Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities

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Abstract | The accumulation of unfolded proteins in the endoplasmic reticulum (ER) represents a cellular stress induced by multiple stimuli and pathological conditions. These include hypoxia, oxidative injury, high-fat diet, hypoglycaemia, protein inclusion bodies and viral infection. ER stress triggers an evolutionarily conserved series of signal-transduction events, which constitutes the unfolded protein response. These signalling events aim to ameliorate the accumulation of unfolded proteins in the ER; however, when these events are severe or protracted they can induce cell death. With the increasing recognition of an association between ER stress and human diseases, and with the improved understanding of the diverse underlying molecular mechanisms, novel targets for drug discovery and new strategies for therapeutic intervention are beginning to emerge.

Molecular chaperone
A molecular chaperone is a protein that aids the folding of other proteins. Some molecular chaperones reside in the lumen of the endoplasmic reticulum, such as GRP78, a member of the HSP70 family, and GRP94, a member of the HSP90 family.

Protein disulphide isomerase
(PDI). A cellular enzyme in the lumen of the endoplasmic reticulum of eukaryotes or the periplasmic region of prokaryotes. This enzyme catalyses the formation and breakage of disulphide bonds between cysteine residues in proteins, which affects protein folding.

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The endoplasmic reticulum (ER) is an organelle that has essential roles in multiple cellular processes that are required for cell survival and normal cellular functions. These processes include intracellular calcium homeostasis, protein secretion and lipid biosynthesis[1–3]. The lumen of the ER constitutes a unique cellular environment. For instance, the highest concentrations of calcium within the cell are found in the ER owing to active transport by Ca^{2+}-ATPases. In addition, because of its role in protein folding and transport in the secretory pathway, the ER is also rich in calcium-dependent molecular chaperones such as glucose-regulated protein, 78 kDa (GRP78; also known as HSPA5), GRP94 (also known as HSP90B1) and calreticulin, which help stabilize protein-folding intermediates. The ER lumen also has an oxidative environment, which is crucial for the formation of disulphide bonds mediated by protein disulphide isomerase (PDI) and for the proper folding of many proteins destined for secretion or for display on the cell surface. In addition, various post-translational modifications of proteins, including glycosylation and lipidation, occur in the ER (reviewed in REFS 3–5). The ER also has vital roles in lipid-membrane biosynthesis and in controlling production of cholesterol and other membrane lipid components. In addition to its biosynthetic capacity, the ER is a signalling organelle due to its ability to release sequestered calcium via ion channels that respond to second messengers (for example, inositol triphosphate; IP_3) as well as protein kinases and other modulators (reviewed in REFS 5,6).

Multiple disturbances can cause accumulation of unfolded proteins in the ER, triggering an evolutionarily conserved response termed the unfolded protein response (UPR)[7,8]. Disturbances in cellular redox regulation caused by hypoxia, oxidants or reducing agents interfere with disulphide bonding in the lumen of the ER, leading to protein unfolding and misfolding[9]. Glucose deprivation also leads to ER stress, probably by interfering with N-linked protein glycosylation in the ER. Aberrations of calcium regulation in the ER also contribute to protein unfolding problems because of the calcium-dependent nature of chaperones such as GRP78, GRP94 and calreticulin[3]. Viral infection may also trigger the UPR owing to the overloading of the ER with virus-encoded proteins, which could represent one of the ancient evolutionary pressures for linking ER stress to cell suicide in avoiding the replication and spread of viruses. Recently, high-fat diet has also been linked to ER stress, at least in some organs such as the liver[10]. In addition, protein-inclusion-body diseases that are typical of most chronic neurodegenerative diseases, as well as disorders such as inclusion-body myositis, indirectly cause accumulation of unfolded proteins in the ER. This occurs perhaps by exhausting proteasome capacity and causing an accumulation of unfolded proteins scheduled for degradation via retrograde translocation from the ER into the cytosol for ubiquitylation[11,12] (reviewed in REFS 13,14).

The consequences of triggering the UPR because of ER stress in mammalian cells can be grouped into three types of effector functions: adaptation, alarm

and apoptosis[15]. The initial intent of the UPR is to re-establish homeostasis and normal ER function, and adaptive mechanisms predominantly involve activation of transcriptional programmes that induce expression of genes that are capable of enhancing the protein folding capacity of the ER and genes for ER-assisted degradation (ERAD). This helps clear the ER of unfolded proteins and export them to the cytosol for degradation. Translation of mRNAs is also initially inhibited for a few hours, thereby reducing the influx of new proteins into the ER until mRNAs encoding UPR proteins are produced. These adaptive aspects of the UPR probably have essential roles in the normal physiology of some types of cells, including professional secretory cells such as pancreatic β cells, plasma cells and hepatocytes[16], which exert high demands on their ER. The UPR-induced alarm refers to signal-transduction events that are commonly associated with cellular stress, including activation of mitogen-activated protein kinases (MAPKs), Jun N-terminal kinase (JNK) and p38 MAPK. Also, kinases responsible for activation of the transcription factor nuclear factor-κB (NF-κB) may be induced in some contexts[17]. This suggests that signalling events that are often associated with innate immunity and host defence are triggered, implying that the cell (and perhaps its surrounding neighbours) is informed that something is wrong. Finally, when the adaptive mechanisms put in to motion by the UPR fail to compensate — for instance, when the primary stimulus causing protein unfolding in the ER is protracted or excessive — cell death is induced, typically by apoptosis. However, ER-stress-induced cell death can proceed even without caspase activity, and it is clear that the cell death mechanisms triggered as a result of ER stress are diverse, involving both caspase-dependent apoptosis and caspase-independent necrosis[18]. Moreover, it is becoming increasingly apparent that ER stress induces autophagy[19,20], a catabolic cellular programme that promotes cell survival in many contexts but which has been associated with induction of non-apoptotic cell death in others (reviewed in REF. 21).

This Review will discuss the current understanding of the complex signalling events induced by ER stress, highlighting the roles of ER stress in disease pathophysiology and emerging opportunities for drug discovery.

UPR signalling mechanisms

When unfolded proteins accumulate in the ER, resident chaperones become occupied with protein folding, which then releases transmembrane ER signalling proteins responsible for the UPR (FIG. 1). These UPR-initiating proteins straddle ER membranes, with their N terminus in the lumen of the ER and their C terminus in the cytosol, thereby bridging these two cellular compartments. One theory states that the N termini of these transmembrane ER proteins are held by the ER chaperone GRP78, which prevents their aggregation. When misfolded proteins accumulate, GRP78 releases these transmembrane signalling proteins, allowing their oligomerization and thereby initiating the UPR. However, other mechanisms probably also contribute to the initiation of the UPR, including direct activation of some UPR signalling proteins by unfolded proteins (reviewed by REF. 22). Among the crucial transmembrane ER signalling proteins are PRKR-like ER kinase (PERK; also known as EIF2AK3), inositol-requiring kinase 1 (IRE1; also known as ERN1) and activating transcription factor 6 (ATF6) (FIG. 1 and below). Together, these proteins induce signal-transduction events that ameliorate the accumulation of misfolded proteins in the ER by increasing the expression of ER chaperones, by inhibiting protein entry into the ER by arresting mRNA translation, and by accelerating the retrograde export of proteins from the ER to the cytosol for ubiquitylation and proteasome-mediated degradation (reviewed in REFS 5,23). Moreover, because ER stress can induce autophagy[19,20], this could activate another mechanism for removing unfolded proteins independently of the ubiquitin/proteasome system[20,24], which may be particularly important when severe protein misfolding results in insoluble protein aggregates that cannot be eliminated by the proteasome.

IRE1. The ~100 kDa IRE1α protein is a type I transmembrane protein that has both a Ser/Thr kinase domain and an endoribonuclease domain. The endoribonuclease domain processes an intron from X-box-binding protein 1 (XBP1; HAC1 in yeast) mRNA, rendering it competent for translation to produce the 41 kDa XBP1 protein, a basic leucine zipper (bZIP) family transcription factor (FIG. 1). XBP1 heterodimerizes with the protein NF-Y, and this heterodimer binds to promoters of several genes involved in UPR and ERAD (reviewed in REFS 8,23). In addition to XBP1, IRE1α is required for cleavage and post-transcriptional degradation of many mRNA that encode secreted proteins, thereby reducing the protein load on the ER[25]. Ablation of IRE1α in mice produces an embryonically lethal phenotype[26]. Mouse fibroblasts from *Ire1α*$^{-/-}$ embryos are defective in activation of UPR element-driven reporter genes, which shows a cause-and-effect linkage of IRE1α to this *cis*-acting element[27]. The equivalent pathway in yeast is responsible for the induction of at least 380 target genes during the UPR[28], so it could be presumed that even more target genes exist in humans.

IRE1 also has an intrinsic activity as a protein kinase, although its substrates are not clear. Similar to many members of the tumour necrosis factor (TNF) receptor family, IRE1 binds the adaptor protein TNF receptor-associated factor 2 (TRAF2), an E3 ubiquitin ligase. Among other functions, IRE1 and TRAF2 collaborate to activate protein kinases that have previously been implicated in immunity, inflammation and apoptosis, in particular apoptosis signal-regulating kinase 1 (ASK1; also known as MAP3K5), which causes JNK activation[29,30].

In addition to serving as a platform for kinase activation, IRE1 may also be a focal point for the ER-targeted activities of some of the B-cell leukaemia/lymphoma 2 (BCL-2) family of proteins, a diverse group of proteins that regulate cell death[31]. For example, the pro-apoptotic proteins BCL-2-associated X protein (BAX) and BCL-2 antagonist/killer (BAK) are reported to physically interact with and activate IRE1α, therefore modulating UPR

Unfolded protein response (UPR). A conserved physiological response involving endoplasmic reticulum (ER)-initiated signal-transduction events, induced by accumulation of unfolded proteins in the lumen of the ER. In mammals, the UPR includes signals initiated by ER membrane-associated proteins: IRE1, PERK and ATF6.

ER stress
An organelle-initiated cell stress condition, typically associated with accumulation of misfolded or unfolded proteins in the lumen of the ER. ER stress is caused by a wide diversity of stimuli.

ER-assisted degradation (ERAD). ERAD involves the retrograde translocation of unfolded proteins from the lumen of the ER to the cytosol, where ER membrane-associated ubiquitin ligases post-translationally modify the translocated proteins thereby targeting them for degradation, usually by the 26S proteasome.

