

The Unfolded Protein Response in Health and Disease

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Abstract

The endoplasmic reticulum (ER) is the principal cellular organelle in which correct folding and maturation of transmembrane, secretory, and ER-resident proteins occur. Research over the past decade has demonstrated that mutations in proteins or agents/conditions that disrupt protein folding adversely affect ER homeostasis, leading to ER stress. This in turn initiates the unfolded protein response (UPR), an integrated intracellular signalling pathway that responds to ER stress by increasing the expression of ER-resident molecular chaperones, attenuating global protein translation and degrading unfolded proteins. Failure to relieve prolonged or acute ER stress causes the cell to undergo apoptotic cell death. Recent groundbreaking studies have provided compelling evidence that ER stress and UPR activation contribute to the development and progression of human disease, including neurodegenerative disorders, diabetes, obesity, cancer, and cardiovascular disease. Furthermore, the ability of the UPR to modulate oxidative stress, inflammation, and apoptosis provides important cellular clues as to how this evolutionarily conserved cellular-stress pathway maintains and responds to both normal physiologic and pathologic processes. In this Forum issue, many aspects of the UPR are reviewed in the context of how ER stress and UPR activation influence human disease. This current information provides a solid foundation for future investigations aimed at targeting the UPR in an attempt to reduce the risk of human disease. *Antioxid. Redox Signal.* 11, 2279–2287.

Overview of the Endoplasmic Reticulum and Its Function

THE ENDOPLASMIC RETICULUM (ER) consists of a network of interconnecting and continuous branched tubules and flattened sacs extending throughout the cytosol. It is the principal cellular organelle responsible for the proper folding and processing of nascent polypeptide chains that encode transmembrane, secretory, or cell-surface proteins. Once synthesized on ER membrane-bound ribosomes, these polypeptide chains cotranslocate across the ER membrane through the Sec61 complex into the ER lumen (64). Unlike the cytosol, the environment of the ER lumen is sufficiently oxidized to allow the oxidation of cysteine residues to form disulfide bonds critical to the correct conformation of many mature proteins (41, 64). To assist in the correct folding of newly synthesized proteins and to prevent aggregation of folding intermediates, the ER contains a high concentration of molecular chaperones including GRP78, GRP94, calnexin, and calreticulin.

The ER is also the major intracellular Ca^{2+} store with a resting concentration of Ca^{2+} of $\sim 400 \mu\text{M}$ (46, 57). These elevated ER Ca^{2+} levels facilitate interactions between Ca^{2+} -dependent chaperones such as calreticulin and calnexin and

nascent polypeptides (71). Depletion of the ER Ca^{2+} stores impairs chaperone activity and inhibits the secretion of specific proteins, demonstrating that proper protein processing requires high levels of Ca^{2+} within the ER lumen (38). The ER lumen is also the cellular site for the addition of sugar moieties to asparagine residues, a process termed N-linked glycosylation. Given that glycosylation is an important component of correct protein folding, underglycosylated proteins have increased association with ER-resident chaperones, leading to decreased cell-surface expression or secretion (16).

Once a protein is successfully modified and folded in its native state, it is transported out of the ER in coatamer protein (COP) II-coated vesicles to the cis-face of the Golgi complex (3). Proteins that are irreversibly damaged and cannot be correctly folded are eliminated *via* the ER-associated degradation (ERAD) pathway. ERAD involves the retrotranslocation of damaged proteins out of the ER into the cytosol *via* the Sec61 translocon (74). Once in the cytosol, these proteins are ubiquitinated and targeted for proteasome-mediated degradation (44). A growing body of evidence demonstrates that the accumulation of mutant proteins or conditions/agents that disturb protein folding/processing cause ER stress and contribute to a wide range of human diseases, including

diabetes, cancer, neurodegenerative disease, and heart disease. Whether this impairment in the cellular secretory pathway is the primary cause or a secondary event in disease progression is yet to be determined.

This Forum issue focuses on the physiologic roles of the unfolded protein response (UPR) pathway and its connection to reactive oxygen species (ROS) production and inflammation, as well as its relation to the development and progression of certain human diseases, such as cancer, atherosclerosis, renal disease, and diabetes (Fig. 1). In addition, unique properties of the ER-resident chaperone, GRP78, are highlighted with respect to its role as a multifunctional cell-surface receptor and as a modulator of early embryonic development.

