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Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress

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Abstract

The ability to respond to perturbations in endoplasmic reticulum (ER) function is a fundamentally important property of all cells, but ER stress can also lead to apoptosis. In settings of chronic ER stress, the associated apoptosis may contribute to pathophysiological processes involved in a number of prevalent diseases, including neurodegenerative diseases, diabetes, atherosclerosis and renal disease. The molecular mechanisms linking ER stress to apoptosis are the topic of this review, with emphases on relevance to pathophysiology and integration and complementation among the various apoptotic pathways induced by ER stress.

The ability of cells to respond to perturbations in ER function, or 'ER stress', is critical for cell survival, but chronic or unresolved ER stress can lead to apoptosis. Factors that perturb ER function and contribute to the development of ER stress include increases in protein synthesis or protein misfolding rates that exceed the capacity of protein chaperones ('client load'), alterations in calcium stores in the ER lumen, oxidative stress and disturbances to the redox balance in the ER lumen[1]. In multicellular eukaryotes, ER stress is sensed by three upstream signalling proteins that, when activated, begin a cascade of corrective actions[1]. The activity of these three pathways collectively constitutes an ER-specific unfolded protein response (UPR).

The evolutionarily oldest branch of the UPR is triggered by the activation of a combined nuclease and kinase called IRE1 (inositol-requiring protein-1), which exists in mammals as two isoforms: IRE1α, which is expressed in all cells, and IRE1β, which is restricted to the gastrointestinal and respiratory tracts. All of the ER-stress transducers, including IRE1, become activated when there is an imbalance of unfolded proteins and chaperones. Activation is probably initiated by both ER-stress-driven changes in heterologous protein interactions, such as dissociation of the protein chaperone BiP (immunoglobulin-binding protein; Grp78), and by direct binding to unfolded proteins[1]. These lumenal events control the linked processes of transautophosphorylation, nucleotide binding and oligomerization[2]. Together they promote the cytosolic effector function of IRE1: highly sequence-specific endoribonucleolytic cleavage and subsequent splicing of an mRNA encoding a critical transcription factor called XBP-1. The spliced form of XBP-1 induces the expression of a large number of genes involved in almost all aspects of the UPR (except the suppression of

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translation initiation), whereas the unspliced form represses such gene expression[1]. Under certain conditions, the IRE1 nuclease can also degrade and thus block the translation of a wide variety of mRNA species, although the possible physiologic and pathologic functions of this action are still being investigated[3].

A second branch of the UPR is initiated by activation of the kinase PERK (protein kinase RNA (PKR)-like ER kinase), which similarly to IRE1, responds to ER stress by autophosphorylation and homomultimerization[1]. PERK phosphorylates the α-subunit of the translation initiation factor eIF2 (eukaryotic translation initiation factor-2), which results in the attenuation of global translation initiation. However, translation of the gene encoding the transcription factor ATF4 (activating transcription factor-4) is favoured by limiting amounts of active eIF2, and thus the expression of ATF4 and its key downstream target, CHOP (C/EBP-homologous protein; also known as GADD153; gene name *Ddit3*), are increased when eIF2α is phosphorylated by PERK[1]. CHOP, usually through interactions with other transcriptional regulators, participates in ER-stress-corrective actions through induction or suppression of a number of genes. A particularly important transcriptional target of CHOP and ATF4 is GADD34 (growth arrest and DNA damage-inducible protein-34), a substrate-specific regulatory subunit of a holo-phosphatase complex that dephosphorylates phosphorylated eIF2α and thus restores global protein translation and suppresses ATF4 translation to basal levels[1,4].

A third branch of the UPR involves the protease-mediated activation of a transcriptional factor called ATF6 (activating transcription factor-6; ref. 1). ATF6 has a major role in chaperone induction and can also transcriptionally induce CHOP. Interestingly, ATF6 is also capable of binding and sequestering the transcription factor CREB, which has the net effect of suppressing hepatic gluconeogenesis[5].

Although all three branches are usually activated by any given ER stress event, the timing of activation can differ[6]. In particular, prolonged ER stress leads to the sequential activation then deactivation of the IRE1α, ATF6 and PERK pathways, respectively[7]. This timing sequence probably has implications for ER-stress-induced apoptosis. It should also be noted that in addition to the suppressive action of CHOP-induced GADD34 on phosphorylated eIF2α, one or more branches of the UPR can be downregulated by other mechanisms specific to certain settings[8–12]. These mechanisms may help cells adapt to physiologic but prolonged ER stress.