Autophagy
Autophagy, or autophagocytosis, is a catabolic celullar process involving the lysosome-dependent degradation of macromolecules, organelles and other cell components. Autophagy plays housekeeping roles in protein degradation, complementing the proteasome-based protein degradation system. Autophagy can also be important for cell survival during times of nutrient deprivation and hypoxia, and is induced in some cases by endoplasmic reticulum stress. Autophagy has also been associated with cell death in some contexts.

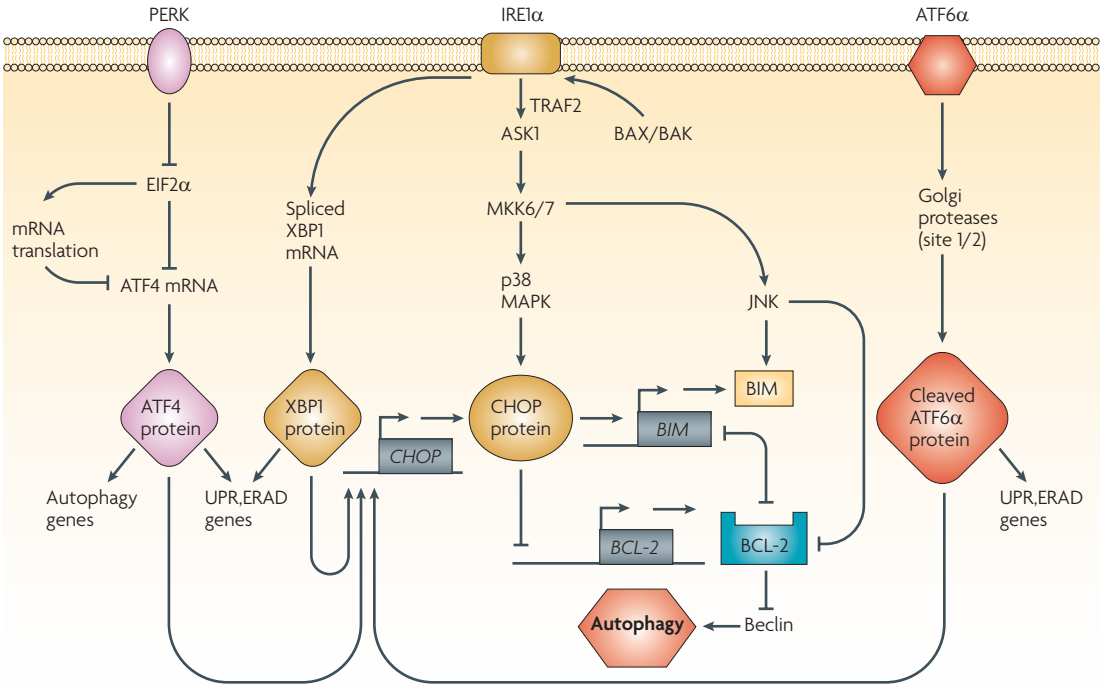

Figure 1 | **UPR events and connection to the cell death machinery.** The major unfolded protein response (UPR) signalling pathways induced by endoplasmic reticulum (ER) stress are depicted, and some connections to the cell death machinery are illustrated. The protein chaperone glucose-regulated protein, 78 kDa (GRP78) binds the termini of inositol-requiring kinase 1 (IRE1α), PRKR-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6) in the lumen of the ER, preventing their activation. Unfolded proteins in the ER cause GRP78 to release IRE1α, PERK and ATF6. On release, IRE1α and PERK oligomerize in ER membranes. Oligomerized IRE1α binds TNF receptor-associated factor 2 (TRAF2), activating apoptosis signal-regulating kinase 1 (ASK1) and downstream kinases that activate p38 MAPK (mitogen-activated protein kinase) and Jun N-terminal kinase (JNK). The intrinsic ribonuclease activity of IRE1α also results in X-box-binding protein 1 (XBP1) production, a transcription factor that induces expression of genes involved in restoring protein folding or degrading unfolded proteins. Oligomerization of PERK activates its intrinsic kinase activity, which results in the phosphorylation of eukaryotic translation initiation factor 2α (EIF2α) and suppression of global mRNA translation. Under these conditions, only selected mRNAs, including ATF4, are translated. ATF4 induces expression of genes involved in restoring ER homeostasis as well as autophagy genes. Release of ATF6α allows this protein to translocate to the Golgi for proteolytic processing to release active ATF6α, which controls expression of UPR and ER-assisted degradation (ERAD) genes. ATF4, XBP1 and ATF6 all converge on the promoter of the gene encoding C/EBP homologous protein (CHOP), which transcriptionally controls expression of the genes encoding BIM (also known as BCL2L11) and B-cell leukaemia/lymphoma 2 (BCL-2). The p38 MAPK stimulates CHOP activity. JNK activates BIM, but inhibits BCL-2. Note that BCL-2-associated X protein (BAX) and BCL-2 antagonist/killer (BAK) are also reported to interact with and activate IRE1α. The autophagy protein Beclin is inhibited by direct binding to BCL-2. MKK, mitogen activated protein kinase kinase.

signalling[32]. The IRE1α–ASK1–JNK signalling pathway is also connected to a cell death mechanism, whereby JNK-mediated phosphorylation has been reported to activate the pro-apoptotic BCL-2 family member BIM (also known as BCL2L11)[33,34], while inhibiting the anti-apoptotic protein BCL-2 (REF. 35). Conversely, BCL-2 and BCL-X_L (also known as BCL2L1) have been reported to inhibit JNK activation and reduce apoptosis induced by the ER stress inducer thapsigargin[36], an irreversible inhibitor of the ER Ca^{2+}-ATPase.

Finally, IRE1 activation is reported to trigger the recruitment of caspase 12 (CASP12)[37], a protein with homology to the caspase family of cysteine proteases involved in apoptosis and inflammatory cytokine processing. However, most humans lack CASP12, owing to a polymorphism that creates a nonsense mutation in the coding region[38], which therefore discounts this protein as a crucial participant in ER-stress signalling. Nevertheless, many reports have claimed functionally important roles for CASP12 in ER-stress-induced apoptosis (reviewed in REF. 39). However, if CASP12 is not present in most humans, then which caspases are involved in cell death signalling following ER stress, and how do they become activated? These are questions that remain largely unanswered. In this regard, human CASP4 is one of the closest paralogues of rodent CASP12, which may associate with the ER[40], raising the possibility that this protease can perform the functions that are normally ascribed to rodent CASP12 in the context of ER stress. CASP4 belongs to the group of pro-inflammatory caspases (that is, CASP1, CASP4 and CASP5), which are best known for proteolytic activation

of cytokines rather than apoptosis (reviewed in REF. 41). Nevertheless, small-interfering-RNA-mediated knockdown of CASP4 in a human neuroblastoma cell line was reported to reduce cell death caused by ER stress inducers (for example, thapsigargin and amyloid-β peptide), but not by inducers of mitochondrion-dependent cell death (for example, UV irradiation and DNA-damaging drugs). This suggests a partial requirement of CASP4 for ER-stress-induced apoptosis; however, it remains uncertain whether CASP4 is vital for ER-stress-induced apoptosis in the majority of cell types and tissues[18].

PERK. PERK is a Ser/Thr protein kinase, the catalytic domain of which shares substantial homology to other EIF2α family kinases[42]. Oligomerization of PERK in ER membranes induces its autophosphorylation and kinase domain activation (FIG. 1). PERK phosphorylates and inactivates EIF2α, shutting off mRNA translation and reducing the protein load on the ER. However, certain mRNAs, including the mRNA encoding transcription factor ATF4, gain a selective advantage for translation under conditions in which EIF2α is phosphorylated (on serine 51)[43]. The 39 kDa ATF4 protein is a member of the bZIP family of transcription factors, and regulates the promoters of several genes implicated in the UPR. Among the documented or putative targets of ATF4 include genes encoding the ER chaperones GRP78 and GRP94; genes that are involved in glutathione biosynthesis and resistance to oxidative stress; and genes involved in amino-acid metabolism and transport[44]. Many of these ATF4 targets therefore increase the levels of chaperones, restore cellular redox homeostasis, and help the ER to either fold proteins or degrade them. The importance of PERK-initiated signals for protection against ER stress has been documented in studies of *PERK*$^{-/-}$ cells and of knock-in cells that express non-phosphorylatable EIF2α (Ser51Ala), both of which are hypersensitive to ER stress[45,46].

The role of the PERK pathway in cell death regulation is unclear. Compounds that sustain phosphorylation of EIF2α (see below) provide cytoprotection during circumstances that induce ER stress[47], implying that maintenance of EIF2α in an inactive state is somehow beneficial. However, prolonged suppression of protein synthesis is typically incompatible with cell survival, and might be expected to induce autophagy. In fact, autophagy induced by at least some types of stimulators of ER stress is known to require PERK and EIF2α phosphorylation, and ATF4 has been shown to stimulate expression of at least one autophagy gene, *ATG12* (REFS 48,49). Autophagy is generally a survival mechanism but it has been associated with induction of non-apoptotic cell death in several contexts[21].

ATF6. ATF6α and ATF6β are bZIP family transcription factors, similar to ATF4, with an interesting mechanism of regulation[8] (FIG. 1). ATF6 proteins have an ER-targeting hydrophobic sequence that tethers them to ER membranes. ER stress triggers a different mechanism of protein activation for ATF6 proteins compared with PERK and IRE1. Instead of oligomerizing, release of GRP78 frees ATF6 proteins to translocate to the Golgi, where resident proteases (site 1 and site 2 proteases) cleave at a juxtamembrane site, releasing these transcription factors into the cytosol and allowing them to migrate into the nucleus to regulate gene expression[50]. ATF6α stimulates ER stress genes as a homodimer or upon heterodimerization with certain bZIP transcription factors, which include XBP1. ATF6α also collaborates with IRE1 to induce XBP1 expression: ATF6α induces the transcription of XBP1 mRNA, which is spliced by the endoribonuclease activity of IRE1. Known or suspected target genes of ATF6α include GRP78, PDI and ER degradation-enhancing α-mannosidase-like protein 1 (EDEM1), resulting in increased ER chaperone activity and degradation of misfolded proteins[51,52]. ATF6β, has considerably weaker transcriptional activity compared with ATF6α[53], which raises the possibility of dominant-negative regulation of ATF6α. However, studies of ATF6α and ATF6β knockout cells have not demonstrated such antagonism[51].