ER-resident chaperone proteins

To assist and regulate the folding process, the ER lumen contains a family of proteins known as ER chaperones that act as foldases and stress sensors in the ER quality-control system. These chaperones include protein disulfide isomerase (PDI), glucose-regulated protein-78/immunoglobulin-binding protein (GRP78/BiP), glucose-regulated protein-94 (GRP94), ERp72, calreticulin, and calnexin (59). ER chaperones target unfolded or misfolded proteins that enter the ER, thereby simultaneously preventing inappropriate aggregate formation and maintaining the proteins in a conformation compatible with correct folding. Several chaperones, including GRP78, PDI, and GRP94, have been identified in a large complex,

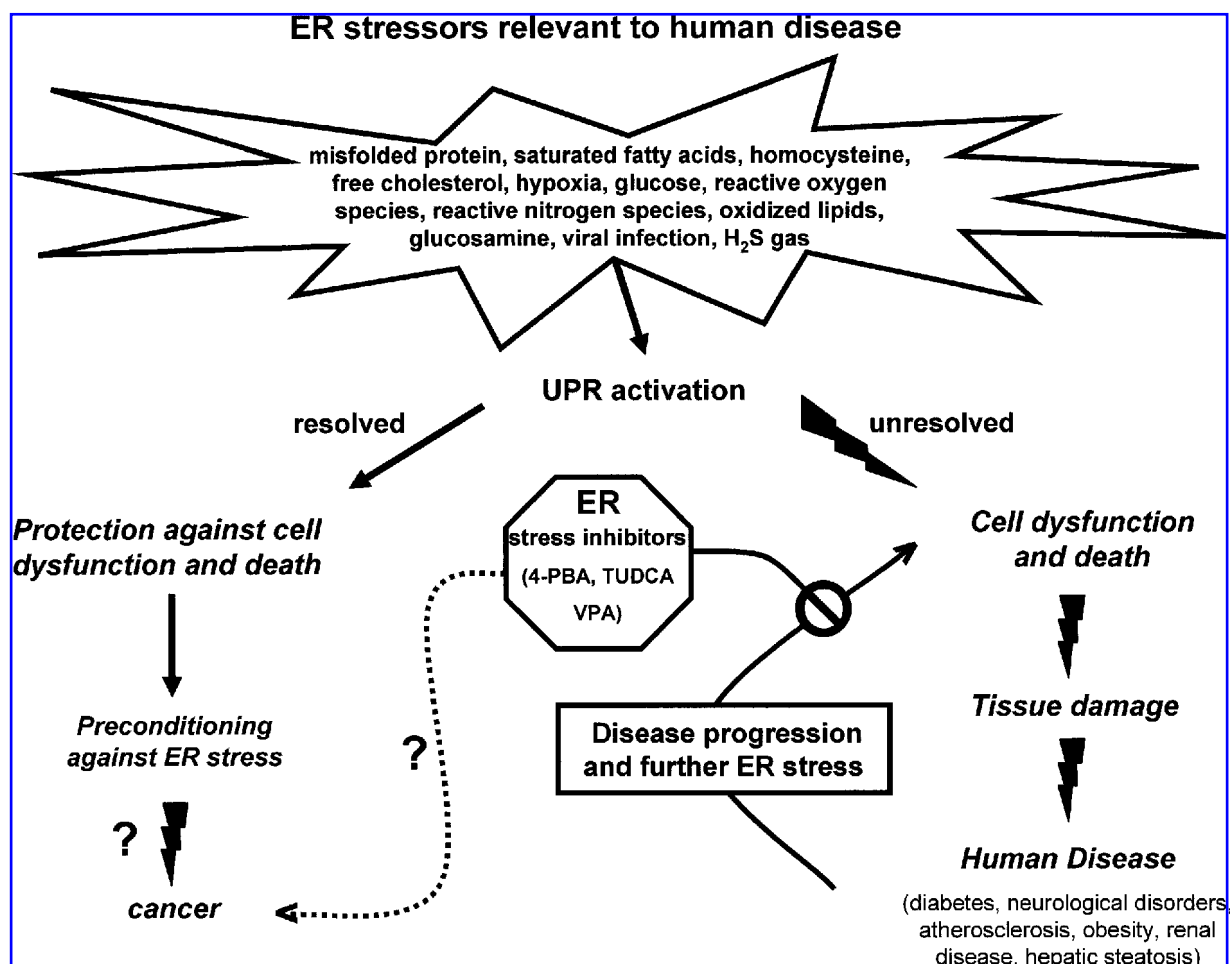


FIG. 1. Current model linking ER stress and UPR activation to the development and progression of human disease. Cell types relevant to human disease, such as neurons, hepatocytes, pancreatic β cells, macrophages, and adipocytes, are exposed to a number of factors that induce ER stress and UPR activation. Resolution of ER stress by UPR activation leads to cytoprotection and preconditioning against further ER stress and oxidative stress. However, severe or unresolved ER stress causes cell dysfunction (*i.e.*, lipid dysregulation, release of pro-inflammatory cytokines, oxidative stress) and death, resulting in tissue damage. This contributes to the development and progression of human diseases such as diabetes, atherosclerosis, neurologic disorders, renal disease, obesity, and hepatic steatosis. Further ER stress and UPR activation can result from disease progression, leading to a continuous cycle of cell death and tissue damage. Recent studies have demonstrated that ER-stress inhibitors, such as 4-PBA, TUDCA, and VPA, can suppress disease progression, including diabetes, obesity, renal disease, and neurologic disorders. However, this strategy may not apply to cancer because sensitivity to ER stress and the activation of UPR-mediated apoptotic pathways would be a beneficial approach of inhibiting tumor cell growth and proliferation.

together with incompletely folded immunoglobulins, suggesting that chaperones form a multimeric network that binds unfolded proteins (45). Neither calreticulin nor calnexin was identified in this multimeric complex, providing evidence of a separation of chaperone systems by function or temporal action (45). Within the ER lumen, chaperones bind to and interact with unfolded proteins through their inappropriately exposed hydrophobic domains or underglycosylated residues (59, 64).

