ENDOPLASMIC RETICULUM STRESS AND APOPTOSIS

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Abstract: Cell death is an essential event in normal life and development, as well as in the pathophysiological processes that lead to disease. It has become clear that each of the main cellular organelles can participate in cell death signalling pathways, and recent advances have highlighted the importance of the endoplasmic reticulum (ER) in cell death processes. In cells, the ER functions as the organelle where proteins mature, and as such, is very responsive to extracellular-intracellular changes of environment. This short overview focuses on the known pathways of programmed cell death triggering from or involving the ER.

Key words: Endoplasmic reticulum, Apoptosis, p53, Scotin

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Abbreviations used: ATF6 – activating transcription factor 6; BAP31 – B-cell receptor-associated protein 31; BiP – luminal binding protein; C/EBP – CCAAT/enhancer-binding proteins; eIF-2α – eukaryotic translation initiation factor; EOR – ER overloaded response; ER – endoplasmic reticulum; GADD 153 – growth arrest and DNA damage; GCN2 – general control of amino acid biosynthesis kinase; GRPs – glucose-regulated protein family; GSK3β – glycogen synthetase kinase-3β; HRI – hemin-regulated inhibitor of protein synthesis; IP3 – inositol 1,4,5-trisphosphate; IRE1 – inositol-requiring gene-1; IRF-1 – interferon regulatory factor 1; MEFs – mouse embryonic fibroblasts; NF-κB – nuclear factor-κB; PDI – protein disulphide isomerase; PERK – PKR-like ER kinase; PKR – interferon-inducible; dsRNA-activated protein kinase; PS1, 2 – presenilin-1, -2; SREBPs – sterol-regulatory element-binding proteins; SRP 72 – 72 kDa component of SRP (signal recognition particle); UPR – unfolded protein response; XBP1 – X-Box protein-1
ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is the cytoplasmic compartment where proteins and lipids are synthesized and modified. The ER provides a unique oxidizing compartment for the folding of membrane and secretory proteins that are transported to the cell surface, and to the lysosomes and the Golgi compartments. Due to the presence of high concentrations of protein, numerous protein chaperones exist in the ER to maintain proteins in a folding-competent state and prevent protein folding intermediates from aggregating. Proteins that do not mature properly are retained in the ER and eventually re-translocated to the cytosol for degradation by the 26S proteasome [1] (Fig. 1).

In response to a range of cytotoxic conditions, including hypoxia, nutrient deprivation, pH change, Ca\(^{2+}\) depletion from the ER lumen, inhibition of asparagine (N)-linked glycosylation, reduction of disulfide bonds and overexpression of some proteins, protein misfolding occurs, leading to unfolded proteins accumulating and aggregating in the ER. These abnormalities in the ER are collectively called ER stress. In order to overcome this, the organelle has

![Fig. 1. Schematic representation of endoplasmic reticulum functions. Proteins are translocated into the endoplasmic reticulum lumen through proteinaceous channels in the ER membrane called translocons. In the environment of the ER lumen, resident chaperones like BiP, calnexin and protein disulphide isomerase (PDI) serve to facilitate the proper folding of the nascent protein by preventing aggregation, monitoring the processing of the highly branched glycans, and forming disulphide bonds. Changes in the ER environment shift the balance from normal to improper folding, leading to the accumulation of unfolded proteins in the ER. This activates ER-stress sensors (IRE1, PERK and ATF6), which initiate the unfolded protein response.](image-url)
specific signalling pathways that have been termed (i) the UPR (unfolded protein response) and (ii) the EOR (ER overloaded response). There is substantial overlap in these responses, both in terms of the activating signals and the response [2, 3].

Recent observations indicate that the ER cooperates with other organelles that are important in the processes of apoptosis, like the mitochondria and the nucleus, and it is suggested that the ER also plays a role in autophagic pathways [4].

THE UNFOLDED PROTEIN RESPONSE (UPR)

The UPR is a signal transduction cascade which serves to limit the accumulation of unfolded proteins. It results in the reduction of general protein synthesis and selectively activates the expression of proteins facilitating chaperone activities. The primary effects of UPR activation are designed to protect the ER, but they also serve to limit damage to other organelles and protect the organism by eliminating cells experiencing prolonged stress. Under conditions of severe ER stress that cells are unable to adapt to, the UPR triggers apoptosis [3, 5].

