Figure 2b, Table 1) (Chae *et al*., 2004; Bailly-Maitre *et al*., 2006).

Lee *et al*. (2007) demonstrated that BI-1 can interfere with all three ER signalling pathways (Figure 2b, Table 1). During ER stress BI-1 overexpression decreased the level of IRE1 expression with downstream repercussions on the activation of XBP-1 and pro-apoptotic signalling through JNK phosphorylation (Lee *et al*., 2007). Moreover BI-1 was shown to bind IRE1 through its C-terminus (Lisbona *et al*., 2009), which regulates its ability to oligomerize, thereby blocking its RNase activity. In addition, the binding allosterically

Table 1 Fold induction of protein expression and signalling events during ER stress

	No ER Stress		*ER Stress*	
	Normal/bi-1 over-expression	*bi-1−/−*	*Normal/bi-1 −/−*	*bi-1 over-expression*
Grp78	NC[a]	NC	× 30	× 15
IRE1	NC	NC	× 22	NC
pJNK	NC	× 3	× 30	× 5
CHOP	NC	× 2	× 15	× 6
XBP-1 splicing	NC	NC	× 25	NC
Cleaved ATF6	NC	× 0.25	× 1.5	× 0.25
p-eIF2a	NC	NC	× 12	× 6

Abbreviation: NC, no change.
ER stress results in the activation of the UPR. The UPR is mediated by IRE1, PERK and ATF6, which are held inactive by ER chaperones (Grp78) until stress is detected. In the event of prolonged or excessive ER stress this induces a signalling cascade (involving JNK), through IRE1, activating transcription factors (that is, CHOP) promoting apoptosis.

inhibits IRE1 interaction with the pro-apoptotic BCL-2 members, Bak and Bax, which stabilise the active form of IRE1 (Hetz *et al*., 2006; Lisbona *et al*., 2009). Moreover, BI-1 is negatively regulated by bi-functional apoptosis regulator, a RING-type E3 ligase. Interaction between bi-functional apoptosis regulator and BI-1 induced ubiquitination of BI-1 and its degradation by the proteasome therefore removing the inhibition of IRE1 by BI-1 during ER stress (Rong *et al*., 2011).

Interestingly, although not shown to directly interact with the other UPR proteins PERK and ATF6, BI-1 affects their signalling pathways (Figure 2b, Table 1). Indeed, the level of phosphorylation of eIF2α is decreased when BI-1 is overexpressed (Lee *et al*., 2007) suggesting that BI-1 inhibits PERK by an unknown mechanism. Moreover, BI-1 overexpression also decreased the cleavage of ATF-6 (Lee *et al*., 2007) and therefore blocks the subsequent signalling pathway.

BI-1 was found to inhibit cell death by promoting ER pro-survival signalling and inhibiting ER pro-apoptotic signalling. In addition to ER-stress signalling events, the ER also regulates cytosolic Ca^{2+} levels and ROS production, both potent inducers of apoptosis if released or produced in excess.

BI-1: a regulator of ROS

ROS are highly reactive oxygen containing molecules, which are a natural by-product of metabolic pathways and have an important role in cell signalling. During times of cellular stress ROS production can dramatically increase, culminating in oxidative stress, damage to cellular architecture and apoptosis. At the ER, ROS are produced by the microsomal mono-oxygenase system composed of cytochrome P450 (CYP) members, NADPH-P450 reductase (NPR) and phospholipids. The cytochrome P450 member CYP2E1 produces a large quantity of ROS, such as H_2O_2, without substrate oxidation (Nieto *et al*., 2002) and has an important role during ER stress. BI-1 is able to regulate ER ROS production and activity by two mechanisms (Figure 3a). Firstly, ROS production is controlled by the redox-sensitive transcription factor Nrf-2. Nrf-2 controls the expression of different anti oxidant enzymes for example, Heme-Oxygenase-1, glutahion-S-transferase, glutamyl cysteine synthetase and phase II detoxifying enzyme. Overexpression of BI-1 in cells increased the activation of Nrf-2, promoting its translocation to the nucleus resulting in an increase in anti-oxidant enzymes, such as, Heme-Oxygenase-1. Heme-Oxygenase-1 is an oxygenase enzyme, which blocks ROS activity and is expressed to counteract ROS accumulation, thereby promoting cell survival (Lee *et al*., 2007). Secondly, BI-1 can directly inhibit the formation of ROS by interacting through its C-terminus with NPR, and to a lesser extent with CYP2E1 (Figure 3a). Interaction between BI-1 and NPR induces destabilisation of the NPR-CYP2E1 complex, blocking electron transfer and ROS production (Kim *et al*., 2009).