Calreticulin and calnexin specifically assist in glycoprotein folding by recognizing underglycosylated residues within the nascent polypeptide chain. As the polypeptide chains enter the ER lumen, they are modified by the addition of glucose moieties that provide binding sites for calreticulin and calnexin, thus retaining these proteins in the ER (65, 71). The glucose moiety is removed by glucosidase I and II, releasing the protein from calreticulin/calnexin and allowing the newly synthesized protein the opportunity to fold correctly (46). During this period, the oxidoreductase PDI catalyzes the correct formation of disulfide bonds by reshuffling incorrect disulfide bonds and ensuring flexibility of the disulfide bonds until all folding is completed (7). Once correctly folded and modified, the protein continues onto the Golgi; however, if folding is impaired, the glycoprotein is reglycosylated by UDP-glucose:glycoproteins glucosyltransferase (UGGT), recreating the calreticulin/calnexin binding site (46). This cycle of addition/removal of glucose moieties and binding and release from calreticulin/calnexin continues until proper protein folding occurs.

Chaperones from the heat-shock 70 family, including GRP78 and GRP94, recognize unfolded proteins by their inappropriately exposed hydrophobic domains (19). The chaperone GRP78 undergoes cycles of protein binding and release mediated by its N-terminal ATPase activity until the protein is folded correctly and no further GRP78 binding motifs are exposed (20). When GRP78 is bound to ATP, the conformation of GRP78 is modified to allow the binding of unfolded proteins (*via* their hydrophobic domains) (20). ERdj1 through 5 are members of the DnaJ family of proteins and co-chaperones of GRP78 that bind to the GRP78/protein complex and stimulate the ATPase domain of GRP78 to hydrolyze ATP to ADP (8, 10, 61, 62). The conversion of ATP into ADP causes a conformational change in GRP78, which increases the binding affinity of GRP78 to the unfolded protein (20). The nucleotide exchange factor BiP-associated protein (BAP) then mediates the exchange of ADP to ATP, thereby weakening the GRP78/protein interaction and releasing the protein from GRP78 (9). These cycles of binding and release between GRP78 and the unfolded protein, mediated by ATP hydrolysis, continue until the protein is correctly folded, and no further hydrophobic residues remain exposed.

GRP78 and other ER chaperones assist in maintaining the intracellular Ca^{2+} balance by sequestering Ca^{2+} within the ER (34). Ca^{2+} does not bind to ER chaperones at specific Ca^{2+} -binding motifs but rather to paired anionic amino acids present in their structure (40). Although GRP78 contains the greatest number of anionic residues of any chaperone, it has a relatively low number of paired anionic residues and thus has a low capacity for Ca^{2+} (1–2 mole Ca^{2+} /mole), compared with calreticulin (20 mole Ca^{2+} /mole) or PDI (23 mole Ca^{2+} /mole) (34, 40). Nevertheless, overexpression of GRP78 has been shown effectively to increase the Ca^{2+} storage capacity of the ER (34), attenuate oxidant-induced alterations in

intracellular Ca^{2+} levels, and decrease apoptotic cell death (36). Conversely, reducing GRP78 expression increases intracellular Ca^{2+} levels in response to the agonists glutamate (79) or H_2O_2 (1). These findings demonstrate that ER-resident chaperones have the capacity to bind Ca^{2+} and play a major role in maintaining cellular Ca^{2+} homeostasis.