ATF6α has cytoprotective activity in various models of cellular stress[53]. Although the specific downstream target genes directly responsible for preserving cell survival have not been entirely defined, one contributor seems to be regulator of calcineurin 1 (RCAN1)[54]. RCAN1 is an endogenous inhibitor of calcineurin (protein phosphatase B), which is a calcium-activated phosphatase whose substrates include the pro-apoptotic BCL-2 family member, BCL-2 antagonist of cell death (BAD). Dephosphorylation of BAD by calcineurin has been reported to restore its ability to dimerize with and inhibit anti-apoptotic BCL-2 family proteins such as BCL-X_L (REF. 55). Interestingly, ATF6α and ATF6β are members of a family of ER-membrane tethered transcription factors that are cleaved and released to translocate into the nucleus upon ER stress, which include BBF2H7 (also known as CREB3L2), cyclic AMP responsive element binding protein 4 (CREB4), CREB-H, Luman (also known as CREB3) and Oasis (also known as CREB3L1) (reviewed in REF. 56). The functions of these other members of the ATF6 family are less well understood.

CHOP. The transcription factor C/EBP homologous protein (CHOP; also known as DDIT3/GADD153) operates as a downstream component of ER-stress pathways, at the convergence of the IRE1, PERK and ATF6 pathways[57] (FIG. 1). CHOP is a member of the C/EBP family of bZIP transcription factors that is induced by ER stress (reviewed in REF. 58). The *CHOP* gene promoter contains binding sites for all of the major inducers of the UPR, including ATF4, ATF6 and XBP1, and various studies have provided evidence that these transcription factors have causative roles in inducing *CHOP* gene transcription. Interestingly *PERK*$^{-/-}$ and *AFT4*$^{-/-}$ cells and EIF2α (Ser51Ala) knock-in cells fail to induce *CHOP* during ER stress[44,46,59]. The IRE1–ASK1–p38 MAPK pathway may also enhance CHOP activity at a post-transcriptional level[60]. In addition to the aforementioned regulators, upstream activators of *CHOP* also include ATF2, which is induced by hypoxia and is required for *CHOP* induction during amino-acid starvation[61].

EIF2α
(Eukaryotic translation initiation factor 2α). The translation initiation complex EIF2 is a heterotrimer of EIF2α, EIF2β and EIF2γ. This complex binds to GTP and Met-tRNA. It transfers Met-tRNA to the 40S subunit of the ribosome to form the 43S pre-initiation complex. Successive rounds of translation and initiation are promoted by exchanging GDP for GTP. Phosphorylation of EIF2α by PERK inactivates EIF2α, resulting in inhibition of cap-dependent translation initiation.

Overexpression of the 29 kDa CHOP protein induces apoptosis through a mechanism that can be inhibited by BCL-2 (REF. 62). In this regard, CHOP can exacerbate ER stress by inducing expression of genes encoding ER client proteins (thereby increasing protein load), and by rendering the ER more oxidative by inducing expression of the oxidase ERO1α[63,64]. In addition, recent data indicate that CHOP binds an element in the promoter of the gene encoding BIM proteins (pro-apoptotic members of the BCL-2 family)[65]. Moreover, BIM-deficient cells withstand cell death that is induced by stimulators of ER stress better than normal cells. In addition, CHOP inhibits expression of the gene encoding the anti-apoptotic protein BCL-2, at least in some cellular contexts[62]. CHOP can function as either a transcriptional activator or repressor, forming heterodimers with other C/EBP family transcription factors via bZIP-domain interactions to inhibit expression of genes responsive to C/EBP family transcription factors, while enhancing expression of other genes containing a specific 12–14 bp *cis*-acting element[66]. Although clearly important for ER-stress-induced apoptosis in many scenarios, CHOP is not uniformly essential for cell death induced by ER stress, as demonstrated by the observation that *PERK*$^{-/-}$ and EIF2α (Ser51Ala) knock-in cells are hypersensitive to ER-stress-induced apoptosis but fail to induce *CHOP* gene expression[44,46].

ER calcium regulation and cell death mechanisms

Disturbances in ER calcium regulation can induce the UPR and also independently perturb cellular events that are critically linked to cell life and death. Perhaps the strongest link between ER calcium regulation and the cell death machinery is found in the BCL-2 family of proteins, many of which reside at least in part in the ER membranes. Although better known for their actions on mitochondria, several BCL-2 family proteins have been reported to modulate ER calcium homeostasis and to control cell death induced by agents known to trigger ER stress. These agents include tunicamycin (an inhibitor of N-linked glycosylation), brefeldin A (an inhibitor of ER–Golgi transport), thapsigargin and oxidants (reviewed in REF. 67). Of potential relevance, mitochondria and ER physically and functionally associate at numerous levels in orchestrating life and death decisions of the cell (reviewed in REF. 2). Anti-apoptotic proteins such as BCL-2 and BCL-X_L reduce basal calcium concentrations in the ER, $[Ca^{2+}]_{er}$ (REF. 68), whereas the pro-apoptotic protein BAX has the opposite effect[69]. Furthermore, knocking out the genes encoding pro-apoptotic BAX and BAK reduces resting $[Ca^{2+}]_{er}$ (REFS 70,71). Although the mechanisms by which BCL-2 family proteins regulate $[Ca^{2+}]_{er}$ are not fully understood, the available data favour a role for IP_3 receptors, whereby the rate of basal calcium leakage from the ER is increased by BCL-2 and BCL-X_L via these calcium channels, apparently in an IP_3-independent manner[72–74]. Experiments suggest that BCL-2 targeted exclusively to the ER (as opposed to both the ER and mitochondria) is more restricted in its anti-apoptotic actions by selectively suppressing cell death induced by ER stress agents, but failing to suppress cell death induced by various other stimuli that kill through mitochondrion-dependent mechanisms[75]. Conversely, ER-targeted BCL-X_L suppresses autophagy[76], unlike mitochondrial targeting of this anti-apoptotic protein, which suggests a specific role for the ER-associated pool of BCL-2 family proteins in regulating autophagy.

ER calcium is also regulated by the 26 kDa BAX inhibitor 1 (BI1; also known as TMBIM6) protein[77]. BI1 contains six or seven transmembrane domains, resides in the ER, and interacts functionally and probably physically with BCL-2 family members[78]. This evolutionarily conserved protein has been reported to block cell death that is induced by either oxidative stress or overexpression of the mammalian pro-apoptotic BAX protein in yeast, plants and animals[79]. This suggests that BI1 regulates a cell death pathway more ancient than that of the BCL-2 family of proteins, which are found only in animal species[80]. Mice lacking BI1 display increased sensitivity to tunicamycin-induced kidney damage and neuronal cell death *in vivo*, implying a role for BI1 in protection from insults known to trigger ER stress[81]. Similar to BCL-2, BI1 regulates $[Ca^{2+}]_{er}$, with overexpression reducing and gene-silencing increasing resting $[Ca^{2+}]_{er}$ via a mechanism involving changes in the rates of basal calcium leakage from the ER[82]. Moreover, the BCL-2 family of proteins seem to require BI1 to regulate $[Ca^{2+}]_{er}$. This concept is based on studies of *BI1*$^{-/-}$ cells showing that BCL-X_L fails to reduce $[Ca^{2+}]_{er}$ in the absence of BI1 protein[82]. Therefore, the evolutionarily conserved BI1 protein operates downstream of the BCL-2 family of proteins to regulate $[Ca^{2+}]_{er}$, making BI1 a potential target for modulating ER calcium homeostasis. It remains to be determined whether BI1 alters $[Ca^{2+}]_{er}$ through effects on IP_3 receptors, as proposed for the BCL-2 family of proteins. Interestingly, not only BI1 but also the ER resident protein B-cell receptor associated protein 31 (BAP31; also known as BCAP31) binds BCL-2 and BCL-X_L to regulate $[Ca^{2+}]_{er}$ and apoptosis[83]. Therefore, BI1, BAP31 and probably other ER resident proteins seem to collaborate with BCL-2 family proteins in regulating $[Ca^{2+}]_{er}$ and cell death.

What do these alterations in $[Ca^{2+}]_{er}$ mean for cell death regulation? One notion is that if the ER has less free calcium to release upon stress, then cytosolic calcium levels will not rise to the critical levels needed for triggering deleterious downstream effectors (FIG. 2). Also of relevance to the issue of ER calcium and cell death is genetic evidence linking ER calcium channels to necrotic cell death in the nematode *Caenorhabditis elegans*, in which mutations in the ryanodine receptor (*unc-68*) and the IP_3 receptor (*itr*) prevent neuronal cell death[84]. Recently, the IP_3 receptor was also shown to be required for autophagic cell death in *Dictyostelium*[85] and to modulate autophagy in mammalian cells, apparently through a calcium-independent mechanism[86]. Moreover, calcium-mediated activation of protein kinase Cθ (PKCθ) and of calmodulin-dependent kinase kinase-β (CaMKKβ) was recently reported to induce autophagy in the context of ER stress[87,88]. In addition to the effects of calcium released from ER into the cytosol, it is also likely that