Overview of ER-stress-induced apoptosis

Prolonged activation of IRE1 and CHOP can trigger apoptosis in cells under certain physiologic and pathophysiologic conditions[13]. In normal physiology, UPR-induced apoptosis may be a means to eliminate the few cells in an ER-stressed environment that remain uncorrected despite the actions of the UPR. Damaged but non-apoptotic cells can elicit inflammation, whereas apoptosis is usually accompanied by efficient phagocytic clearance that both prevents post-apoptotic necrosis and induces an anti-inflammatory response[14]. Another possible role for physiologic ER-stress-induced apoptosis may be as a host defence mechanism against the intracellular organism *Mycobacterium tuberculosis*[15].

In contrast to these scenarios of limited and selective apoptosis, chronic ER stress can induce widespread pathologic apoptosis[16]. Non-resolving ER-stress-induced apoptosis is becoming increasingly recognized as an important pathogenic factor in a number of widespread and devastating diseases, including neurodegenerative diseases, diabetes, atherosclerosis and renal disease[16].

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In the sections below, we will discuss molecular mechanisms of ER-stress-induced apoptosis, meaning cell death mediated directly by signalling pathways responsive to ER stress.

Molecular mechanisms of apoptosis triggered by IRE1α signalling

In organisms such as yeast and worms, the IRE1 branch of the UPR has an essential role in defending cells and tissues against the lethal consequences of ER stress[17,18]. Thus, when the IRE1 branch is silenced in these organisms, they become more sensitive to agents that perturb protein folding in the ER. In contrast, mammalian cells lacking the widely expressed IRE1α and its major downstream effector XBP-1 are not conspicuously hypersensitive to ER stressors that perturb protein folding in the ER. Rather, these cells seem to alter their development in a manner that places them at less risk for ER stress, that is by failing to acquire the ability to synthesize and fold large amounts of secreted proteins[19–21].

Thus, IRE1-deficient mammalian cells not only survive, but they may actually survive better than wild-type cells under prolonged ER stress. *In vitro*, the RNase activity of IRE1 is highly sequence specific[22,23], and at physiologically low levels of signalling, the endoribonuclease of IRE1 is exquisitely sequence specific *in vivo* as well[24]. At higher levels of stress signalling, however, there is evidence in cultured cells that IRE1 may contribute to rather promiscuous degradation of membrane-associated mRNAs through a process referred to as regulated IRE1 dependent decay or RIDD[3,25]. Although RIDD may help to defend cells against ER stress by degrading ER-associated mRNAs and thus limiting new protein translation, it may also be a mechanism of apoptosis in the setting of severe ER stress. In experiments in which IRE1 activity was manipulated towards RIDD in an insulin-producing pancreatic beta cell line (INS-1), ER-stress-induced apoptosis was enhanced[26] (Fig. 1). Moreover, similar findings were observed with induced overexpression of IRE1α, which is a way to activate IRE1 RNase activity through transphosphorylation in the absence of ER stress. However, when IRE1 was allosterically activated through a pseudokinase mechanism, generalized mRNA degradation and apoptosis were not observed, despite intact *XBP1* splicing[26]. Whether these findings are applicable to pancreatic beta cell death in the setting of diabetes or in other cases of pathologic ER-stress-induced cell death remains to be explored *in vivo*.

Other possible links between IRE1 and ER-stress-induced apoptosis may entail interaction of IRE1 with proteins involved in apoptosis signalling. For example, co-immunoprecipitation experiments suggest that mammalian IRE1α binds Bak and Bax, proteins involved in the mitochondrial pathway of apoptosis (Fig. 1). This interaction seems to be important for IRE1α activation[27].