ER stress and the UPR

ER stress is defined as a disruption in ER homeostasis that leads to the accumulation of unfolded or misfolded proteins in the ER (58). Mutations in secretory proteins or receptors that alter their proper folding induce ER stress and cause human disease (81). Furthermore, a large number of pharmacologic agents or physiologic conditions can alter ER homeostasis, thereby inducing ER stress. For example, depletion of ER Ca^{2+} stores by Ca^{2+} ionophores, such as A23187, thapsigargin, or ionomycin, induces ER stress by impairing Ca^{2+} -dependent chaperone activity (38). The addition of reducing agents such as dithiothreitol (DTT) or homocysteine induces ER stress by impairing disulfide bond formation, leading to protein misfolding (39). Tunicamycin or 2-deoxyglucose induces ER stress *in vitro* by antagonizing N-linked glycosylation, an important posttranslational modification required for proper protein folding (17).

ER stress also is induced by many physiologic conditions or insults that overwhelm the folding/secretory capacity of the ER. Viruses exploit the host cell's translational machinery to synthesize their viral proteins, leading to increased folding pressure on the ER and chaperone upregulation (66). Differentiation of B cells to antibody-producing plasma cells activates the UPR to accommodate the dramatic increase in the expression of IgG (30).

To relieve the accumulation of misfolded/aggregated proteins and reduce ER stress, the cell initiates the activation of a series of integrated stress pathways collectively known as the unfolded protein response (UPR). The UPR consists of three mechanistically distinct but integrated pathways that include the IRE1, ATF6, and PERK pathways. One of the first responses to ER stress involves activation of the PERK pathway. PERK is an ER transmembrane sensor bound to GRP78 under nonstressed conditions (5). With ER stress, GRP78 dissociates from PERK to bind the accumulating unfolded proteins in the ER lumen (5). After its dissociation from GRP78, PERK phosphorylates the α subunit of the eukaryotic translation-initiation factor 2 (eIF2 α), thereby leading to the attenuation of general protein translation (60). This prevents any further increase in ER protein load during the initial stages of ER stress. Although eIF2 α phosphorylation attenuates translation in a global manner, transcription and translation of ER chaperones such as GRP78 and GADD153/CHOP can occur through translation of the transcription factor ATF4 (23, 60). Similar to PERK, ATF6 is an ER transmembrane-bound transcription factor with its ER luminal domain bound to GRP78 under nonstressed conditions (26, 41). Dissociation of GRP78 under ER-stress conditions releases ATF6 from the ER, allowing it to transit to the Golgi, where it is cleaved by site-1 and site-2 proteases (S1P and S2P) (76). Cleavage of the transcriptionally active domain of ATF6 from the Golgi membrane allows it to migrate to the nucleus, where it binds to the promoter of UPR-inducible genes, specifically ER chaperones and XBP-1 (33, 78).

The final pathway activated with ER stress is the IRE1 pathway. Similar to the PERK and ATF6 arms of the UPR, IRE1 is an ER transmembrane sensor protein that is bound to GRP78 under nonstressed conditions (5). After its dissociation from GRP78, activated IRE1 exhibits endoribonuclease activity, resulting in unconventional splicing and subsequent translation of the mRNA encoding X-box-binding protein (XBP1). The removal of a 26-bp intron from the XBP1 message by IRE1 produces an active XBP1 transcription factor that drives the expression of several ER chaperones, including GRP78.

Although the UPR is orchestrated to alleviate ER stress, severe or prolonged ER stress can lead to apoptotic cell death. One mechanism by which ER stress can induce cell death is through activated IRE1, which recruits TRAF2 to the ER membrane (70). TRAF2 activates apoptosis-signaling kinase 1 (ASK-1), which in turn activates JNK and caspases in a mitochondria-mediated manner (70). Recruitment of TRAF2 to the ER membrane by IRE1 also releases TRAF2 from procaspase 12 in the cytosol, thus allowing the active form of caspase 12 to activate downstream executioner caspases (77). Furthermore, release of ER Ca^{2+} stores can activate calpain, which in turn activates procaspase 12 and other caspases, leading to cell death (51).