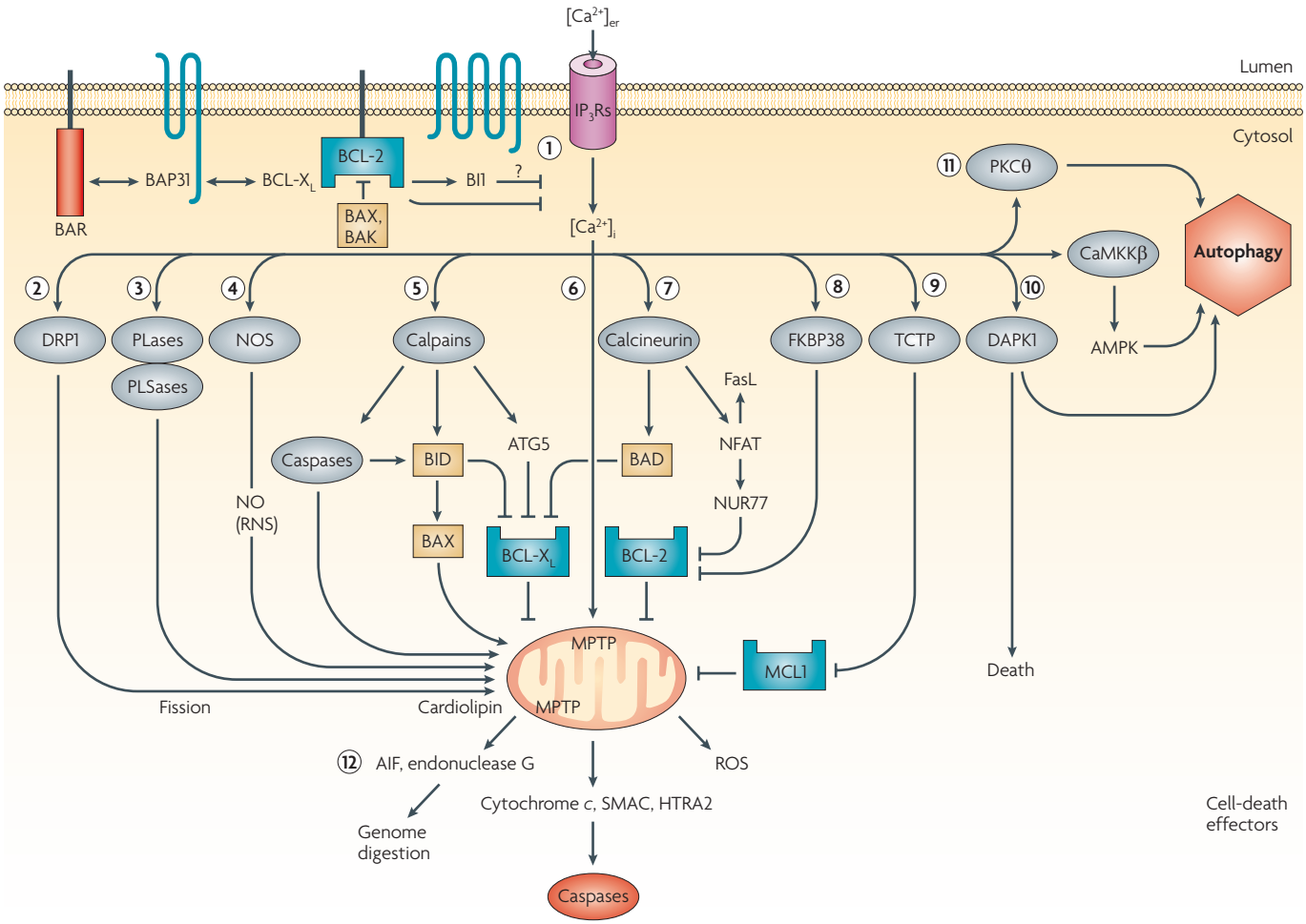

Figure 2 | **Calcium-induced pathways relevant to cell death and ER stress.** Several of the proposed regulators of basal concentrations of calcium in the endoplasmic reticulum, $[Ca^{2+}]_{er}$, are depicted. Inositol triphosphate receptors (IP_3Rs) represent the most likely ER calcium channel of relevance (**1**). IP_3Rs are negatively regulated by B-cell leukaemia/lymphoma 2 (BCL-2) and BCL-X_L (also known as BCL2L1), which require BAX inhibitor 1 (BI1). BI1 is inferred to inhibit IP_3R, but this has not been directly demonstrated. BCL-2-associated X protein (BAX) and BCL-2 antagonist/killer (BAK) oppose BCL-2 and BCL-X_L. BCL-2 and BCL-X_L also interact with ER resident proteins B-cell receptor-associated protein 31 (BAP31) and bifunctional apoptosis regulator (BAR), which interact with each other (**1**). Calcium released into the cytosol, $[Ca^{2+}]_i$, is capable of stimulating numerous pathways with links to the cell death machinery, particularly mitochondrion-dependent cell death mechanisms. Some of these pathways are depicted here (**2–10**). Activating calcium-sensitive mitochondrial fission protein dynamin related protein 1 (DRP1)[224], which has been implicated in BAX-induced release of cytochrome *c* from mitochondria (**2**). Calcium-dependent phospholipases (PLases) and phospholipid scramblases (PLSases), which have been suggested to transfer cardiolipin from the inner to the outer membrane of mitochondria; a signal for targeted insertion of pro-apoptotic BCL-2 family proteins BH3-interacting domain death agonist (BID) and BAX into mitochondrial membranes[225,226] (**3**). Stimulating calcium-sensitive isoforms of nitric oxide synthase (NOS), thereby generating reactive nitrogen species (RNS) and contributing to oxidative stress (reviewed in REF. 227) (**4**). Calpain family cysteine proteases, calcium-dependent proteases implicated in many pathological cell death scenarios and whose substrates include BAX and BID (which are activated)[228,229]; BCL-2 and BCL-X_L (which are inhibited); several caspases; and autophagy protein ATG5, which binds and inhibits BCL-X_L upon cleavage[230] (**5**). Calcium-induced mitochondrial membrane permeability transition, releasing apoptogenic proteins from mitochondria and stimulating reactive oxygen species (ROS) production[231] (**6**). Calcium-sensitive phosphatases such as calcineurin (protein phosphatase B), which regulates activity of pro-apoptotic BCL-2 family protein BCL-2-associated agonist of cell death (BAD)[232], and which dephosphorylates nuclear factor of activated T-cells (NFAT) family transcription factors, allowing entry into the nucleus and transactivation of pro-apoptotic genes encoding Fas ligand (FasL) and NUR77 (also known as NR4A1)[233] (**7**). Calcium/calmodulin-induced activation of the peptidyl proylyl isomerase, FKBP38, which binds to BCL-2 and induces apoptosis[234] (**8**). Regulating the calcium-binding protein TCTP (fortilin), a putative modulator of anti-apoptotic BCL-2/BAX family proteins such as myeloid cell leukaemia sequence 1 (MCL1)[235] (**9**). Activating death-associated protein kinase 1 (DAPK1) and its close relative DRP1, which contain calmodulin-binding domains (reviewed in REF. 236), in which DAPK1 can induce either apoptosis or autophagy (reviewed in REF. 237) (**10**). Also depicted are calcium-activated pathways that trigger autophagy (**11**). Downstream of mitochondria are both caspase-dependent (cytochrome *c*)-dependent and caspase-independent cell death mechanisms. These include release of endonuclease G and apoptosis-inducing factor (AIF), inducers of genomic digestion, and ROS from disrupted electron-chain transport (**12**). Not depicted are the impact of these calcium-activated events on the ER, such as calpain-mediated activation of BAX, an activator of inositol-requiring kinase 1α (IRE1α) and antagonist of BCL-2/BCL-X_L with respect to regulation of $[Ca^{2+}]_{er}$; PLases that degrade plasma membrane and ER membranes; nitric oxide (NO) and RNS, which inhibit protein disulphide isomerase and exacerbate protein misfolding in the ER; and other effects on ER membranes, ER-resident proteins and ER calcium regulation. AMPK, 5′-AMP-activated protein kinase; CaMKKβ, calmodulin-dependent protein kinase kinase-β; PKCθ, protein kinase Cθ.

altered $[Ca^{2+}]_{er}$ directly affects UPR signal transduction events that are of relevance to cell death regulation, a subject that warrants further investigation. Several additional candidate regulators of ER-initiated cell death have been described (BOX 1).

ER stress, disease and drug discovery

ER stress has been associated with a wide range of diseases, including neurodegeneration, stroke, bipolar disorder, cardiac disease, cancer, diabetes, muscle degeneration and others (reviewed in REF. 58) (TABLE 1), some of which we discuss below. Attempts to exploit knowledge about mechanisms linking ER stress to disease for drug discovery purposes are largely in their infancy, but several targets for potential drug discovery are beginning to emerge (TABLE 2).

Neurodegeneration. Amyloid-β peptide, a proteolytic product of amyloid-β precursor protein (APP), has been causally associated with Alzheimer's disease. Autopsy studies suggest that the PERK–EIF2α pathway is hyperactive in the brains of patients with Alzheimer's disease[89], implying that ER stress is activated. APP undergoes complex proteolytic processing that is mediated by the integral membrane γ-secretase protease complex, which includes the Presenilin proteins as core components. Interestingly, mutant versions of Presenilin 1 (PS1) are associated with Alzheimer's disease and interfere with the UPR[90]. These mutant versions of PS1 blunt signalling by IRE1α, PERK and ATF6α, as well as induce altered ER calcium homeostasis and render cultured neurons more susceptible to cell death that is induced by ER stress[91]. The brains of mice harbouring Alzheimer's-disease-associated mutants of PS1 also have increased levels of CHOP, which suggests the presence of ER stress[92]. Furthermore, PS1 was reported to induce cleavage of IRE1α in cultured cells, releasing the cytosolic domain to translocate to the nucleus. This suggests that there are further interactions between molecules involved in Alzheimer's disease and ER stress responses[93].

Hereditary mutations in the ER-associated E3 ubiquitin ligase Parkin have also been associated with ER-stress-induced cell death, and are found in patients with juvenile-onset Parkinson's disease[94,95]. Overexpression of wild-type Parkin suppresses cell death that is induced by several ER-stress-inducing pharmacological agents and by α-synuclein — the principal component of Lewy bodies, representing the pathopneumonic lesion of Parkinson's disease[95–97]. Parkin expression is induced by ER stress, which suggests a role for this E3 ubiquitin ligase in adaptation to ER stress, presumably functioning in ERAD to clear misfolded proteins[96]. Neurons from *PERK*$^{-/-}$ embryos display increased sensitivity to 6-hydroxydopamine[98], suggesting that at least some neurotoxins of relevance to Parkinson's disease kill dopaminergic neurons through mechanisms modulated by or involving ER stress and UPR signalling components.

Several additional neurodegenerative diseases associated with inclusion-body formation and protein aggregation have also been linked to ER stress, including amyotrophic lateral sclerosis with mutant superoxide dismutase (SOD) and disorders associated with proteins that contain expanded glutamate homopolymeric sequences such as Huntington's disease (reviewed in REFS 58,99,100). It has been proposed that these protein aggregates exhaust cellular proteasome activity in a failed attempt to degrade them, resulting in a secondary accumulation of misfolded proteins in the ER, thereby triggering ER stress (reviewed in REF. 13). However, more complicated mechanisms may contribute to the pathogenesis of these aggregation-prone proteins. SOD mutants associated with amyotrophic lateral sclerosis, for instance, were reported to bind a component of the ERAD machinery (Derlin 1), thereby directly interfering with ERAD while not inhibiting global proteosome activity[100]. Also, while Huntingtin protein (HTT) variants with polyglutamine expansions induce classical signal-transduction events associated with the UPR[101], as well as cause global reductions in proteasome activity[102], more intricate mechanisms may apply[103–108] (BOX 2).