Phosphorylated, activated mammalian IRE1 also interacts with the adaptor protein TRAF2 (tumour necrosis factor receptor (TNFR)-associated factor-2) and promotes a cascade of phosphorylation events that ultimately activates Jun amino-terminal kinase (JNK, ref. 28; Fig. 1). Given the links between sustained JNK activity and cell death[29,30], JNK activity may link IRE1-mediated ER stress signalling to cell death in certain settings[31]. Genetic tools to address the physiological significance of this hypothesized link are not available, as there are no known mutations in IRE1 or TRAF2 that selectivity disrupt this signalling node. However, an intriguing study suggests that ER-localized Bim and Puma selectively activate the TRAF2–JNK arm of IRE1 signalling, biasing the response away from XBP-1 activation in favour of JNK[32]. The experimental system described relies on overexpression of modified versions of these BH3-only apoptosis effectors, with no contribution of luminal protein misfolding stress, and thus it is unclear how these events might tie in with cell death mediated by ER stress.

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A key goal for future studies of the role of IRE1 in apoptosis is to define mechanisms and show relevance *in vivo*. This goal may be achievable through mutations in IRE1 that could drive a wedge between potential cell death pathways (that is, RIDD and apoptotic protein interaction) and cell-survival pathways (that is, XBP-1 splicing), but such mutations have not yet been reported. However, the use of chemical tools may prove helpful in achieving this goal. *In vitro* studies with the yeast IRE1 enzyme have revealed that certain flavenols can stabilize the IRE1 dimer, promoting XBP-1 splicing in the absence of IRE1 phosphorylation[33]. Although flavenols are too pleiotropic to be of use *in vivo*, it is intriguing to consider that more selective compounds acting on the mammalian enzyme may favour XBP-1 splicing, which enhances the ability of cells to cope with ER stress, over JNK activation, which may be linked to cell death. Given the powerful negative feedback that exists between XBP-1 and IRE1 (ref. 1), even partial biasing of the outputs of IRE1 could have significant effects on physiology.

Molecular mechanisms of CHOP-induced apoptosis

Studies using *Chop*-null mice have established the role of CHOP in ER-stress-induced apoptosis in a number of disease models, including renal dysfunction[34], diabetes[35–37], ethanol-induced hepatocyte injury[38], Parkinson's disease[39], experimental colitis[40], advanced atherosclerosis[41,42] and cardiac-pressure overload[43]. However, the molecular mechanisms remain incompletely understood.

One of the more widely cited mechanisms of CHOP-induced apoptosis is suppression of the pro-survival protein Bcl-2 (Fig. 2), which was based initially on a study showing correlations among CHOP expression, oxidative stress, apoptosis and downregulation of Bcl-2 in a CHOP-transfected rat fibroblast cell line[44]. Most importantly, genetic restoration of Bcl-2 rescued the CHOP-transfected cells from both oxidative stress and apoptosis. The mechanism may involve the ability of CHOP to interact with one or more transcriptional repressors to decrease *Bcl2* transcription[44]. In thapsigargin-treated MEFs, CHOP nuclear translocation, *Bcl2* transcriptional suppression and apoptosis were shown to require CHOP interaction with an isoform of C/EBPβ called liver inhibitory protein (LIP)[45].

A correlation between CHOP-mediated apoptosis and downregulation of Bcl-2 *in vivo* was shown in a mouse model of cardiomyocyte apoptosis, where there was a small but statistically significant decrease in cardiomyocyte Bcl-2 in ER-stressed wild-type mice but not in *Chop*$^{-/-}$ mice[43]. However, a direct molecular link between CHOP and Bcl-2 in this setting remains to be explored, and in general there has been no documentation that Bcl-2 restoration *in vivo* rescues mice from CHOP-induced apoptosis and tissue dysfunction.

Bcl-2 mediates cell survival through sequestration of BH3-only proteins, such as Bad, Bim, Noxa and Puma, which are necessary for Bax–Bak-mediated mitochondrial permeabilization and apoptosis[46]. With regard to BH3-only proteins, a study using multiple ER stressors demonstrated the importance of Bim in ER-stress-induced apoptosis[47] (Fig. 2). Moreover, *Bim*$^{-/-}$ mice were protected from tunicamycin-induced renal epithelial cell apoptosis. ER stress increased Bim levels through both decreased proteasomal degradation and CHOP–C/EBPα-mediated gene induction. In another study, palmitate-induced ER stress was shown to be associated with CHOP–AP-1-dependent upregulation of Puma, Bax activation and apoptosis[48]. The role of Bax in CHOP-induced apoptosis was suggested initially from studies using cultured macrophages[42,49,50] and then later shown in the aforementioned model of ER-stress-induced cardiomyocyte apoptosis, where Bax levels increased with ER stress in a CHOP-dependent manner[43] (Fig. 2).