The UPR is essential for a range of normal physiological and developmental processes. These include the regulation of insulin secretion by pancreatic islet \( \beta \)-cells [6], the differentiation of immunoglobulin-secreting plasma cells [7], B-cell differentiation [8], and possibly the proliferation of hepatocytes [9]. In these cases, activation of the UPR is essential to maintain homeostasis in the cell, and genetic disruption of the UPR can lead to diabetes, a block in plasma-cell differentiation, or hepatocellular death [3]. The UPR was first studied in yeast; it was later found that mammals have preserved the basic components of the yeast UPR and greatly expanded it.

ER stress and the survival response

In mammals, three ER transmembrane proteins, IRE1, ATF6 and PERK, respond to the accumulation of unfolded proteins in the lumen. They are normally kept in an inactive state through an association between their N-terminal luminal domains and the chaperone BiP (luminal binding protein). A number of experiments suggest that BiP negatively regulates the UPR. In both yeast and mammalian cells, overexpression of BiP down-regulates the UPR, and reduction of BiP levels is sufficient to induce the UPR [5]. BiP was identified as a member of a glucose-regulated protein family (GRPs) that is expressed at a high level in virally transformed cells and under conditions of glucose deprivation. BiP is present in the ER in monomeric and oligomeric states, and its oligomers are modified by phosphorylation and ADP [10, 11]. BiP interacts transiently with exposed hydrophobic areas on protein-folding intermediates, and is thought to prevent their aggregation while maintaining the protein in a folding-competent state. BiP interaction ensures that only properly folded and assembled proteins exit the ER compartment. Under conditions of ER stress, BiP dissociates (allowing binding to unfolded proteins), and IRE1 and
PERK undergo homo-oligomerization and stimulate trans-autophosphorylation within their serine/threonine kinase domains [5]. IRE1 (inositol-requiring gene-1) exists in two homologues; IRE1α and IRE1β [12, 13]. An activated IRE1 cytosolic endonuclease domain cleaves XBP1 (X-Box protein-1) mRNA. This act allows the synthesis of a highly active bZIP transcription factor that stimulates the transcription of ER chaperone genes [14-16]. The mRNA for IRE1α appears to be auto-regulated in that the endonuclease activity of IRE1α degrades its own mRNA. IRE1α was shown to colocalize with RanGAP1, a component of the nuclear pore complex, suggesting that IRE1 is an ER membrane protein preferentially localized to the nuclear envelope. Overexpression of wild-type IRE1α or β constitutively activates the UPR [12].

Activated PERK (PKR-like ER kinase) phosphorylates eIF-2α (eukaryotic translation initiation factor), resulting in an inhibition of general protein synthesis and G1 arrest [17, 18]. PERK is also required for NF-κB (nuclear factor-κB) activation, which positively regulates anti-apoptotic proteins like BCL-2 during ER stress, thus contributing to the balance between survival and death signals [19].

ATF6 (activating transcription factor 6) contains an ER luminal stress-sensing domain and a cytosolic domain that encodes a transcription factor. During ER stress, BiP dissociates from its luminal domain and allows ATF6 to traffic to the Golgi, where it is cleaved by two proteases, S1P and S2P [20]. The cytosolic transcription-factor domain is uncovered and migrates to the nucleus, where it transactivates downstream promotors. ATF6 induces XBP1 transcription, which is then spliced by the activated IRE1 endonuclease domain and stimulates ER chaperone gene transcription [21, 22] (Fig. 2).

The solid arrows represent the survival response, in which the specific transmembrane sensors ATF 6, IRE 1 and PERK (and PKR) are activated by dissociation of the chaperone BiP, caused by the accumulation of unfolded/misfolded proteins in the ER. ATF6 is proteolytically cleaved after the dissociation of BiP, and acts as a transcription factor in the nucleus, where it transactivates the transcription of XBP1, among others. The cytosolic endonuclease domain of IRE1 initiates XBP1 mRNA splicing into an activated transcription factor, bZIP. PERK and PKR are protein kinases promoting the phosphorylation of eukaryotic initiation factor 2 (eIF-2), which suppresses ER protein synthesis, decreases delivery of newly formed proteins to the ER lumen, and attenuates the unfolding stress, thus preserving nutrients and energy. PERK also activates the transcription factor NFκB. All three ER transmembrane receptors are thus responsible for initiating the expression of proteins that enable the cell to survive in stress conditions.
Fig. 2. Unfolded protein response pathways. When the conditions of ER stress persist, apoptotic pathways are activated (empty arrows). eIF-2 phosphorylation by PERK outbalances NFκB activation. All three signaling pathways (ATF 6, PERK and IRE 1) can initiate CHOP transcription. CHOP is a nuclear transcription factor that negatively regulates cell growth, leading to cell cycle arrest and apoptosis. Moreover, evidence has been presented for the involvement of caspase-12. A proposed model suggested that caspase-12 (probably represented by caspase-4 in humans) is activated by IRE1 and triggers the activation of a downstream cascade of effector caspases, but more recent data questions the role of caspase-12 in ER stress-dependent apoptosis (see text for details).