In summary, BI-1 overexpression promotes cell survival by increasing the production of anti-oxidant enzymes as well as destabilising the complex responsible for ROS production. These two mechanisms have a major role in the anti-apoptotic activity of BI-1.

BI-1: a regulator of Ca^{2+}

The main cellular store for Ca^{2+} is the ER; release of Ca^{2+} from the ER will determine the sensitivity of cells to apoptosis by determining mitochondrial permeability (Shiraishi *et al*., 2006). Due to the negative (inside) membrane potential of mitochondria, they can efficiently accumulate Ca^{2+}, which acts to increase their metabolic activity and permeability. However, excessive Ca^{2+} leads to mitochondrial membrane hyper-permeability resulting in the influx of cytosolic components in exchange for mitochondrial proteins, lysis and release of pro-apoptotic proteins (Lebiedzinska *et al*., 2009). Cytosolic Ca^{2+} is regulated at the ER by both the inositol 3 phosphate receptor (IP3R), a channel which passively releases Ca^{2+}, and the sarcoplasmic/ER Ca^{2+} ATPase (SERCA), which actively pumps Ca^{2+} into the ER from the cytosol (Figure 3b).

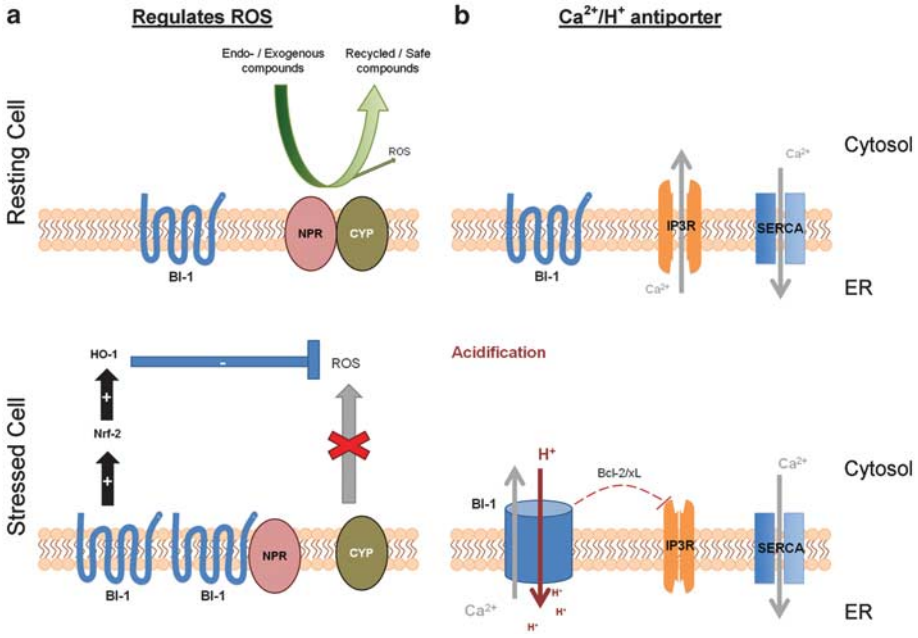

Figure 3 (**a**) BI-1 regulates reactive oxygen species (ROS) production and functions as a Ca^{2+}/H^{+} antiporter. In a resting cell, the microsomal monooxygenase system, composed of NADPH-P450 reductase (NPR) and cytochrome P450 members (CYP), inclusive of CYP2E1 recycle or neutralise endogenous and exogenous compounds. Apoptotic pathways can convert harmful endogenous or exogenous compounds to ROS, which promote apoptosis and cytosolic acidification. BI-1 inhibits the ROS production by destabilizing the MMO complex thereby blocking electron transport. In addition, BI-1 promotes heme oxygenase 1 (HO-1) production, which counteracts the ROS that have been produced (**a**). (**b**) Resting cells have largely monomeric forms of BI-1, which do not act as a Ca^{2+}/H^{+} antiporter. Oligomerization of BI-1 during stress promotes the Ca^{2+}/H^{+} antiporter activity. Oligomerized BI-1 releases ER stored Ca^{2+} hence reducing cytosolic acidification and promoting cell survival. The binding Bcl2/xl to BI-1 brings them in close proximity to IP3R, which blocks the channel, directing the control of the ER Ca2+ flux to BI-1.