Prions are known to form protein aggregates, including polymeric structures, and are causally involved in the transmissible spongiform encephalopathies of Creutzfeldt–Jakob disease and Kuru, among others[109]. Interestingly, the brains of patients succumbing to Creutzfeldt–Jakob disease show increased levels of ER chaperones[110], implying an induction of ER stress. In cell culture, prion-treated cells show evidence of induction of ER-stress markers. Overexpression of ER chaperones suppresses prion-induced neuronal cell death, whereas knockdown of ER chaperones by RNA interference increases sensitivity[111], implying that elements of the UPR are protective against prion-mediated neurotoxicity. Therefore, modulators of ER stress provide a strategy for counteracting the prion-based brain diseases.

Targets for drug discovery in the context of these neurodegenerative diseases are not well validated. A screen for compounds that block cell death of a neuronal cell line induced by tunicamycin resulted in the discovery of salubrinal, a compound that prevents dephosphorylation of EIF2α through inhibition of a multi-protein protein phosphatase complex containing GADD34 (also known as protein phosphatase 1). This results in increased EIF2α phosphorylation and inactivation, thereby generating stronger PERK responses[47]. Salubrinal was reported to protect neuronal cells from cell death triggered by various ER stress inducers, including toxicity induced by α-synuclein mutants associated with Parkinson's disease[112] and polyglutamine-expanded HTT[113]. However, this inhibitor of EIF2α activity also impairs long-term memory in a mouse model[114], suggesting that this approach would not be acceptable as a direction for chronic therapies. Recently, mutants of SOD associated with amyotrophic lateral sclerosis were shown to induce cell death via an ASK1-dependent mechanism, and ASK1 deficiency in mice was reported to reduce motor-neuron loss *in vivo* and extend survival of mice harbouring a SOD (Gly93Ala) mutant[100]. This suggests that chemical inhibitors of ASK1 might provide cytoprotection in neurodegenerative diseases involving protein inclusion bodies. Chemical inhibitors of ASK1 have been described[115], although little is

Box 1 | **More players in ER-initiated cell death: clues without closure**

Various additional candidate regulators of cell death that could be connected to the endoplasmic reticulum (ER) have been described, often with little detail in terms of molecular mechanisms or pathophysiological importance. For example, the protein tyrosine kinase ABL has been reported to translocate from the surface of the ER to mitochondria in response to ER stress[210]. Moreover, *ABL*$^{-/-}$ fibroblasts display resistance to cell death induced by calcium ionophores, brefeldin A and tunicamycin[210]. How ABL promotes apoptosis is unknown and how important it is in the context of tissue injury involving ER stress is unclear. SCOTIN is another ER-targeted apoptosis inducer[211]. The gene encoding SCOTIN (*SHISA5*) is a direct target of p53, which suggests that there is a way to link DNA damage to ER-mediated cell death mechanisms. BRUCE (also known as APOLLON/BIRC6) is a member of the inhibitor of apoptosis protein (IAP) family of anti-apoptotic proteins, which associates with trans-Golgi and possibly ER membranes[212]. The BIR domain of BRUCE binds caspase 9 (CASP9), as well as IAP antagonists SMAC (also known as DIABLO) and HtrA serine peptidase 2 (HTRA2). The ubiquitin-conjugating (UBC) enzymatic domain of BRUCE then polyubiquitylates SMAC and HTRA2, targeting them for proteasomal destruction[213–215]. Therefore, BRUCE may play cytoprotective roles in the context of caspase-mediated processes spatially targeting the Golgi and ER. Conversely, in insect cells, at least one example of a protein that is normally sequestered in the lumen of the ER, but released into the cytosol in response to certain apoptosis stimuli has been uncovered, namely Jafrac2 (REF. 216). Like most proteins imported into the ER, the N-terminal leader peptide of Jafrac2 is removed by proteolysis. This proteolytic processing exposes an IAP-binding motif (IBM) in Jafrac2, poising it for attack of anti-apoptotic IAP family proteins upon entry into the cytosol and thereby (presumably) freeing caspases from the grip of IAPs. The proteolytic activation of Jafrac2 is analogous to the strategy used by mitochondria for readying IAP antagonists for promoting apoptosis, therefore preventing unanticipated cell death during protein translation in the cytosol by concealing the IBM within an internal region of the protein. So far, no examples of apoptogenic protein release from the ER of mammalian cells have been described.

known about their efficacy in animal models of disease. As JNK is activated downstream of ASK1, and JNK is known to activate BID while inhibiting BCL-2, it would be interesting to explore whether chemical inhibitors of JNK have cytoprotective activity in such contexts[116]. Another potential strategy for ameliorating ER stress induced by inclusion bodies is to stimulate autophagy, therefore clearing insoluble protein aggregates from cells more efficiently. Indeed, chemical screens for enhancers of autophagy have been described, which have identified compounds that improve clearance of polyglutamine protein aggregates, α-synuclein aggregates, and other types of protein inclusions from cultured cells[117,118]. Among the compounds purported to increase autophagy, without signs of cellular toxicity, are several drugs already approved by the US Food and Drug Administration (FDA). These include antipsychotics (such as fluspirilene, trifluoperazine, pimozide) and calcium-channel modulators (such as nicardipine, niguldipine, amiodarone), acting through mechanisms that are distinct from that of rapamycin (a mammalian target of rapamycin (mTOR) inhibitor)[117]. Although not tested, salubrinal would also be expected to increase autophagy, given that autophagy induction is dependent on PERK and phosphorylation of EIF2α in the context of polyglutamine-protein-induced stress[48]. With respect to clearance of protein inclusions by autophagy, three types of autophagy have been described, including chaperone-mediated autophagy, micro-autophagy and macro-autophagy (reviewed in REF. 119). It remains to be determined which of these types of autophagy is most relevant to removal of protein inclusions and amelioration of chronic ER stress.

Another proposed strategy for removing insoluble protein inclusions is to increase chaperone activity in cells, especially the cytosolic heat-shock protein 70 (HSP70) family of proteins (reviewed in REF. 120), to maintain the culprit proteins in a soluble, presumably properly folded, state. In this regard, it should be noted, however, that some studies have suggested that sequestering aggregation-prone proteins into insoluble inclusions may actually provide a means of reducing cellular cytotoxicity[121]. Nevertheless, it would be interesting to explore the efficacy of chemical chaperones that serve as ligands to stabilize protein structure and promote protein folding, analogous to what has been described for compounds such as SR121463A (1-[4-(Ntert-butylcarbamoyl)-2-methoxybenzene sulphonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane] indol-2-one, fumarate)[122], and others[123–125].

One obvious approach for dealing with protein inclusions is to inhibit production of the offending proteins. With respect to mutants of SOD associated with amyotrophic lateral sclerosis, for instance, proof-of-concept data have been obtained in mouse models using both antisense oligodeoxynucleotides and nanoparticle-formulated siRNA molecules that target SOD mRNAs delivered into brain ventricular fluid[126,127]. FDA-approved medical devices (Medtronic) for brain delivery of drugs makes such a strategy clinically feasible, although less than ideal compared with orally bioavailable small-molecule drugs.

One of the underlying causes of protein unfolding in the ER identified in the context of neurodegeneration and brain ischaemia is nitrosylation of PDI, which inhibits protein disulphide-bond formation[128]. Cysteines in the ER-associated E3 ubiquitin ligase Parkin are also targets of *S*-nitrosylation, resulting in altered Parkin activity in terms of auto-ubiquitylation and ubiquitylation of its substrates, such as synphilin 1 (REF. 129). Electrophilic compounds that induce endogenous antioxidant pathways may prove beneficial in these circumstances (see below).

Stroke and ischaemia–reperfusion injury. Reduced blood flow resulting from arterial occlusion or hypotension leads to tissue hypoxia and hypoglycaemia, which cause protein misfolding and ER stress. Reperfusion of the affected tissues then triggers oxidative stress, with production of nitric oxide (NO), a mediator of protein nitrosylation, and other reactive oxygen species (ROS) that alter cellular redox-dependent reactions, interfere with protein disulphide bonding and result in protein misfolding (reviewed in REF. 130). Consequently, overexpression of the ER-inducible protein PDI shows protective activity in rodent stroke models[131]. NO and other reactive molecules may also modify oxidizable residues (cysteine, tyrosine) in ER-associated calcium channels, including ryanodine receptors (*S*-nitrosylation) and sarco/endoplasmic reticulum Ca^{2+}-ATPases (by tyrosine nitration), causing ER calcium depletion, which is another cause of protein misfolding[132–134].

S-nitrosylation
S-nitrosylation describes the covalent attachment of a nitrogen monoxide group to the thiol (-SH) of cysteines in proteins. It is a post-translational modification of proteins that can modulate cellular signalling, which provides a mechanism for redox-based physiological regulation.

Table 1 | **ER stress-related diseases**

Disease	Role of ER stress	Target protein*	Refs
Alzheimer's disease (AD)	• Exact implications of ER stress in AD is unclear (AD brains show increase of protective UPR proteins) • AD-associated mutant Presenilin 1 induces ER stress response with suppression of protective UPR signalling	Presenilin, PERK–EIF2α	89–93
Parkinson's disease (PD)	• Unclear whether ER stress in PD is mainly protective or contributory to disease • Parkin suppresses ER-stress-induced cell death • Parkin expression is controlled by ER stress • Parkin mutants associated with PD	Parkin, α-synuclein and others	94–98
Amyotrophic lateral sclerosis	• Mutant SOD interferes with ER-assisted degradation machinery and activates ASK1	SOD, ASK1	100
Polyglutamine disease	• Polyglutamine induces the UPR and suppresses proteasomal activity	Huntingtin‡, SCA§, androgen receptor‖	101,102
Prion disease	• Brains affected with prions show induction of ER chaperones, implying protective UPR against ER stress	Prion protein	109–111
Stroke	• Ischaemia induces ER stress in neurons, activates the UPR and finally leads to neuronal apoptosis associated with CHOP induction and ASK1 activation	PERK–EIF2α, ASK1	131–137
Bipolar disease	• Medications for treating bipolar disease induce the UPR	XBP1 polymorphisms (controversial)	152–154
Heart disease	• Induction of ER stress by ischaemia in the heart leads to degeneration of cardiac myocytes • Transaortic constriction induces expression of ER stress • Myocardial infarction induces the UPR	ASK1	158–163
Atherosclerosis	• Oxidized lipids and homocysteins induce ER stress in vascular cells, cholesterol in macrophages	IRE1 pathway	173–176
Type 1 diabetes	• Impaired PERK pathway is responsible for type 1 diabetes (Wolcott–Rallison syndrome)	PERK–EIF2α	46, 177–183
Type 2 diabetes	• Obesity (a cause of type 2 diabetes) induces ER stress, leading to insulin resistance	XBP1, JNK	10
Type 2 diabetes	• Fatty acids (palmitate) induce apoptosis of β cells	CHOP	186
Cancer	• Protective UPR proteins are upregulated in cancer cells subjected to hypoxic environments	GRP78, XBP1, PERK	187–191
Autoimmune disease	• ER protein overload may contribute to autoantigen production • GRP78 can be an autoantigen	GRP78, HLA-B27 and others	22,207,208

*Agonists or antagonists of target desired, depending on context. ‡For Huntington's disease. §For spinocerebellar ataxia. ‖For spinobulbar muscular atrophy. ASK1, apoptosis signal-regulating kinase 1 (also known as MAP3K5); CHOP, C/EBP-homologous protein (also known as DDIT3); EIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; IRE1, inositol-requiring kinase 1 (also known as ERN1); JNK, Jun N-terminal kinase; PERK, PRKR-like endoplasmic reticulum kinase (also known as EIF2AK3); SOD, superoxide dismutase 1; UPR, unfolded protein response; XBP1, X-box-binding protein 1.