ER stress also can induce death through the PERK- and IRE1-mediated activation of proapoptotic proteins, specifically the growth arrest-DNA damage gene (GADD153/CHOP), which can induce apoptosis by decreasing Bcl-2 expression (41, 58). Recent studies by our group have shown that ER stress can induce the expression of TDAG51 (27), a member of the pleckstrin homology-related domain family having proapoptotic characteristics. Overexpression of TDAG51 leads to dramatic alterations in cell shape and increases detachment of cells from their appropriate matrix (27), supporting a mechanism involving detachment-induced PCD or anoikis. The observation that TDAG51-induced shape changes are independent of caspase activation and occur prior to apoptosis is consistent with this mechanism. Therefore, both caspase-dependent and -independent pathways of cell death are activated in response to prolonged or severe ER stress.

Cytoprotective effects of the ER chaperone GRP78

Although GRP78 plays a critical role in ER protein quality control and regulation of the activation of the ER transmembrane signaling molecules, it also confers protection against cytotoxicity and apoptosis (36, 55, 72). GRP78 overexpression protects cells from injury induced by increases in intracellular Ca^{2+} or oxidative stress by sequestering Ca^{2+} and in turn inhibiting oxidant-induced increases in intracellular Ca^{2+} (1, 36). GRP78 has also been shown to be protective against apoptotic cell death induced by topoisomerase inhibitors such as etoposide, doxorubicin (Adriamycin), and camptothecin (55).

A proportion of GRP78 was found to be expressed on the cytosolic side of the ER membrane in a complex with procaspase 7, suggesting that GRP78 exerts its antiapoptotic effects through the sequestration of caspase 7 and thus the prevention of further caspase activation and caspase-mediated cell death (55). Caspase 12 also may be involved, because it has been identified in complex with GRP78 and caspase 7 (54). That deletion of the ATP-binding domain of GRP78 prevents complex formation between procaspase 7

and GRP78 suggests that binding ATP is essential for its antiapoptotic properties (55).

GRP78 also has been shown in a cell-free system to inhibit cytochrome *c*-mediated caspase activation and subsequent cell death (54). An interaction between GRP78 and cytochrome *c* is postulated to occur in the cytosol, based on observations that after prolonged ER stress, GRP78 is redistributed from the ER lumen to the ER transmembrane and the cytosol.

The cytoprotective property of GRP78 also has been demonstrated to contribute to tumor survival and chemotherapeutic drug resistance (15, 72). The tumor microenvironment of hypoxia and glucose starvation induces UPR activation and the subsequent upregulation of GRP78. This provides an antiapoptotic benefit to tumor cells, which contributes to resistance to chemotherapeutic drugs such as etoposide (15).

ER luminal versus cell-surface GRP78

GRP78 is sequestered in the ER lumen by its C-terminal KDEL (lys-asg-glu-leu) sequence, an ER-retention signal that binds to the KDEL receptor. However, GRP78 as well as other KDEL-containing chaperones, including PDI and calreticulin, have been identified on the surface of several human cancers, including prostate and breast cancer (2, 22, 72). GRP78 also associates with the cytosolic surface of the ER membrane, suggesting that the structure of GRP78 is consistent with that of a transmembrane protein (55). However, it is unclear as to how GRP78 is orientated in the plasma membrane; whether it is a true integral membrane protein or whether its cell-surface expression is dependent on its association with other cell-surface proteins.

It also remains unclear as to how GRP78 (and other ER chaperones) escapes ER retrieval mechanisms mediated by the KDEL sequence, especially given that at least one study has demonstrated that GRP78 retains its KDEL sequence when expressed on the cell surface (55). Two possible explanations are that the KDEL receptors on the ER membrane are saturated (possible during ER stress and GRP78 upregulation) or that the KDEL signal on GRP78 is masked by its interaction with other proteins such as MTJ-1 (49).

Physiologic functions of cell-surface GRP78

Although the chaperone function of GRP78 is well characterized, current studies indicate that the physiologic function of cell surface GRP78 appears to be that of a receptor (22). GRP78 can associate with MHC class I molecules on the cell surface and acts as a co-receptor for virus internalization (69). Absence of MHC class I molecules increases GRP78 expression, thereby compensating for the loss of MHC molecules and suggesting that GRP78 may represent a unique antigen-presentation system (69). Cell-surface GRP78 also is the receptor for α_2 -macroglobulin ($\alpha_2\text{M}^*$), mediating its downstream signaling and regulating cell-survival/cell-death pathways (47–49). Binding of $\alpha_2\text{M}^*$ to cell-surface GRP78 regulates the Ras-dependent MAPK and PI3 kinase pathways, leading to increases in cell proliferation, IP_3 synthesis, intracellular Ca^{2+} levels, and expression of the docking proteins Grb2, Shc, and Sos (48). Binding of $\alpha_2\text{M}^*$ to GRP78 also mediates macrophage motility through the recruitment of p21-activated protein kinase-2 (PAK-2) to the plasma membrane *via* the adaptor protein NCK. PAK-2 is activated by tyrosine phosphorylation