Another mechanism implicated in CHOP-induced apoptosis is oxidative stress. Prolonged ER stress can both hyperoxidize the ER lumen, which may result in H_2O_2 leakage into the

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cytoplasm, and directly induce cytotoxic reactive oxygen species (ROS) in the cytoplasm. Oxidation of the ER lumen is induced by the CHOP transcriptional target ER oxidase 1α (ERO1α)[51]. In normal physiology, this promotes disulfide bond formation in newly translated proteins, but partial silencing of *ero-1* in *Caenorhabditis elegans* protected the organism from tunicamycin-induced death[51]. This has led to the speculation that with prolonged ER stress, ERO1 may promote a hyperoxidizing environment that leads to cell death. In the setting of diabetes, CHOP deficiency suppresses pancreatic beta cell apoptosis, and this protection was associated with decreased ERO1α, suppression of oxidative-stress markers and induction of anti-oxidant genes[36].

Recent work has suggested a specific molecular mechanism that might link ERO1α to CHOP-induced apoptosis (Fig. 2). Recent *in vitro* and *in vivo* data has shown that CHOP-induced apoptosis involves activation of pro-apoptotic cytoplasmic calcium signalling pathways[52–54]. In particular, UPR-CHOP-induced apoptosis can be blocked by buffering cytoplasmic calcium[52]. Cytoplasmic calcium triggers apoptosis by activating the calcium-sensing kinase CaMKII, which in turn triggers a number of downstream apoptosis pathways[53,54]. A causative role for CaMKII in ER-stress-induced apoptosis was observed in a number of different cell types exposed to various ER stressors and in tunicamycin-treated mice[53,55]. The role of ERO1α is suggested by the observation that CHOP-induced ERO1α activates the ER calcium-release channel IP3R1, which is crucial for the signalling events triggered by cytoplasmic calcium[55]. ERO1α-induced IP3R1 activation may involve disulfide bond formation in a lumenal loop of IP3R1 (refs 51, 56).

In addition to directly promoting hyperoxidizing conditions in the lumen of the ER, the CHOP–ERO1α pathway can induce pro-apoptotic oxidative stress in the cytoplasm. Indeed, one of the consequences of the CHOP–ERO1α–IP3R1–CaMKII pathway is induction of the NADPH (nicotinamide adenine dinucleotide phosphate) oxidase subunit Nox2 and generation of ROS, which is not only essential for apoptosis but which may amplify CaMKII activation as part of a positive feedback loop[54,57,58]. Interestingly, ROS induced by NADPH oxidase is also part of a positive feedback cycle that activates PKR and thus amplifies CHOP expression, and ER-stress-induced apoptosis is suppressed by *Nox2* or *PKR* deficiency in cultured cells and *in vivo*[54]. ROS induced by NADPH oxidase can also be triggered by chronic ER stress secondary to protein misfolding, and oxidative stress in this setting may be exacerbated by high levels of mitochondrial electron transport and by consumption of glutathione[48]. For example, CHOP-induced death of hepatocytes in a mouse model of protein misfolding was associated with increased oxidative stress and was relieved by the anti-oxidant butylated hydroxyanisole (BHA)[59].

The ability of cells to slow protein translation through eIF2α phosphorylation is a key mechanism to prevent oxidative stress and apoptosis in certain settings of physiologic prolonged ER stress[60]. In those settings, the CHOP transcriptional target GADD34 (Fig. 2), which promotes the dephosphorylation of phosphorylated eIF2α and thus restores protein translation, represents another pro-apoptotic mechanism of prolonged CHOP expression. *In vivo* support for this concept came from a study showing that tunicamycin-treated mice homozygous for a disabling *Gadd34* mutation are protected against renal epithelial cell apoptosis[51]. In addition, elevated levels of GADD34 may mediate CHOP-induced apoptosis by other mechanisms[61,62]. However, the role of phosphorylated eIF2α in cell viability may be more complex, as illustrated by a study showing that translational suppression by phosphorylated eIF2α in ER-stressed cultured insulinoma cells blocks the expression of the cell survival protein Mcl-1 and promotes cell death[63].