**ER stress and the death response**

When stress conditions persist, apoptotic signalling pathways are initiated and damaged cells are eliminated. During ER stress, three death-inducing signals are generated. The first triggers the transcriptional induction of CHOP/GADD153, the second is mediated through phosphorylation of the translation initiation factor eIF-2, and the third involves activation of caspases.

**ER stress and CHOP/GADD153**

CHOP was identified as a member of the C/EBP (CCAAT/enhancer-binding proteins). It serves as a dominant negative inhibitor of the C/EBP, and is also known as a gene induced on growth arrest and DNA damage (GADD 153) [23, 24]. CHOP is a nuclear transcription factor that represses the BCL-2 promoter.
and may sensitize mitochondria to the proapoptotic effects of BH3-only proteins. Overexpression of CHOP can lead to cell cycle arrest and apoptosis [25, 26]. Expression of CHOP is mainly regulated at the transcriptional level, and it is one of the most highly induced genes during ER stress. For maximal induction of CHOP, all three ER stress-signalling pathways are required, but the PERK/eIF2α signalling pathway plays an essential role and is dominant over the ATF6 and Ire1/XBP-1 pathways [6, 27, 28]. CHOP is also regulated at the post-translational level by MAP kinase phosphorylation, which enhances transcriptional activation and elicits a maximal apoptotic effect of CHOP [29]. Overexpression of CHOP leads to cell cycle arrest, elevated BiP attenuates the induction of CHOP in ER stress, and CHOP−/− mice exhibit reduced levels of apoptosis in response to ER stress. Thus, it is supposed that CHOP plays an important role in ER stress-induced apoptosis [30, 31].

**ER stress-signalling through eIF-2α kinases**

In addition to changes in the transcriptional machinery, the immediate response to the accumulation of unfolded proteins in the ER occurs at the translational level with the inhibition of translational initiation, thereby protecting cells from further accumulation of unfolded proteins, as well as preserving nutrients and energy. The most frequently used mechanisms for translation control are the reversible phosphorylation of eIF-4E and eIF-2 [1]. The original observations demonstrated that treating cells with agents that disrupt ER function leads to protein synthesis inhibition, which correlates with increased eIF-2α phosphorylation. Small increases in the phosphorylation of eIF-2α immediately inhibit additional initiation events [32]. The status of eIF-2α phosphorylation is controlled by several Ser/Thr protein kinases that primarily recognize eIF-2α as a substrate. Four specific eIF-2α kinases have been identified: HRI (hemin-regulated inhibitor of protein synthesis), GCN2 (general control of amino acid biosynthesis kinase), PKR (interferon-inducible, dsRNA-activated protein kinase), and PEK/PERK [17, 18]. Although all known eIF-2α kinases are activated by different cellular stress conditions, current evidence indicates that PERK and possibly PKR respond to stress in the ER [17].

PKR is the interferon-inducible, dsRNA-activated protein kinase that is ubiquitously expressed in all mammalian cells [33]. The transcription of PKR is induced upon treatment with interferon α and β [34]. PKR displays two well-known activities: autophosphorylation, and phosphorylation of its physiological substrate, the α subunit of eIF-2. As a consequence of PKR activation, viral replication and cell growth are inhibited and cells may undergo differentiation. Increasing evidence supports an additional role for PKR in the transcriptional regulation of dsRNA-activated genes such as interferon β through activation of IRF-1 (interferon regulatory factor 1) and NF-κB [35, 36]. PKR is associated with rough ER membranes, and this localization suggested that PKR might signal in response to ER stress [37-39].
Activation of caspases