(49). PAK-2 activation leads to LIM kinase activation, which in turn phosphorylates cofilin, an actin-binding protein, and inhibits its depolymerization, thus regulating actin assembly and motility (49). Cell-surface GRP78 also was shown to act as a receptor for kringle 5 of plasminogen and to mediate its antiangiogenic properties by inducing apoptosis of proliferating endothelial cells (12). Cell-surface GRP78 is also a major autoantigen in several forms of cancer, including prostate, ovarian, and gastric cancer (22). Recent studies suggested that the effects of anti-GRP78 IgG on tumor-cell proliferation, survival, or apoptosis is dependent on the epitope binding domain. In general, autoantibodies directed against the N-terminal region of GRP78 enhance cell survival and proliferation, whereas C-terminal-specific antibodies inhibit cellular proliferation and induce apoptosis (21). In addition to its effects as a receptor, a role for cell-surface GRP78 in thrombosis has been reported (6, 73). This study identifies GRP78 on the cell surface and demonstrates that anti-GRP78 antibodies can increase TF procoagulant activity (6). Although no specific mechanism was addressed, a direct interaction between GRP78 and TF exists on the cell surface was proposed (6). These results support previous findings that GRP78 plays an important role in the regulation of TF PCA (73) and provides evidence that cell-surface GRP78 may affect TF directly. Together these findings demonstrate that cell-surface GRP78 displays unique topology that allows it to interact with and regulate a diverse group of signaling pathways and surface proteins that control the balance between cell proliferation and cell death.

ROS Production, Inflammation, and the UPR

Unlike the cytosol, the ER contains a unique oxidizing environment that allows the formation of intra- and interchain disulfide bonds critical for the correct folding and assembly of nascent polypeptides. The formation or rearrangement of these disulfide bonds involves a cascade of disulfide-bond transfers that are catalyzed by protein disulfide isomerase (PDI) and ER oxidoreductase 1 (ERO1), with molecular oxygen serving as the terminal electron acceptor (63). Recent studies suggested that ROS generated as a byproduct of disulfide bond formation in the ER cause oxidative stress and contribute to apoptotic cell death (24, 42, 43). As a response to the increase in oxidative stress, a UPR-mediated integrated stress response involving the Perk pathway is activated to preserve cell function and survival (24). Activation of the Perk pathway by the accumulation of misfolded proteins in the ER, amino acid starvation, or oxidants leads to the phosphorylation of eIF2 α . This inhibits general protein synthesis while promoting translation and expression of ATF4, a transcription factor that regulates amino acid import, glutathione biosynthesis, and resistance to oxidative stress. Thus, lack of ATF4 results in reduced GSH levels, accumulation of ROS, and apoptotic cell death (24).

Furthermore, ATF4-deficiency significantly reduces cystathionine γ -lyase (CGL) protein expression, a critical enzyme in the transsulfuration pathway that regulates cysteine synthesis (Austin and Dickhout, unpublished data). Supplementation of the media with high-dose amino acids necessary for GSH synthesis showed cytoprotection by decreasing ROS levels. Consistent with these studies, Malhotra and colleagues (43) demonstrated that antioxidant treatment in CHO cells

overexpressing factor VIII (coagulation factor sensitive to misfolding) reduces UPR activation, oxidative stress, and apoptosis, as well as increasing factor VIII secretion *in vitro* and *in vivo*. These studies imply that persistent oxidative stress and protein misfolding act synergistically to increase apoptotic cell death and tissue damage that occurs in several human diseases such as diabetes, neurologic disorders, and atherosclerosis. Thus, antioxidant treatment may improve the clinical outcome in patients with congenital diseases associated with protein misfolding (81).

In addition to ROS production, ER stress can cause activation of nuclear factor- κ B (NF- κ B), a key transcription factor that regulates inflammation (32). Recent studies also demonstrated that ER stress causes a biphasic, bidirectional regulation of NF- κ B that can modulate the expression of inflammation-associated genes encoding cytokines, chemokines, adhesion receptors, and various enzymes, leading to initiation, amplification, and perpetuation of inflammatory responses. Given the importance of the inflammatory process in a host of human disease, such as atherosclerosis and cancer, future experimentation will likely focus on the bidirectional roles of ER stress in the inflammatory processes so that approaches to suppress the proinflammatory response can be developed.