Other CHOP-induced molecules that have been implicated in apoptosis include death receptor-5 (DR5; TRAIL-R2) and Tribbles-related protein 3 (TRB3; Fig. 2). DR5 has been

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shown to be a mediator of ER-stress-induced death in a number of cultured cancer cell lines[64], and TRB3 is necessary for the full apoptotic response in cultured 293 and HeLa cells exposed to tunicamycin[65]. Data evaluating the importance of DR5 and TRB3 in CHOP-induced apoptosis and tissue dysfunction *in vivo* are lacking. However, a recent study showed an increase in pancreatic beta cell apoptosis in mice overexpressing a hyper-stable form of TRB3 associated with decreased beta cell function in humans[66].

It is intriguing to consider how pathologic CHOP-induced cell death is avoided when prolonged ER stress is an appropriate adaptive response to increased client load, for example in macrophages and other cell types exposed to LPS (lipopolysaccharide) and in B-cell maturation[11,67]. In both of these examples, a prolonged ER stress response through the XBP-1 and ATF6 chaperone branches is necessary, and yet CHOP-induced apoptosis is avoided[11,68–70]. Indeed, forced expression of CHOP in an *in vivo* sepsis model leads to inappropriate cell death and tissue dysfunction[11]. With LPS, *in vitro* and *in vivo* data support a mechanism in which Toll-like receptor signalling leads to 'resistance' to the translational effects of phosphorylated eIF2α, resulting in selective suppression of the ATF4–CHOP axis. Thus, CHOP-induced apoptosis is avoided while the protective arms of the UPR remain engaged[11]. This mechanism appears to be engaged when fitness requires sustained protein synthesis, despite the physiological ER stress imposed, and contrasts markedly with the circumstances reviewed above[51,60]. In other settings, the eIF2α kinases may be checked by a UPR-induced inhibitor $P58^{IPK}$ (refs 8, 9). However, the relevance of $P58^{IPK}$ to eIF2α activity is called into question by recent evidence that it resides in the ER lumen[71,72]. Finally, CHOP- and IRE1α-induced apoptosis may be avoided by a phenomenon called pre-conditioning, in which all three branches of the UPR are partially suppressed in cells subjected to low-level ER stress before being exposed to a robust UPR activator[10]. In this setting, the downregulation of pro-apoptotic proteins is greater than that of pro-survival proteins, such as chaperones, owing to differential mRNA stability[10]. Pre-conditioning is likely to be highly context-dependent and, in certain pathological settings, cells subjected to even low-level ER stress may undergo CHOP-induced apoptosis if exposed to other factors that suppress compensatory cell-survival pathways in ER-stressed cells[73].

Integration among UPR branch apoptosis pathways

Most of the studies investigating ER-stress-induced apoptosis have tended to neglect molecular and/or functional links among the different UPR branch apoptosis pathways. It is possible that two or more UPR branches may induce different steps of a single linear apoptosis pathway (epistasis). In another setting, different UPR branches may separately induce the same pro-apoptotic effector or response, which would amplify that effector or response. Finally, different, complementary UPR branches may promote entirely different apoptosis pathways in a manner that could increase the intensity or chronicity of the cell death response[7] (Fig. 3a). Indeed, in most studies where one UPR branch or effector is experimentally silenced, ER-stress-induced apoptosis is not suppressed completely.

The fact that CHOP is a transcriptional target of not only ATF4 but also XBP-1 and ATF6 (ref. 1) provides an obvious possible link among all three branches. A caveat here is that the IRE1α–XBP-1 and ATF6 branches may be at relatively low levels of activation compared with the PERK–CHOP branch during the later, apoptotic phase of prolonged ER stress[7]. Indeed, as noted above, prolonged, pathologic activation of IRE1 may favour RIDD over XBP-1 splicing. Thus, experiments in which XBP-1 and/or ATF6 are silenced in various settings of CHOP-mediated cell death are needed to fully explore this issue.