Brain ischaemia–reperfusion injury caused by transient cerebral artery occlusion has been shown to activate the PERK–EIF2α pathway, and induce *CHOP* expression in rodents[135,136]. Moreover, *Chop*$^{-/-}$ mice suffer less tissue loss after stroke injury, implying a causal role for this mediator of ER stress in neuronal cell death *in vivo*[137]. NO, a known mediator of brain injury during stroke, induces *CHOP* expression in cultured neurons. Furthermore, an NO synthase (NOS) inhibitory compound shows protective effects in a rodent model of global brain ischaemia[138]. In addition, mice lacking the gene encoding inducible NOS (*Nos2*) have reduced CHOP induction and display decreased sensitivity to brain ischaemia[139], suggesting a causal role for this ER stress inducer in stroke damage.

Given the documented role for CHOP in brain ischaemia, neutralizing this transcription factor is an attractive therapeutic strategy. As p38 MAPK augments CHOP activity, small-molecule antagonists of p38 MAPK that are currently in development for inflammatory diseases might find utility as cytoprotective agents in clinical scenarios involving ER stress[140,141]. Downstream effectors of CHOP, such as BIM_{EL} — the pro-apoptotic BCL-2 family member whose expression is directly induced by CHOP — are also possible therapeutic targets[65]. In this regard, the basis for a high-throughput screening assay for BIM_{EL} inhibitors is found in its interaction with an antagonistic endogenous 24-amino-acid peptide, Humanin[142,143]. Thus, screens for compounds that bind the same site on BIM_{EL} as Humanin, thereby displacing

Table 2 | **Emerging drug discovery opportunities for ER stress**

Target(s)/pathway(s)	Compound(s)	Potential indications	Potential risks	Comments/refs
Cytoprotective ER-stress-modulating compounds				
ER chaperone inducers	Valproate, lithium	Neuroprotection	GRP78 is an autoantigen in RA	Mood stabilizers that induce ER chaperones[155–157]
ER chaperone inducers	BIX	Cerebral ischaemia, stroke	GRP78 is an autoantigen in RA	Small-molecule inducer of GRP78 (REFS 145,146)
PERK–eIF2α pathway	Salubrinal	Parkinson's disease, polyglutamine diseases, stroke	Impairs memory, promotes β-cell killing by fatty acids	Agonist of PERK–eIF2α pathway[46,47,113,135,177,178]
p38 MAPK (CHOP activity)	p38 MAPK antagonists	Stroke, diabetes	Anti-platelet and neurological	Used to inhibit p38-dependent CHOP phosphorylation[60,92,136,137,140,141,182,183]
ASK1	Fused heterocyclic compounds	Neuroprotection, cardioprotection	Unknown	ASK1 genetically implicated in ER stress-induced cell death; bioactivity of ASK1 inhibitory compounds undefined[238]
Superoxide dismutase (SOD); $SOD^{r/h333611}$, $SOD^{r/h146144}$, $SOD^{r/h146145}$, $SOD^{r146192}$	ISIS #333611	Amyotrophic lateral sclerosis	Inflammation and infection from delivery device	Antisense oligodeoxynucleotides, chemically modified RNA interference[126,127]
Nitric oxide signalling pathway	L-NNA, ONO-1714, L-NAME, L-NMMA	Stroke	Vascular instability	Chemical antagonists of nitric oxide synthase[138,148]
NRF2–KEAP1 pathway	Carnosic acid, tri- terpenoids, sulphoraphane, tert-butylhydroquinone	Stroke, seizure, neurodegeneration	Compound-dependent	Activates the NRF2 antioxidant pathway[218–220,222]
Autophagy inducers	Fluspirilene, trifluoperazine, pimozide, nicardipine, niguldipine, loperamide, amiodarone	Polyglutamine diseases, other inclusion-body diseases	Neurological damage, cardiac arrythmias, blood-pressure instability	REF. 117
Autophagy inducers	Rapamycin and SMERs	Polyglutamine diseases, other inclusion-body diseases	Immunosuppression	REF. 118
Cytotoxic ER-stress-modulating compounds				
GRP78	Versipelostatin	Cancer	Increases ER stress in vulnerable organs	REF. 192
Proteasome	Bortezomib, nelfinavir, atazanavir	Cancer, autoimmune disorders	Immunodeficiency from plasma-cell killing	Used to kill multiple myeloma cells and autoantibody-producing plasma cells[195,198,199,203]
ADP-ribosylation factor	Brefeldin A, breflate	Cancer (leukaemia)	Increases ER stress in vulnerable organs	REFS 200,201

ASK1, apoptosis signal-regulating kinase 1 (also known as MAP3K5); BIX, BIP inducer X (1-(3,4-dihydroxyphenyl)-2-thiocyanate-ethanone); CHOP, C/EBP-homologous protein (also known as DDIT3); eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; GRP78, glucose-regulated protein, 78 kDa (also known as HSPA5); KEAP1, Kelch-like ECH-associated protein 1; L-NAME, N^{ω}-nitro-L-arginine methyl ester; L-NMMA, L-N^{G}-monomethyl arginine; L-NNA, N^{ω}-nitro-L-arginine; MAPK, mitogen-activated protein kinase; NRF2, NF-E2-related factor 2 (also known as NFE2L2); PERK, PRKR-like endoplasmic reticulum kinase (also known as EIF2AK3); RA, rheumatoid arthritis; SMERs, small-molecule enhancers of rapamycin.

this peptide, could result in the discovery of compounds that mimic Humanin and suppress BIM_{EL} activation. Antagonism of the downstream effectors of the BIM_{EL} protein, BAX and BAK (reviewed in REF. 144), might also provide a path forward in terms of drug discovery.

An alternative strategy to inhibiting CHOP or its downstream effectors is to induce expression of protective ER proteins such as GRP78. In this regard, small-molecule compounds have been described that selectively induce GRP78 expression without triggering the production of other UPR genes[145]. In focal ischaemia stroke mouse models, intracerebroventricular injection of the prototype compound 1-(3,4-dihydroxyphenyl)-2-thiocyanate-ethanone (BIX), before middle cerebral artery occlusion, reduces infarct volume and numbers of apoptotic neurons in the penumbra region of infarcts[146]. Acting upstream of CHOP, salubrinal protects against normal cell death induced by kainic acid, a glutamate receptor agonist that mimics the neuroexcitotoxicity thought to occur during stroke and seizures[147], both in cell culture and *in vivo*.

Activating endogenous antioxidant pathways is another strategy for protecting against reperfusion injury (BOX 3). Chemical inhibitors of NOS have been

described[138,148], which may provide an approach to mitigating ER stress and neuronal cell death in the context of stroke injury, provided side effects from vascular instability are not an issue. Chemical inhibitors of caspases might also be considered for stroke given that mice lacking CASP1, CASP2 and CASP11 (a candidate orthologue of human CASP4 or CASP5) display resistance to stroke injury[149], a condition in which ER stress is likely to participate in the mechanism of cell death. However, it is unclear whether the protection afforded by ablation of these caspase-encoding genes is due to primary neuroprotective mechanisms versus a secondary consequence of reducing inflammation after stroke injury. It seems doubtful that inhibitors of the caspase family of cell death proteases would be adequate to preserve cell survival in the face of ER stress, given that cell culture experiments have shown that non-apoptotic cell death still occurs in the presence of compounds such as benozyl-valinyl-alaninyl-aspartyl-fluoromethylketone (zVAD-fmk), at least when strong pharmacological inducers of ER stress are used, such as tunicamycin and thapsigargin[18,81,150]. However, while zVAD-fmk is a broad-spectrum inhibitor of caspases, it is not particularly effective against some members of the family, particularly CASP2. Finally, one might consider targeting downstream targets of caspases activated in the context of ER stress and stroke injury, such as BID, which is cleaved and activated by caspases. In this regard, chemical antagonists of BID with neuroprotective activity have been described[151].

Bipolar disorder. ER stress with associated markers of UPR has been implicated in bipolar disorder (manic–depressive disease). Genetic polymorphisms in the promoter region of the *XBP1* gene have been linked to bipolar disorder in Japanese patients[152], although not apparently in persons of European origin[153]. The XBP1 allele associated with this mood disorder (–116G) is expressed at lower levels and is less inducible in response to ER stress stimuli compared with the more common allele (–116C)[152]. In addition, polymorphisms associated with bipolar disorder have been found in the promoter region of the *GRP78* gene, which correlates with reduced expression of this ER chaperone[154]. Medications proven effective in bipolar disorder, including lithium and valproate, have been shown to increase expression of ER chaperones — GRP78, GRP94 and calreticulin — providing protection against ER stress[155–157].

Heart disease. The role of ER stress in heart disease is under active investigation, with relevance to myocardial ischaemia, cardiac hypertrophy, heart failure and possibly atherosclerosis (reviewed in REFS 56,158). For example, cardiac hypertrophy models wherein transaortic constriction (banding) is used to increase blood pressure and induce heart strain have consistently provided evidence of ER stress occuring[159]. Myocardial infarction (ischaemia–reperfusion injury) also induces markers associated with the UPR, including expression of XBP1, GRP78 and PDI[160]. Conversely, it has been proposed that induction of ER chaperones, particularly of GRP78, underlies the phenomenon of preconditioning in the heart, in which exposure to a transient episode of brief ischaemia provides subsequent protection from a sustained ischaemic challenge[161]. Induction of GRP78 expression stimulated by endothelin 1 may also underlie the cardioprotective activity observed for this protein[162]. In addition, overexpression of the ER chaperone GRP94 reduces cardiomyocyte cell death in ischaemia-simulating conditions[163].