Caspase-12 is located in the ER membrane and participates in apoptosis under ER stress. After the initiation of apoptotic pathways, procaspase-12 is cleaved and becomes activated by an unknown mechanism. In mice, procaspase-12 is localized to the cytosolic face of the ER membrane, placing it in a position to respond to ER stress as a proximal signalling molecule [40]. Fischer et al. [41] reported that the human caspase-12 gene has acquired deleterious mutations that prevent the expression of a functional protein. Despite these discoveries, two reports showed that antibodies against murine caspase-12 detect an appropriately sized protein in human cells that is processed following ER stress. Caspase-12 is remarkably specific to factors that elicit ER stress, and is not proteolytically activated by other death stimuli. Recent data indicates that in humans, there is perhaps a caspase-4 that is homologous to murine caspase-12, and that it is activated in an ER stress-specific manner [42]. It is suggested that caspase-12 activation is linked to IRE1 signalling. The cytosolic tail of IRE1 can recruit TRAF2 [43], and when overexpressed, TRAF2 can interact with caspase-12 and weakly induce its oligomerization and cleavage [44]. Activated caspase-12 (lacking its protodomain) may directly process downstream caspases in the cytosol, or it may target other as-yet unidentified substrates that influence the progression of apoptosis. Two groups reported that caspase-12 can directly trigger caspase-9 activation and apoptosis independently of the mitochondrial cytochrome c/Apaf-1 pathway [45, 46]. However, the exact mechanism of caspase-12 function is still unknown. There is some contradictory evidence about its function in the latest literature. Several recent papers have confirmed that caspase-12 is activated in ER stress conditions in the caspase-12-/- mouse model, and in cortical neurons [47, 48]. On the other hand, using the same model, Saleh et al. [49] demonstrated the role of caspase-12 in modulating the inflammatory response by the inhibition of caspase-1. Moreover, they argued against an apoptotic caspase-12 function, proposing that caspase-11 rather than caspase-12 might have a role in ER stress-induced apoptosis in some settings [50].

THE ER OVERLOADED RESPONSE (EOR)

Very similar signals to those induced by the UPR are triggered during viral infection when viral glycoproteins are produced in massive amounts. The ER then sends a signal to activate the transcription factor NF-κB to induce expression of inflammatory genes and immune response genes, such as interferons and cytokines. This signalling pathway was termed the ER overloaded response (EOR), because it is activated by the accumulation of membrane proteins in the ER [51, 52]. A number of stimuli that disrupt protein folding, such as 2-deoxyglucose, tunicamycin and calcium ionophores, activate both the UPR and the EOR [1].
THE ER AND OTHER ORGANELLES INVOLVED IN APOPTOSIS

It is no exaggeration to say that cells can die by a thousand deaths, as current knowledge on cell death pathways shows. A pattern emerges, however, that each major cellular structure can initiate its own set of unique signals to induce apoptosis. These signals are often associated with specific damage or perturbation to the organelle involved. Although the ER can trigger unique apoptotic pathways as a reaction to different stress signals, there is evidence that the ER can also cooperate with other apoptotic pathways [4].

The ER and mitochondria

The BCL-2 family and the ER

Members of the BCL-2 family are also located on the ER, and several lines of evidence suggest that mitochondria are important components of the ER stress-induced apoptotic pathway.

1. ER stress agents cause mitochondrial release of cytochrome c and loss of mitochondrial transmembrane potential [53, 54].
2. BCL-2/BCL-XL inhibit ER stress-induced apoptosis [53, 55, 56].
3. Bax−/− and Bak−/− MEFs (mouse embryonic fibroblasts) are resistant to tunicamycin (which inhibits N-linked glycosylation), thapsigargin (which disrupts ER Ca2+ stores) and brefeldin A (which blocks ER to Golgi transport), three pharmacological agents that can induce ER stress [57].

The ER is the main intracellular storage compartment for Ca2+, which is an important secondary messenger that is required for numerous cellular functions. Apoptosis occurs upon the perturbation of cellular Ca2+ homeostasis, such as cytosolic Ca2+ overload, ER Ca2+ depletion, and mitochondrial Ca2+ increase. The close physical contact of mitochondria and the ER results in the mitochondria being exposed to more Ca2+ than the rest of cytosol when Ca2+ is released from the ER. BAX and BAK localized to the ER can directly affect ER Ca2+ stores [58].