Alterations in the expression and/or localization of Bcl-2 family proteins represent another possible area of integration (Fig. 3b). The IRE1α branch can affect BH3-only proteins like

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Puma and Bid[74,75], and CHOP can induce another member of this family, Bim[47]. Thus, the two branches might have additive effects on activation of Bax and Bak, the functional targets of the BH3-only proteins. Moreover, other processes associated with CHOP-induced apoptosis — notably downregulation of Bcl-2 and Mcl-1, which are de-activators of BH3-only proteins, and Bax translocation to the mitochondria — represent additional opportunities for synergy in this pathway, particularly because they are likely to involve different mechanisms from Bim induction[47]. Pro-apoptotic ER stress is also associated with sustained JNK activation (see above), and a recent *in vitro* study linked apoptosis under these conditions with activation of Bad[31]. Integration at the functional level is also likely, because the major pro-apoptotic action of Bax–Bak is through mitochondrial permeabilization[46], which is also a key pro-apoptotic end effect of the CHOP–ERO1α–IP3R–calcium–CaMKII pathway[42,49,53]. Whether Bax and Bak are actually components of the calcium pathway or part of a separate, complementary ER stress–Bax–Bak–mitochondrial pathway is an important question for the future.

In certain cell types, species, and conditions, ER-stress-mediated apoptosis involves caspase-12 activation[76]. Studies to date suggest that the mechanism involves the interaction of pro-caspase-12 with the IRE1–TRAF2 complex[77], but the significance of this interaction remains to be determined. Caspase-12 can also be activated by the calcium-activated protease calpain in settings of ER-stress-induced apoptosis[78]; therefore it is possible that a CHOP–ERO1α–IP3R–calcium–calpain pathway might also contribute to caspase-12 activation. To date, there is little information on how the silencing of various branches of the UPR affects caspase-12 activation and apoptosis in those settings in which this caspase has a role.

Sustained activation of JNK has been implicated in a number of UPR apoptosis pathways[13]. IRE1α has been linked to JNK activation in apoptosis settings through TRAF2 and ASK1 (ref. 79). In addition, one of the major downstream effectors of the CHOP–CaMKII pathway is JNK activation, which is essential for ER-stress-induced apoptosis through at least two mechanisms: induction of Fas and induction of Nox2 and subsequent oxidative stress[53,54]. Therefore, in settings in which both pro-apoptotic IRE1α activation and CHOP expression are prolonged, additive or synergistic activation of JNK may play a crucial role in apoptosis (Fig. 3c). More specifically, this crosstalk may favour prolonged JNK activation, favouring its pro-apoptotic effector function[30,31].

The functional importance of crosstalk among various UPR branch-apoptosis pathways needs to be assessed in one and the same *in vitro* or *in vivo* model. Moreover, in those cases in which different branches contribute different components in the same pathway, lack of temporal coordination may preclude functional significance[7]. Careful studies using disease-relevant *in vitro* and *in vivo* models in which several branches and sub-branches of the UPR are manipulated simultaneously will be needed to define those situations in which functionally significant integration occurs.

Therapeutic implications

Evidence for a role of ER-stress-induced apoptosis in a variety of prevalent chronic diseases make this process a tempting target for therapeutic intervention. A conceptually straightforward strategy would be to globally relieve ER stress itself through the use of so-called proteostasis regulators, including ‘chemical chaperones’[80,81], such as 4-phenyl butyric acid (PBA) and tauroursodeoxycholic acid (TUDCA; Fig. 4). Both have been used in humans for a variety of disorders and have beneficial effects in various mouse models of pathologic ER stress[82–84], but definitive proof that these beneficial effects are

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mechanistically linked to suppression of ER stress and ER-stress-induced apoptosis is lacking. For example, PBA possesses histone deacetylase inhibitor activity.[85]

To circumvent these potential problems, researchers have turned their attentions to more focused manipulations of the pro-apoptotic UPR. As covered in this review, cell culture studies have suggested strategies in which modulatory ligands would be able to promote pro-survival over pro-apoptotic functions of IRE1 (refs 26, 33; Fig. 4a). Several drug strategies targeting the PERK–CHOP pathways of apoptosis have also been tried in mouse models of chronic ER stress (Fig. 4b). Salubrinal is a compound that blocks the dephosphorylation of eIF2α[86] and at certain doses can promote survival in certain cell culture and mouse disease models associated with prolonged ER stress[87–89], although the mechanism is unknown. With regard to the CHOP-oxidative stress-calcium pathway of apoptosis, compounds that inhibit ERO1 have also been shown to protect MEFs from tunicamycin-induced apoptosis in culture[90], and genetic or pharmacologic CaMKII inhibition blocks ER-stress-induced apoptosis in a number of disease models[53,91,92].