As for potential drug targets, ASK1 activity is increased in mice following aortic constriction as well as during myocardial infarction. *Ask1*$^{-/-}$ mice show reduced numbers of apoptotic cardiomyocytes in addition to better preservation of left-ventricular function compared with wild-type animals[164]. Thus, ER-stress-mediated activation of ASK1 may be an important contributor to myocardiocyte cell death, for which various mechanisms have been proposed (reviewed in REF. 165).

In addition, ER stress induces expression of PUMA (also known as BBC3), a pro-apoptotic member of the BCL-2 family[166]. Studies in mice lacking *Puma* gene expression in the heart[167] argue that agents that nullify the downstream targets of PUMA (for example, BAX/BAK) may be useful for cardioprotection (see above). The *PUMA* gene is p53-responsive, raising the possibility of involvement of the related transcription factor p73, in as much as p73 is reportedly induced by ER stress[168–170].

In contrast to the destructive actions of ASK1 and PUMA, ATF6α is protective in the heart[171]. For example, when hearts from transgenic mice with elevated ATF6α are subjected to ischaemia–reperfusion *ex vivo*, they display better functional recovery and have significantly less evidence of apoptotic and necrotic cell death[171]. The ER-stress-inducible protein PDI is also cardioprotective, as demonstrated by viral-vector-mediated gene transfer in a mouse model of coronary artery ligation[172].

Hypercholesterolaemia and elevated triglycerides may also induce ER stress in vascular cells[56]. For example, oxidized lipids stimulate the UPR in endothelial cells, and UPR components ATF4 and XBP1 have been implicated in ER-stress-induced cytokine production by these vascular cells[173]. Also, loading macrophages with cholesterol was shown to induce ER stress, triggering expression of cytokines in a CHOP-dependent manner[174], which further implicates the UPR in atherosclerosis mechanisms. Homocysteine, a by-product of high-protein diets, which frequently accompanies obesity, induces ER stress in vascular endothelial cells, triggering apoptosis through an IRE1-dependent mechanism[175]. Additional connections between ER stress and cholesterolaemia have been uncovered by studies of XBP1. Ablation of XBP1 expression in the liver results in hypocholesterolaemia and hypotriglyceridaemia, demonstrating that XBP1 is a regulator of lipogenesis and implying that XBP1 activation following ER stress in the liver may contribute to dyslipidaemias[176]. Therefore, ER stress may contribute to atherosclerosis.

Box 2 | **An interesting twist on HTT and ER stress**

Normal Huntingtin (HTT) protein shuttles from the endoplasmic reticulum (ER) to the nucleus, whereas polyglutamine-expanded HTT tends to accumulate in nuclei. There is evidence that HTT may control nuclear gene expression[103], which suggests the possibility that normal HTT participates in an ER-initiated signal-transduction mechanism. This mechanism presumably becomes dysfunctional in the context of polyglutamine expansions. Also, HTT binds HTT-interacting protein (HIP), which interacts with HIP-protein interactor (HIPPI), which in turn binds caspase 8 (CASP8)[104]. Polyglutamine-expanded HTT reportedly has reduced affinity for HIP compared with normal HTT, the consequence of which is release of HIP to bind HIPPI and trigger CASP8 activation. Interestingly, HIP and HIPPI contain a homologous domain with weak similarity to death effector domains (DEDs) found in the prodomain of CASP8 (DED-like domain); although this domain has a different three-dimensional fold than DEDs[105]. The BCL-2/BCL-X_L-interacting proteins B-cell receptor-associated protein 31 (BAP31) and bifunctional apoptosis regulator (BAR) also contain this homologous domain[106] — which forms dimeric coiled-coil interactions — and they bind to each other and to HIP and HIPPI[107]. Together, these findings raise the possibility that HTT coordinates the activity of several apoptosis-regulating proteins. The physiological consequences of these results are unclear, but could underlie to some extent the severe developmental defects seen in *Htt*$^{-/-}$ mouse embryos[108].

Diabetes. ER stress is a relevant pathogenic mechanism for both type 1 and type 2 diabetes. In the case of type 1 diabetes, in which insulin-producing β cells are lost, pancreatic β cells have an extremely well-developed ER, which reflects their function in secreting large amounts of insulin and other glycoproteins. This secretory function of β cells may explain why mice lacking PERK are susceptible to diabetes, showing apoptosis of their β cells and progressive hyperglycaemia with ageing[177]. Moreover, *PERK* gene mutations in association with infant-onset diabetes have been described in humans with the autosomal recessive disorder Wolcott–Rallison syndrome[178], in which patients at autopsy exhibit massive β-cell loss, resembling the pathology of *Perk*$^{-/-}$ mice. Similarly, EIF2α (Ser51Ala) knock-in mice suffer from β-cell depletion beginning *in utero*, suggesting a more rapid course than *Perk*$^{-/-}$ mice[46]. The failure of *Perk*$^{-/-}$ to phenocopy EIF2α (Ser51Ala) raises the possibility that other kinases besides PERK participate in inhibition of EIF2α during ER stress. Another hereditary disorder in which type 1 diabetes develops is Wolfram syndrome, in which defects in the ER-stress-inducible WFS1 protein occur[179]. WFS1 protein expression is normally induced by stimuli that trigger insulin secretion, and silencing of the WFS1-encoding gene induces ER stress and apoptosis of β cells[180,181]. Pancreatic β-cell apoptosis induced by NO, a mediator of inflammation of relevance to autoimmune (type 1) diabetes, has been shown to be CHOP-dependent, which further implicates ER stress as an instigator of β-cell death[182]. Also, in a rodent model of diabetes caused by a non-secreted insulin mutant (Akita mice), homozygous deletion of *Chop* causes a marked delay in disease onset[183], implying a central role for this gene in β-cell depletion *in vivo*. Therefore, compounds that target the UPR pathways leading to CHOP expression or CHOP phosphorylation (for example, p38 MAPK inhibitors) might be considered for type 1 diabetes treatment before irreversible loss of β cells. However, care must be taken to target the right spot in UPR pathways. It was noted that ATF4 and CHOP were markedly upregulated by the activation of PERK branch of the UPR in pancreatic β cells. In this regard, the neuroprotective agent salubrinal, which inactivates EIF2α by preventing its dephosphorylation, paradoxically promotes apoptosis of pancreatic β cells and increases their sensitivity to fatty-acid-induced ER stress[184].

With respect to type 2 diabetes, in which insulin resistance is the underlying problem often in association with obesity, it is now recognized that a high-fat diet causes ER stress. In this regard, signs of ER stress (for example, PERK phosphorylation, EIF2α phosphorylation and GRP78 upregulation) have been found in liver and adipose tissue of obese mice and mice fed high-fat diets[10]. Moreover, JNK activation resulting from ER stress contributes to insulin resistance, correlating with phosphorylation of insulin receptor substrate 1 (IRS1) on Ser307 and with reduced tyrosine phosphorylation of IRS1 in insulin-stimulated cells and organs, such as liver and adipose tissue. XBP1 seems to blunt the ER stress pathway leading to JNK activation. This observation is based on experiments using *Xbp1*$^{+/-}$ heterozygous mice, which are more sensitive to diabetes caused by obesity and a high-fat diet, developing insulin resistance more easily than their wild-type counterparts[10]. Pharmacological agents that inhibit ER signalling pathways that drive JNK activation (that is, the IRE1 pathway) therefore may be beneficial in the treatment of type 2 diabetes by reducing insulin resistance. Assessments of chemical chaperones in animal models of diabetes have suggested another strategy for alleviating stress on the ER and improving insulin sensitivity. Several chemicals, including 4-phenyl-butyric acid, trimethylamine *N*-oxide dihydrate and dimethylsulphoxide, as well as endogenous bile acids and their derivatives, stabilize protein conformation and improve ER folding capacity. These chemical chaperones increase insulin sensitivity in liver, muscle and adipose tissue, improving glucose homeostasis in obese and diabetic mice[185].

Progression of type 2 diabetes to insulin dependence occurs when pancreatic islets burn out, and β-cell mass declines after the initial β-cell hyperplasia that is typically associated with obesity-related type 2 diabetes. ER stress seems to play a major role in the β-cell loss that occurs during progression of type 2 diabetes, with contributing stressors that include increased demand for insulin secretion (protein load) and toxic effects of fatty acids (high-fat diet). In several mouse models of genetically induced or high-fat-diet-induced diabetes, *Chop* gene ablation preserves β-cell mass, prevents apoptosis and improves glycaemic control[64]. How fatty acids cause ER stress and apoptosis of β cells is unclear, but an early step involves degradation of carboxypeptidase E (CPE). Ablation of the *CPE* gene results in β-cell apoptosis, whereas overexpression protects β cells against palmitate-induced ER stress and apoptosis (lipotoxicity)[186].

Box 3 | **The endogenous antioxidant pathway: KEAP1–NRF2**

NRF/MAF family transcription factors induce the expression of diverse genes containing conserved *cis*-acting elements — antioxidant response elements (AREs) — and encode proteins with antioxidant activity, including haem oxidase 1 (HO1) (reviewed in REF. 217). The transcription factor NRF2 (also known as NFE2L2) is sequestered in the cytosol by KEAP1, a redox-sensitive protein. Upon binding of electrophiles to regulatory cysteines in KEAP1, NRF2 is released. Electrophilic compounds that bind KEAP1, such as oxidized forms of carnosic acid (a prominent constituent of the herb rosemary), certain prostaglandins and semi-synthetic triterpenoids, among others, activate the KEAP1–NRF2 antioxidant pathway, leading to production of HO1 (REFS 218–221). Several of these electrophilic compounds are effective deterrents of reperfusion injury, as demonstrated by their neuroprotective activity in rodent stroke models[218,222]. Interestingly, the cytoprotective, endoplasmic reticulum resident protein BAX inhibitor 1 (BI1; also known as TMBIM6) also activates the NRF pathway[223], and a role for BI1 in intrinsic protection from cerebral ischaemia has been demonstrated in studies of *Bi1*$^{-/-}$ mice, which suffer larger infarcts following transient middle cerebral artery occlusion compared with wild-type mice[81]. However, a strategy for pharmacologically inducing BI1 expression or BI1 protein activation has not yet emerged.