Different reports suggest that BI-1 can regulate the concentration of Ca^{2+} in the ER, $[Ca^{2+}]_{ER}$, and the cytosol, $[Ca^{2+}]_{cytosolic}$. Overexpression of BI-1 induced a decrease in the $[Ca^{2+}]_{ER}$, because of ER Ca^{2+} release, while knockdown of BI-1 increased $[Ca^{2+}]_{ER}$ (Kim *et al*., 2008). The ability of BI-1 to decrease the $[Ca^{2+}]_{ER}$ was discovered using Ca^{2+} filled liposomes containing BI-1. This approach demonstrated that BI-1 acted as an ER membrane Ca^{2+}/H^{+} antiporter allowing Ca^{2+} release into the cytoplasm (Kim *et al*., 2008; Ahn *et al*., 2009).

Interestingly, anti-apoptotic BCL-2 members, Bcl-2 and Bcl-xL, blocked the passive ER IP3R Ca^{2+} efflux (Rong *et al*., 2009), but increased the oligomerization and the Ca^{2+}/H^{+} antiporter activity of BI-1 (Figure 3b) (Ahn *et al*., 2010). A similar effect has been observed with anionic phospholipids such as cardiolipin and phosphatidylserine (Ahn *et al*., 2010). Cardiolipin, phosphatidylserine, Bcl-2 and Bcl-xL all increase the Ca^{2+} efflux and H^{+} influx activity of BI-1. The interaction between BI-1 and Bcl-2 or Bcl-xL (Xu and Reed, 1998; Ahn *et al*., 2010) may be important in regulating ER Ca^{2+} release by bringing Bcl-2 and Bcl-xL in proximity to IP3R, thereby directing control of cytosolic $[Ca^{2+}]$ to BI-1. Moreover, BI-1 Ca^{2+}/H^{+} antiporter activity is increased by cytosolic acidification (Kim *et al*., 2008) with elevated cytosolic H^{+} occurring during apoptosis. Cytosolic acidification is detected by the C-terminus of BI-1 (Ahn *et al*., 2009), resulting in BI-1 oligomerization and ER Ca^{2+} release, but also a decrease in cytosolic $[H^{+}]$. This was observed during BI-1 overexpression and suggests that BI-1 may inhibit apoptosis by decreasing cytosolic $[H^{+}]$ as well as promoting mitochondrial metabolic activity to restore the ATP level and help to restore the neutral pH in cells (Ahn *et al*., 2010).

BI-1 and disease

BI-1 tightly regulates intrinsic apoptotic pathways. However, deregulation of BI-1 can be associated with the apparition of cancer, or can be used during the infection process by pathogenic bacteria.