One of the major concepts developed in this review, namely integration and complementation among various UPR branch-apoptosis pathways, suggests that targeting only one branch pathway may not be as efficient as targeting two branches. Combining two or more drugs might accomplish this objective, but a number of potential targets — including the pro-apoptotic Bcl-2 family members and JNK — are common to both the pro-apoptotic IRE1α and CHOP pathways and thus may require only one drug. In particular, JNK inhibitors represent an area of high interest; they show promise in a number of pre-clinical models, including those associated with prolonged ER stress, and are currently being investigated in phase I clinical trials for inflammatory disorders[93] (http://www.celgene.com/pdfs/product_pipeline.pdf). Continued progress in pre-clinical models and a favourable safety profile in humans may pave the way for early phase trials in humans with diseases driven by pathologic ER-stress-induced apoptosis.

Conclusion and perspectives

There is increasing evidence that apoptosis triggered by ER stress is involved in the pathogenesis or exacerbation of a number of widespread disease processes. Research in this area has provided rich mechanistic insight into how the IRE1 and PERK–CHOP branches can lead to apoptosis and has suggested promising and innovative areas of potential translational application. However, as reviewed herein, many of the studies use one or just a few ER stressors and cell types, and thus rapid progression to more pathophysiologically relevant *in vitro* and animal models is essential. This gap is particularly apparent for studies on IRE1-mediated pathways of apoptosis — a challenging area given the dichotomy between the effects of XBP-1 versus JNK and RIDD on cell viability. In addition, the possibility of molecular crosstalk between the various pathways of the UPR or consideration of how their separate but complementary actions may have critical roles in apoptosis has not been adequately addressed by most of the published studies in this area. Progress in these areas is essential to gain a more global understanding of this topic and to optimize the design of new therapeutic strategies. Given the prevalence and devastating nature of the diseases in which ER-stress-induced apoptosis is involved, and the fact that many of these diseases are favoured by the increasingly prevalent trends of increased longevity, decreased physical activity and over-nutrition, the impetus for achieving these goals is clear.

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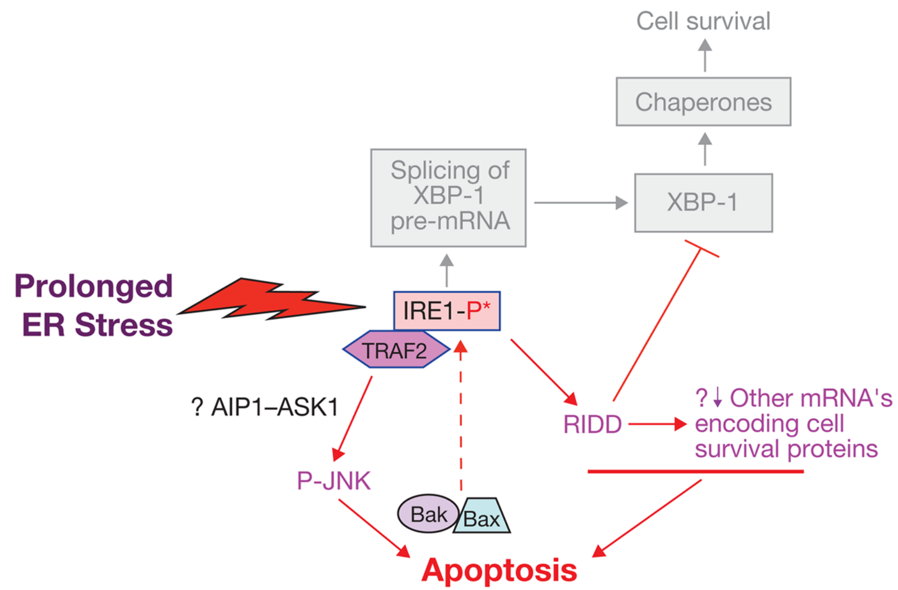

Figure 1.