When a cell is committed to apoptosis, BAX and BAK undergo conformational changes, leading to their oligomerization and localization in the mitochondrial outer membrane [59]. This process is followed by the release of cytochrome c from the mitochondria to the cytosol. Cytochrome c, once released into the cytosol, interacts with APAF-1, leading to the activation of caspase-9 proenzymes. Active caspase-9 then activates the rest of the caspase cascade [57, 60, 61]. ER stress probably induces conformational changes and oligomerization of BAX and BAK on the ER membrane, which may lead to damage of ER membrane integrity. Expression of BAK in the ER results in ER Ca2+ depletion and ultimately cell death. These findings indicate that, in addition to their roles in the mitochondria, BAX/BAK proteins may also be involved in initiating apoptosis from the ER [61].

The anti-apoptotic properties of BCL-2 and BCL-XL have been attributed to their ability to antagonize BAX/BAK by forming heterodimers that prevent their oligomerization and apoptosis initiation. Bcl-2 expression also regulates
intracellular Ca\(^{2+}\) homeostasis and thus contributes to the protective role of this oncogene against programmed cell death [62]. At normal physiological levels, Ca\(^{2+}\) released from the ER during cell activation is taken up by mitochondria to stimulate oxidative phosphorylation and enhance ATP production. Sustained and complete release of ER Ca\(^{2+}\) can initiate Ca\(^{2+}\)-dependent forms of apoptosis by triggering the opening of the mitochondrial permeability transition pore.

Foyouzi-Youssefi et al. [63] showed that Bcl-2 decreases the free Ca\(^{2+}\) concentration within the ER lumen by increasing the Ca\(^{2+}\) permeability of the ER membrane, and thereby protects cells from apoptotic stimuli. Conversely, Bax enhances the loading of the ER Ca\(^{2+}\) store and thus boosts the Ca\(^{2+}\) load to which the apoptotic effector systems (including the mitochondria) are exposed upon physiological and/or pathological challenges. This effect of Bax coincides with gross perturbation of mitochondrial structure and function and finally results in apoptotic progression [64].

In mitochondria-initiated apoptosis, BAX and BAK activation involves members of the BCL-2 family named BH3-only proteins. The presence of several BH3-only proteins, such as tBID, BIM, NOXA and PUMA, in the mitochondria when apoptosis is triggered suggests that their activation of BAX/BAK may take place at this site [65, 66]. Recent models suggest that the BH3-only subset of the proapoptotic BCL-2 family senses diverse death signals and initiates caspase activation [67]. BH3-only molecules may achieve this by binding and inhibiting anti-apoptotic BCL-2 family members or by directly activating pro-apoptotic BAX and BAK. Most BH3-only proteins localized to the mitochondria trigger cytochrome c release, which in all cases is inhibited by BCL-2 overexpression [68]. It is possible that some of the BH3-only, such as BIK/NBK, are localized to the ER and trigger BAX and BAK activation at the ER. BIK mRNA and protein are induced by p53 in response to DNA damage or oncogenic stress, and induce cytochrome c release independent of an association with the mitochondria or zVAD-sensitive caspases. BIK activates factors in both the ER and cytosol to induce mitochondrial transformations [69]. This activity of BIK is influenced by BCL-2. High BIK:BCL-2 ratios change the set of proteins that BCL-2 interacts with at the ER, resulting in Ca\(^{2+}\) release, cytochrome c release from the mitochondria, and apoptosis. ER-localized BCL-2 is able to protect against BIK-induced apoptosis [5].

ER stress-induced cytochrome c release is apparently dependent on the c-ABL tyrosine kinase. c-ABL is relocalized from the ER to the mitochondria, which parallels an increase in its kinase activity. c-ABL may function with JNK kinases, which are recruited and activated by IRE1 during ER stress, and are essential for mediating cytochrome c release in other cell death pathways [43, 70]. Therefore, stress in the ER evokes either mitochondrial-dependent apoptosis or mitochondrial-independent pathways that include activation of relevant caspases. These two arms of the ER stress response apparently operate independently of each other.
Caspase substrates located at the ER

Several ER proteins have now been identified as caspase substrates. The majority seem to be caspase-3 targets; therefore, their cleavage likely contributes to the coordinated shutdown of normal cellular processes during the execution phase of apoptosis.