UPR and Human Disease

Many human diseases, including cancer (72), atherosclerosis (67), diabetes (31), neurologic disorders (72), and renal disease (14), are associated with ER stress and UPR activation (Fig. 1), prompting the hypothesis that ER stress contributes to the pathology of these diseases. This is particularly relevant in the development and progression of atherosclerosis, which is highlighted below.

UPR and atherosclerosis

UPR activation has been reported during all stages of atherosclerosis (82), and pathophysiologic conditions known to induce ER stress contribute to lesion development, plaque rupture, and thrombogenicity (67). Furthermore, a significant correlation between ER stress markers, plaque vulnerability, and lesion apoptosis was reported in human coronary artery plaques (50). Peroxynitrite, a byproduct of the reaction between nitric oxide (NO) and superoxide, elicits ER stress in cultured endothelial cells, resulting in cell dysfunction and apoptosis (13). Several cardiovascular risk factors, including hyperhomocysteinemia (HHcy), diabetes, and hyperlipidemia, are known to enhance the intracellular production of superoxide, which is rapidly scavenged by NO to form peroxynitrite. Recent studies demonstrated that HHcy increases superoxide production in atherosclerotic lesions from apoE^{-/-} mice (75), thereby contributing to peroxynitrite formation. The fact that peroxynitrite induces ER stress and colocalizes with UPR markers in atherosclerotic lesions from apoE^{-/-} mice (13) suggests a role for UPR-induced lesion development and plaque necrosis. In support of this concept, Tabas and colleagues (18) reported that the ER is the site of free cholesterol-induced cytotoxicity in cultured macrophages. The additional observations that cholesterol-induced apoptotic cell death was attenuated in CHOP/GADD153-deficient macrophages (18) and CHOP/GADD153 was markedly elevated in advanced atherosclerotic lesions

from apoE^{-/-} mice (82) suggests that activation of the CHOP/GADD153 arm of the UPR is a key signaling step in cholesterol-induced apoptosis in macrophages. This is supported by recent studies showing reduced apoptosis and plaque necrosis in advanced atherosclerotic lesions from apoE-deficient or LDL receptor-deficient mice lacking CHOP/GADD153 (68). These findings suggest that therapeutic approaches that target the UPR may have beneficial effects on reducing atherosclerotic lesion progression and rupture.

Potential strategies aimed at reducing human diseases by blocking ER stress and UPR activation

Numerous reports support an important role for ER stress and UPR activation in the pathogenesis of a wide range of human diseases, including diabetes, obesity, renal disease, cancer, atherosclerosis, and neurologic disorders. This suggests that ER stress represents a unifying mechanism that contributes to a number of different human disease states and provides a unique opportunity for the development of novel therapeutic approaches aimed at reducing the effects of ER stress. Small chemical chaperones, such as 4-PBA and TUDCA, have been used successfully to reduce the effects of ER stress in mouse models of obesity (4), diabetes (52), and Alzheimer disease (56). Based on these findings, it is possible that the use of these chemical chaperones or their derivatives may provide a unique approach to treating congenital diseases resulting from the improper folding and processing of target proteins. This would provide several potential benefits: it could assist in proper folding, thereby rescuing protein function, and decrease the adverse effects of ER stress in the target tissue due to the accumulation of misfolded proteins.

Cell preconditioning with activators of the UPR has been examined as a potential protective approach against several diseases. Preconditioning with several ER stress-inducing agents/conditions, including ischemia, tunicamycin, or DTTox protects cultured renal epithelial cells, cardiomyocytes, or neurons from subsequent oxidative stress, ischemia/reperfusion, and nephrotoxins (25, 28, 37, 53, 80). *In vivo* studies have demonstrated that preconditioning with tunicamycin or thapsigargin ameliorates passive Heymann nephritis (11) and mesangioproliferative glomerulonephritis (29) in rats. Furthermore, induction of GRP78 by ischemic preconditioning was shown to reduce ER stress and delay neuronal cell death (25). Ischemic postconditioning also protects the heart from ischemia/reperfusion injury (37). Although it is still at an early stage for potential therapeutic use, alteration in UPR activation has emerged as a promising and novel approach for reducing disease progression.