Prolonged activation of IRE1 may promote apoptosis. Studies with cultured cells have identified a pro-apoptotic IRE1–TRAF2–JNK pathway that can be activated by prolonged ER stress. Signal transduction between IRE1–TRAF2 and phosphorylation of JNK may be mediated in certain settings by the MAP kinase kinase kinase (MAPKKK) ASK1 and its activator, AIP1. JNK-induced apoptosis may involve the pro-apoptotic Bcl-2 family members, Bax and Bak, which in turn, can amplify the IRE1 signal. Prolonged IRE1-mediated activation of the RIDD pathway may promote apoptosis by degrading mRNAs encoding essential cell-survival proteins, including *XPB1* itself.

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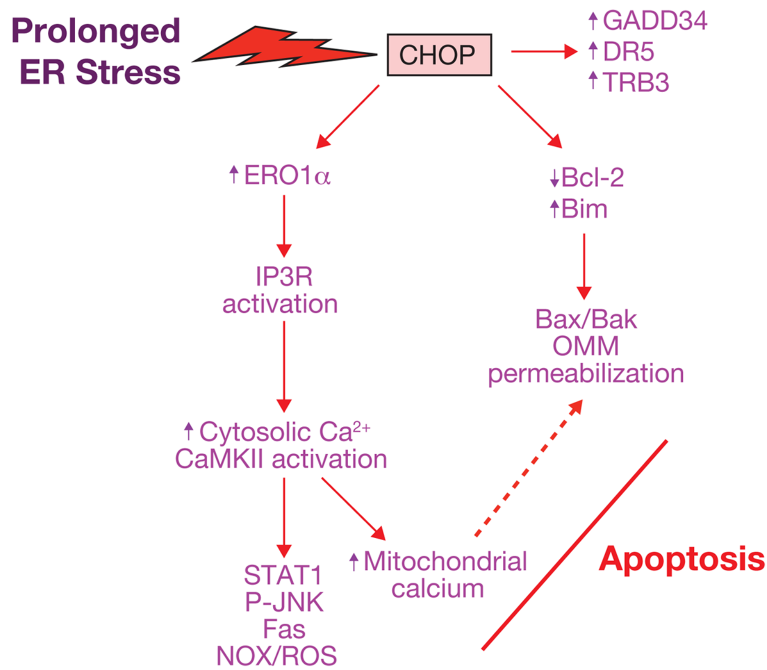

Figure 2.

Pathways through which prolonged activation of CHOP may promote apoptosis. Studies with both cultured cells and gene-targeted mouse models have identified a number of possible pro-apoptotic actions of prolonged CHOP expression, as depicted here and explained in detail in the text. Note that two of the major cell death pathways — the ERO1α–IP3R–Ca^{2+}–CaMKII pathway and the Bcl-2 family member pathway — may lead to a common pro-apoptotic endpoint of outer mitochondrial membrane (OMM) permeabilization (dotted arrow; see text for details).

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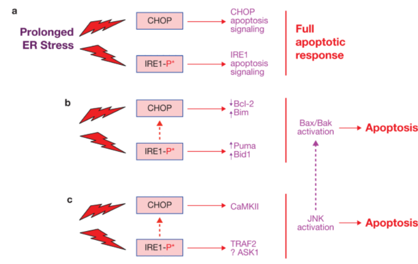

Figure 3.

Examples of integration among the UPR apoptosis pathways. (**a**) In the simplest scenario, differential apoptotic signalling downstream of CHOP and IRE1 may lead to only partial cell death responses, and so the full apoptotic response may require activation of both pathways. (**b**) Both pathways can promote changes in Bcl-2 family protein expression or activity that favour cell death. (**c**) Both pathways can promote JNK activation, which when prolonged, can trigger cell death. Note that one of the mechanisms by which activated JNK leads to apoptosis is through Bax–Bak activation, possibly leading to further integration (purple dotted arrow). See text for details and for other possible areas of integration.

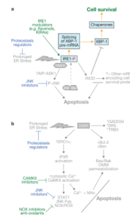

Figure 4.

Examples of therapeutic strategies to prevent cell death in the setting of pathologic, prolonged ER stress. (**a**, **b**) Strategies directed towards IRE1- and CHOP-mediated apoptotic pathways, respectively. Note that several of the approaches are applicable to both (blue font). The common approaches include mitigation of ER stress itself by so-called proteostasis regulators and inhibition of JNK. See text for details.

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