Cancer. Emerging evidence of roles of ER stress and the UPR in tumour biology suggests opportunities for new therapeutic strategies for cancer. In this regard, proteins associated with the UPR, including GRP78 and XBP1, are reportedly overexpressed in the presumptive ischaemic regions of tumours *in vivo* and are induced in cultured cancer cells subjected to hypoxia *in vitro*[187,188]. Therefore, in rapidly growing tumours that outstrip their vascular supply, hypoxia and other stimuli (for example, hypoglycaemia and oxidative stress) may induce ER stress, followed by a protective UPR. Conceivably, therefore, agents that inhibit key UPR signalling molecules could define a new type of cancer therapy, preventing adaptation of tumours to hostile environments. Supporting this notion, GRP78-deficient fibrosarcoma cells as well as XBP1-deficient and PERK-deficient transformed mouse fibroblasts were reported to have significantly reduced tumorigenic activity in mice[189–191].

These observations suggest that compounds that inhibit druggable targets such as PERK and IRE1 should be considered for cancer therapy, provided that their therapeutic index is acceptable. Another approach to blunting the UPR in cancers has resulted in the identification of versipelostatin, a compound that inhibits the rise in GRP78 protein levels induced by some activators of the UPR. This compound shows antitumour activity in tumour xenograft models[192]. Indeed, various chemical inhibitor approaches to antagonizing the GRP78 protein have been preclinically investigated as possible cancer therapies (reviewed in REF. 193), although truly selective inhibitors have yet to be described. Alternatively, a large-molecule strategy for antagonizing GRP78 and inducing ER stress in cancers may be found in interleukin 24 (IL24), a cytokine that associates with GRP78 and shows antitumour activity in mouse models of cancer[194].

Proteasome inhibitors have also recently been shown to induce ER stress, contributing to their cytotoxic activity against cancer cells. ER stress is probably particularly relevant to the clinical activity of the proteasome inhibitor bortezomib (Velcade; Millennium/Janssen-Cilag) in multiple myeloma[195]. This is because plasma cells are highly secretory cells (they produce immunoglobulins) and are known from mouse gene-knockout studies to be particularly vulnerable to disturbances in the UPR machinery (see below). The presumptive basis for ER stress induction by proteasome inhibitors is indirect interference with ERAD, whereby the homeostatic mechanisms for elimination of unfolded proteins in the ER become defective. Preclinical studies have also shown antitumour activity for bortezomib against pancreatic cancers[196,197], which is interesting with regards to the high demands on the ER of exocrine cells. Recently, it has been suggested that HIV protease inhibitors may also inhibit the proteasome, thereby inducing ER stress. Nelfinavir (Viracept; Pfizer) and atazanavir (Reyataz; Bristol–Myers Squibb), for example, induce expression of the UPR markers GRP78 and CHOP in malignant gliomas, and trigger apoptosis that is enhanced by RNA-interference-mediated reductions in GRP78 and inhibited by silencing of CASP4 expression[198]. Nelfinavir also induces ER stress and kills non-small-cell lung cancer cell lines through both caspase-dependent and caspase-independent mechanisms[199]. Clinical trials to determine the maximum tolerated dose of these HIV drugs are currently underway as a prelude to their possible therapeutic application in oncology (National Cancer Institute (NCI) protocol ID NCI-07-C-0047; see Further information). Another ER-stress-inducing agent being explored as a cancer therapy is brefeldin A and water-soluble prodrug analogues thereof, particularly breflate (NCI compound NSC656202)[200,201]. These compounds directly inhibit an ADP-ribosylation factor involved in vesicle trafficking between Golgi and endosomes, inducing defects in protein secretion and subsequent ER stress. Brefeldin A induces GRP78, CHOP and other markers of ER stress in leukaemic B cells, triggering apoptosis through p53-independent mechanisms[201]. Last, given that xestospongins bind IP_3 receptors and have been reported to inhibit their interactions with the anti-apoptotic BCL-2 family of proteins, without directly triggering calcium release from the ER[86], one wonders whether these or analogous chemical modulators of IP_3 receptors could find utility as cancer therapeutics that target ER-based survival mechanisms. The use of this approach depends on an acceptable therapeutic index, which may be challenging for anything that modifies these crucial ER calcium channels.

Immune disorders. Given their robust secretory activity, immunoglobulin-producing plasma cells are especially dependent on UPR signalling. This might explain why mice with XBP1 deficiency lack plasma cells, and why PERK deficiency impairs B-cell homeostasis[202]. Also, IRE1α-deficient B cells fail to develop beyond the pro-B-cell stage[26]. A therapeutic opportunity for exploiting this dependence on the UPR may reside in the proteasome inhibitor bortezomib, which (similar to multiple myeloma) eliminates plasma cells by triggering ER stress. In mouse models of autoimmune disease,

bortezomib prolonged survival, reduced autoantibody levels and protected against autoimmune kidney disease[203].

Like plasma cells, dendritic cells were recently shown to be dependent on UPR signalling for their survival *in vivo*, perhaps due to their extensive processing of peptides for antigen presentation and secretion of cytokines. The IRE1 axis is constitutively activated in dendritic cells under normal physiological conditions, similar to plasma cells, and XBP1 deficiency markedly reduces dendritic cell numbers in mice[204]. Thus, pharmacological agents that place more stress on the ER may reduce dendritic cell function, which could be advantageous or disadvantageous depending on the circumstances. Some bacteria have evolved mechanisms to exploit the dependence of immune cells on the UPR. For example, AB_5 toxins — which include *Shigella*, cholera and *Pertussis* toxins — and subtilase cytotoxin cleave GRP78, which removes the breaks on the UPR and stimulates ER-stress-induced cell death[205].

Hints of a role for accumulation of unfolded proteins associated with the UPR in autoimmune diseases have been observed in several contexts, including autoimmune type 1 diabetes, rheumatoid arthritis, inflammatory bowel diseases and multiple sclerosis (reviewed in REF. 22). Among the proposed mechanisms is the creation of autoantigens as a result of protein misfolding and aberrant modification. Cells with a high degree of secretory activity such as pancreatic β cells may be particularly vulnerable, suggesting that agents that improve ERAD mechanisms might be beneficial. Inflammatory myositis may represent an example in which improving ERAD with a pharmacological agent would provide relief, given the emerging evidence that ER stress contributes to such myopathies[12,206]. ER-stress-induced cell death or dysfunction may also underlie the autoimmune disease ankylosing spondalitis, which is strongly associated with HLA-B27, a class I major histocompatability antigen that appears to be prone to misfolding (reviewed in REF. 22). This raises the possibility that inherited variations in the folding competency of certain proteins directed to the ER could contribute to disease.

Further links between the UPR and autoimmunity are found in GRP78, which is a documented autoantigen in rheumatoid arthritis[207,208]. However, while perhaps contributing to rheumatoid arthritis, the UPR protects against autoimmunity in some contexts. For example, PERK activation in oligodendrocytes reduces the severity of multiple-sclerosis-like pathology in an experimental autoimmune encephalomyelitis model. Thus, a challenge for future research is to empirically define for which autoimmune diseases inhibiting versus enhancing UPR signalling would be beneficial[209].

Next steps and conclusions

With so many UPR mechanisms implicated in so many diseases, how do we begin to translate this knowledge into therapeutic opportunities? To begin, one might divide the landscape for ER-stress-targeting therapies into acute versus chronic diseases. Long-term pharmacological perturbation of UPR mechanisms in a broad systemic manner may be problematic for some tissues and organs, but hopefully acute interventions could be undertaken with acceptable risks of side effects. Such examples might include stroke and myocardial infarction for which short-term application of cytoprotective agents that modulate UPR signalling could be envisioned, and cancer for which cytotoxic agents that affect UPR signalling could be delivered in short courses (cycles) of chemotherapy. Autoimmune disorders might also be attacked with acute courses of UPR-targeting therapies aimed at killing plasma cells, the source of autoantibodies, in an attempt to induce remissions in patients for whom disease severity is life-threatening. Alternatively, some targets that are fundamental to aspects of UPR signalling may be dispensable for daily ER homeostasis and therefore suitable for chronic therapies. In this regard, while most UPR signal-transduction pathways are essential for survival of at least certain types of cells with high demands on their ER, the branch of the IRE1 pathway that activates stress kinases may not be. Within this pathway, ASK1 is a particularly attractive target because its genetic ablation in mice appears to be without detriment, and yet ASK1 contributes importantly to cell death induced by ER stress. Thus, chronic neurodegenerative diseases, such as amyotrophic lateral sclerosis in which ASK1 has been implicated, might be considered, in addition to chronic heart failure in which animal model data support an important role for ASK1. ASK1 presumably is also an obligatory upstream activator of JNK and p38 MAPK in the context of ER stress, and therefore might be considered for chronic diseases for which these kinases exacerbate disease progression. Among these is type 2 diabetes associated with obesity, in which a high-fat diet, leading to ER stress and JNK activation, is thought to cause insulin-receptor signalling defects. Novel classes of agents that increase the folding capacity of the ER (for example, chemical chaperones, inducers of GRP78 and other ER chaperones) and compounds that selectively stimulate ERAD activity could also be envisioned for several chronic diseases in which UPR is pathologically induced.

ER stress has been implicated in several diseases, and multiple pathways linking ER stress to cell death have been reported. The principal challenge with any strategy for blocking cell death caused by ER stress lies with the multitude of parallel pathways that potentially lead to downstream cell death mechanisms. Therefore, blocking only one cell death pathway emanating from the ER may be inadequate to preserve cell survival. For strategies to induce cell death, particularly for cancer, by exploiting ER mechanisms, several candidate approaches have emerged. However, the therapeutic index remains unknown, especially as it applies to potentially vulnerable cells such as pancreatic β cells, cardiomyocytes and neurons. Moreover, modulating ER stress mechanisms may also convert apoptotic cell death to an autophagy response, with unpredictable consequences. Further preclinical studies of various genes and gene products involved in ER-initiated cell death are needed to more fully validate targets for drug discovery.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES

UniProtKB: http://ca.expasy.org/sprot
ATF6α | ATF6β | calreticulin | CHOP | GRP78 | GRP94 | IRE1 | PERK

FURTHER INFORMATION

John C. Reed's homepage:
http://www.burnham.org/default.asp?contentID=215
NCI protocol ID NCI-07-C-0047: http://www.cancer.gov/search/ViewClinicalTrials.aspx?cdrid=529905&version=HealthProfessional&protocolsearchid=5313256

ALL LINKS ARE ACTIVE IN THE ONLINE PDF