BI-1: promoting bacterial survival

It has recently been demonstrated that bacterial pathogens have developed mechanisms to target BI-1 signalling to promote survival of infected cells. Enteropathogenic and enterohaemorrhagic *Escherichia coli* (EPEC and EHEC, respectively) inject into the mammalian cell multiple effector proteins that target and subvert diverse signalling pathways involved in tight junction disruption, mitochondrial lysis, adherent junctional and actin disruption (Crane *et al*., 2001; Caron

et al., 2006). This coordinated assault triggers early symptoms of apoptosis, yet infected cells do not show late apoptotic markers, such as nuclear condensation, membrane blebbing and activation of caspase 3. Among the effector proteins, EPEC and EHEC inject into the cell are proteins called NleH. These proteins have recently been shown to bind BI-1 and to inhibit apoptosis for the benefit of the pathogen (Hemrajani *et al*., 2010). For example, NleH binds BI-1 and decreased the cytoplasmic Ca^{2+} released during infection, thereby blocking Ca^{2+}-mediated apoptotic signalling. NleH homologues have been found in *Salmonella* species, however, it has not yet been tested whether these homologues also bind BI-1 and inhibit apoptosis.

BI-1 and cancer

Levels of BI-1 expression differ in different human cancers—while breast, glioma, prostate, uterine and ovarian cancers show two and above fold upregulation (Schmits *et al*., 2002; del Carmen Garcia Molina Wolgien *et al*., 2005; Grzmil *et al*., 2006), stomach, colon, kidney, lung and rectal cancers exhibit twofold downregulation of BI-1 (Grzmil *et al*., 2006). It is unknown whether these changes are relevant to the cancerous state or are simply a reflection of different levels of BI-1 in these tissues during development (for example, BI-1 expression is inhibited during lung development and was found to be downregulated in lung cancer). The relevance of BI-1 expression in these tissues during normal cell growth and cancerous growth has not yet been fully elucidated and requires further study.

Tumour development is promoted by the formation of a patho-physiological microenvironment characterised by hypoxic conditions, nutrient deprivation and acidification. This acidic environment is regulated by ion channels and transporters, for example, Na^+/H^+ (NHE) or Cl/HCO_3 exchangers (Plopper *et al*., 1995; Lee *et al*., 2010b). Elevated NHE levels enhance the acidification of extracellular space by extruding excess protons from tumour cells (Tannock and Rotin, 1989; Wahl *et al*., 2000), which by activating proteases for example, can promote the invasiveness of carcinoma cells (Reshkin *et al*., 2000; Lee *et al*., 2010b). BI-1 overexpression has been shown to increase glucose uptake, causing downregulation of pyruvate dehydrogenase activity and accumulation of lactate inducing acidification, through an as yet unknown mechanism. These metabolic changes promoted tumourigenic cells to take up glucose and convert it to lactate, despite being in an oxygenated environment (Lee *et al*., 2010b). Moreover, BI-1 overexpression increased cell motility and invasiveness imposing an increased acid load on tumour cells, with this surplus removed by NHE, resulting in extracellular acidification. Further understanding of how an acidic extracellular pH, increased NHE activity and altered glucose metabolism is important in tumorigenesis and metastases is required before we can understand the role of BI-1 in creating the microenvironment for tumour development.