They are:
- BAP31 (B-cell receptor-associated protein 31), which has been suggested as a possible caspase-8 substrate. Breckenridge et al. reported that BAP31 regulates ER-mediated apoptosis through the promotion of membrane fragmentation and cytochrome c release from the mitochondria [71-73].
- SREBPs (sterol-regulatory element-binding proteins). Caspase-3 cleavage of SREBPs upregulates sterol response genes, which may affect lipid rearrangements and/or morphological changes of the plasma membrane during apoptosis [74].
- SRP 72 (72-kDa component of SRP (signal recognition particle)), which is thought to help translocation of polypeptides across the ER membrane. Caspase cleavage of SRP 72 might shut down or alter the translation of secretory proteins during apoptosis [75, 76].
- IP₃R-1, -2 and -3 (inositol 1,4,5-trisphosphate receptors 1, 2 and 3). IP₃R1 and 2 are cleaved by caspase-3 and IP₃R-3 undergoes calpain proteolysis during apoptosis, which removes IP₃R-induced Ca²⁺ flow. Caspase-mediated proteolysis might represent functional down-regulation of this channel during the execution phase of apoptosis. Similarly, calpain proteolysis of the Ca²⁺-binding ER chaperone GRP94 may also affect Ca²⁺ signalling during apoptosis [77, 78].
- PS1 and 2 (presenilin-1 and -2), which are highly homologous integral membrane proteins of the ER and Golgi, and which are mutated in most aggressive early-onset forms of familial Alzheimer’s disease (AD). Caspase cleavage of presenilins creates an apoptotic amplification cycle, accelerating cell death in the neurons of AD patients [79].

p53 and stress in the ER

ER stress was found to inhibit p53. Qu et al. [80] demonstrated that ER stress, induced by ER-specific protein folding inhibitors such as thapsigargin and tunicamycin, or by glucose starvation, inhibits the ability of p53 to induce apoptosis triggered from the nucleus in response to DNA damage. ER stress targets wild-type p53 into the cytoplasm, where it is degraded, providing a mechanistic basis for the inhibition of the apoptotic activity of p53 by ER stress. A pathway by which ER stress modulates p53 intracellular localisation and function involves GSK3β (glycogen synthetase kinase-3β), which can phosphorylate p53 on Ser 376 and 315. In GSK3β −/− cells, p53 did not translocate to the cytoplasm in response to ER stress, and its apoptotic activity was not inhibited. The effect of ER stress on the p53 phosphorylation of Ser 376 is opposite to that of the DNA damage checkpoint, which facilitates
dephosphorylation and activation of p53 function. In the absence of ER stress, wild-type p53 localized predominantly in the nucleus. Thus, Ser 315 and Ser 376 phosphorylation appear to dictate the intracellular localization of p53 only in cells with ER stress, implying that some other post-translational modification is required. It is concluded that inactivation of p53 is a protective mechanism utilized by cells to adapt to ER stress [81, 82].

A further link between the ER, p53 and apoptosis proceeds from the recent identification of a novel p53-regulated ER membrane protein named scotin. Further studies showed that scotin is conserved between mouse and human and is detected in the ER and in the nuclear envelope, but not in the Golgi apparatus or the mitochondria. Scotin is directly transactivated by p53 in response to stresses that evoke apoptosis [83]. It would be interesting to know whether scotin acts downstream of p53 in a transcription-dependent or independent manner. Another question is how scotin activates caspases, because the predicted protein does not contain a specific domain crucial for the activation of caspases. In addition, the inhibition of endogenous scotin protein expression reduced the p53-dependent apoptosis induced by UV irradiation strongly, but not completely, suggesting that other proapoptotic pathways were activated. It also appears that the human scotin gene has several splice variants that are selectively expressed in response to different stress conditions, or occur in neoplastic human tissues, suggesting specific roles in cancer development (author’s unpublished data).

CONCLUSION

Although it is well known that ER stress can lead to apoptosis, very little is known about the mediators involved in ER-controlled apoptosis. It is clear that the ER has its own complement of apoptotic accessories that independently activate caspases and mitochondrial dysfunction. A better understanding of the ER in apoptosis may come after the clarification of BCL-2, scotin and p53 function at this location. Since deregulation of BCL-2 family members at the ER affects cell survival outcomes, an understanding of their functions at this site could be important for developing new therapeutics for treating diseases such as cancer.

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