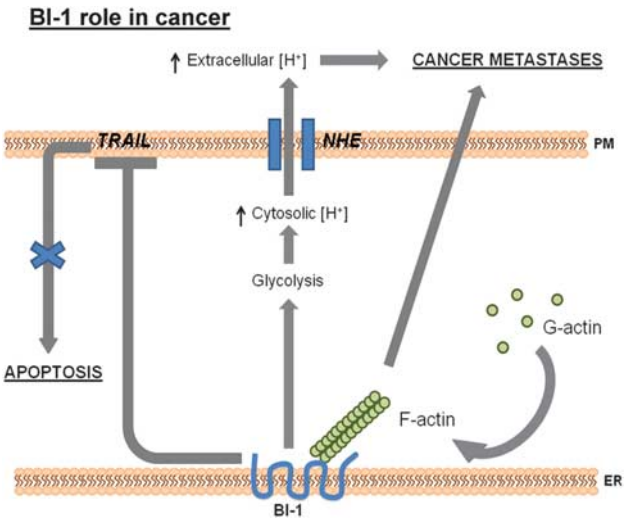

Figure 4 BI-1 is overexpressed in many cancer types, including breast. BI-1 enhances cancer metastases by acting as a docking site to polymerize globular actin (G-actin) into filamentous structures (F-actin). Actin polymerization promotes epithelial to mesenchymal transition and metastases. Furthermore, BI-1 increases glucose uptake, downregulation of pyruvate dehydrogenase activity and lactate accumulation. The increased cytosolic acidification results in H^+ ions being extruded into the extracellular milieu by Na^+/H^+ exchangers (NHE). Enhanced glycolysis and extracellular acidification provided a twofold advantage to promote cancer metastases by promoting cell motility and creating an environment undesired by normal cells. Furthermore, neoplastic cells are sensitive to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), with most normal cells exhibiting resistance. BI-1 was indicated to act in concert with other proteins to inhibit TRAIL-induced apoptosis in tumour cells.

During metastasis, tumour cells migrate to new sites of attachment, leading to the development of secondary cancers. Metastatic cancer requires both cell adhesion and migration, with initial loss of attachment and actin protrusions enabling migration. Actin exists as monomers and polymers, and forms dynamic structures in the cell permitting dramatic changes in cell shape. Overexpression of BI-1 increases cell adhesion and lamellipodia formation (Lee *et al*., 2010a) by binding to actin and modifying actin dynamics and cell shape (Figure 4). This interaction occurs through an LxxL motif in one of BI-1's c-terminal helices; mutations in either leucine residue negate BI-1 actin interaction (Lee *et al*., 2010a). Furthermore, BI-1 overexpression increased levels of focal adhesion kinase expression, which acts as a scaffold for adhesion components regulating apoptosis, cell spreading, proliferation and migration (Lee *et al*., 2010a).

BI-1 and breast cancer

Breast cancer is the most common cancer in women and the second most common cause of female deaths in

Western countries (Grzmil *et al*., 2006). Analysis of the proteome in breast cancer showed elevated levels of anti-apoptotic proteins for example, Bcl-2, Bcl-xL, IAPs and BI-1 providing chemoresistance (Krajewski *et al*., 1999; Grzmil *et al*., 2006). RNA interference (antisense) of the anti-apoptotic Bcl-2 and XIAP sensitized cancerous cells to therapeutic agents. BI-1 antisense in MD-MBA-231 breast cancer cells resulted in spontaneous apoptosis, which was also the case in prostate cancer, indicating BI-1 is essential for survival in these cancers (Lima *et al*., 2004; Grzmil *et al*., 2006). However, BI-1 antisense is not immediately lethal in all breast cancer cell lines, presumably because Bcl-2 and Bcl-xL are overexpressed to varying degrees in 40–80% of human breast tumours (Grzmil *et al*., 2006). Furthermore, IAP levels, a caspase inhibitor, are elevated in certain cell lines thereby blocking caspase activation irrespective of BI-1 expression (Srinivasula *et al*., 1998; Kurokawa *et al*., 1999; Kim *et al*., 2002; Yang *et al*., 2003; Grzmil *et al*., 2006).

BI-1: an inhibitor of TRAIL-induced apoptosis?

The TNF-related apoptosis-inducing ligand (TRAIL) is a potent, pro-apoptotic cytokine and a member of the TNF superfamily (Burns and El-Deiry, 2001). TNF superfamily members are type 2 membrane proteins bearing conserved C-terminal and extracellular domains; with several members inducing apoptosis upon ligand interaction for example, TNFα, Fas ligand and TRAIL. TRAIL uniquely has wide ranging cytotoxic effects against tumour cell lines whereas most normal cell lines exhibit TRAIL resistance. TRAIL-induced apoptosis requires BAX activation (Ravi and Bedi, 2002) and is inhibited by TRAIL decoy receptors, TRID and TRUNDD as well as FLICE-inhibitory proteins, Bcl-2, Bcl-xL and BI-1 (Scaffidi *et al*., 1998; Burns and El-Deiry, 2001). However, when overexpressed alone, BI-1 did not significantly protect against TRAIL-induced cell death in colonic cell carcinomas (SW480 and HCT 116 cell lines) (Burns and El-Deiry, 2001) indicating that BI-1 must act in concert with other TRAIL inhibitors to block TRAIL-induced apoptosis (Figure 4). BI-1 cytoprotectivity may be orchestrated by many proteins and pathways exemplified by the inhibition of TRAIL-induced apoptosis. The exact mechanism by which BI-1 inhibits TRAIL-induced apoptosis is not known. However, Bax is critical for mediating TRAIL-induced apoptosis and BI-1 is known to be a Bax inhibitor, thereby blocking its activation. For this reason, BI-1 may be able to exclusively inhibit cell death to TRAIL, but not TNFα or Fas, which do not require Bax.

Concluding remarks

Considerable progress has been made in recent years in understanding the role of BI-1 in apoptosis; as well as highlighting its role in disease. Although we are beginning to understand the importance of BI-1 in the cell, the way in which it functions remains ambiguous.

BI-1 cytoprotectivity seems to be a balancing act, with possible feedback mechanisms helping it to regulate its own function. This is exemplified by the activity of BI-1 as an Ca^{2+}/H^{+} antiporter during acidification. If released in excess Ca^{2+} is an inducer of apoptosis through mitochondrial lysis, however, balanced Ca^{2+} levels promote mitochondrial swelling, which enhance metabolic pathways, promoting receptor activity and removing cytosolic H^{+}.

It is particularly interesting to observe that BI-1 is able to block Bax-induced apoptosis without directly interacting with Bax. The mechanisms by which BI-1 achieves this require further study, but overlapping pathways emerge when the two proteins are reviewed in parallel. Both proteins interact with IRE1; IRE1 interaction with Bax promotes apoptosis, IRE1 interaction with BI-1 decreases this response allowing the cells to adapt to the stress. Both proteins are regulators of Ca^{2+}: Bax indirectly increases the release of calcium by the IP3R, inducing cell death, whereas BI-1 decreases this flux. Both proteins have a direct, but opposing effect on cytosolic pH: Bax induces a decrease in cytosolic pH leading to apoptosis whereas BI-1 promotes an increase in cytosolic pH allowing cells to adapt to the stress. Moreover, Bax can induce a burst of ROS while BI-1 leads to the decrease of ROS production and an increase in anti oxidant enzymes. By various methods, BI-1 seems to be able to block many features of Bax-induced apoptosis and we predict that other functions of BI-1 will be identified in the future as anti-Bax cell signalling.

Nonetheless, understanding the function of BI-1 is limited to our understanding of apoptosis. Many publications in recent years highlight the fundamental importance of BCL-2 family members in apoptotic pathways. It is now understood that mitochondrial outer membrane permeabilization, and release of pro-apoptotic proteins, can be overcome if there are increased levels of BCL-2 anti-apoptotic members in the cell. Although details of the relationship between BI-1 and BCL-2 family members remain unclear, understanding these interactions may explain how cells are able to overcome severe stresses. In summary, there are many fundamental questions still to be answered about how BI-1 regulates life and death, and we anticipate that further understanding may pave the way for therapeutic manipulation.

Conflict of interest

The authors declare no conflict of interest.

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