Conclusions and Future Perspectives

Recent groundbreaking studies provide compelling evidence that ER stress and UPR activation contribute to a wide range of human diseases. Components of the UPR as well as cellular factors that regulate the UPR are expressed in a cell- and tissue-type-specific manner. It is well established that uncontrolled ER stress induces UPR-mediated apoptotic cell death and tissue damage. However, a detailed understanding of the cellular and molecular events controlled by each arm of the UPR and their contribution to the disease process is lacking. This is based on recent studies indicating that differential activation of the PERK and IRE1 pathways may de-

termine life-or-death decisions after ER stress (35). Future studies will be designed to evaluate the contribution of these UPR pathways to other cellular stress responses and how they affect disease progression. Furthermore, the development of mouse models having tissue-specific deletions in specific components of the UPR should yield important information on disease progression that is more relevant to the human condition. The discovery of cell-surface GRP78 in cancer cells, as well as in cells from pathologic states, suggests additional novel functions distinct from chaperone activity that affect disease development and progression. Ultimately, future investigations will provide a solid foundation for the development of novel inhibitors of ER stress or activators of the UPR or both that modulate a variety of different human disease and their complications.

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References

1. Aoki M, Tamatani M, Taniguchi M, Yamaguchi A, Bando Y, Kasai K, Miyoshi Y, Nakamura Y, Vitek MP, Tohyama M, Tanaka H, and Sugimoto H. Hypothermic treatment restores glucose regulated protein 78 (GRP78) expression in ischemic brain. *Mol Brain Res* 95: 117–128, 2001.
2. Arap MA, Lahdenranta J, Mintz PJ, Hajitou A, Sarkis AS, Arap W, and Pasqualini R. Cell surface expression of the stress response chaperone GRP78 enables tumor targeting by circulating ligands. *Cancer Cell* 6: 275–284, 2004.
3. Barlow C. Signals for the COPII-dependent export from the ER: what's the ticket out? *Trends Cell Biol* 13: 295–300, 2003.
4. Basseri S, Lhotak S, Sharma AM, and Austin RC. The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response. *J. Lipid Res* 2009 May 21. [Epub ahead of print]
5. Bertolotti A, Zhang Y, Hendershot LM, Harding HP, and Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nature Cell Biol* 2: 326–332, 2000.
6. Bhattacharjee G, Ahamed J, Pedersen B, El-Sheikh A, Mackman N, Ruf W, Liu C, and Edgington TS. Regulation of tissue factor-mediated initiation of the coagulation cascade by cell surface GRP78. *Arterioscler Thromb Vasc Biol* 25: 1737–1743, 2005.
7. Chakravarthy S, Jessop CE, and Bulleid NJ. The role of glutathione in disulphide bond formation and endoplasmic-reticulum-generated oxidative stress. *EMBO Rep* 7: 271–275, 2006.
8. Chevalier M, Rhee H, Elguindi EC, and Blond SY. Interaction of murine BiP/GRP78 with DnaJ homologue MTJ1. *J Biol Chem* 275: 19620–19627, 2000.
9. Chung KT, Shen Y, and Hendershot LM. BAP, a mammalian BiP-associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP. *J Biol Chem* 277: 47557–47563, 2002.

10. Cunnea PM, Miranda-Vizuet A, Bertoli G, Simmen T, Damdimopoulos AE, Hermann S, Leinonen S, Huikko MP, Gustafsson J-A, Sitia R, and Spyrou G. ERdj5, an endoplasmic reticulum (ER)-resident protein containing DnaJ and thioredoxin domains, is expressed in secretory cells or following ER stress. *J Biol Chem* 278: 1059–1066, 2002.
11. Cybulsky AV, Takano T, Papillon J, Khadir A, Liu J, and Peng H. Complement C5b-9 membrane attack complex increases expression of endoplasmic reticulum stress proteins in glomerular epithelial cells. *J Biol Chem* 277: 41342–41351, 2002.
12. Davidson DJ, Haskell C, Majest S, Kherzai A, Ega DA, Walter KA, Schneider A, Gubbins EF, Solomon L, Chen Z, Lesniewski R, and Henkin J. Kringle 5 of human plasminogen induces apoptosis of endothelial and tumor cells through surface-expressed glucose-regulated protein 78. *Cancer Res* 65: 4663–4672, 2005.
13. Dickhout JG, Hossain GS, Pozza LM, Zhou J, Lhoták S, and Austin RC. Peroxynitrite causes ER stress and apoptosis in human vascular endothelium: implications in atherogenesis. *Arterioscler Thromb Vasc Biol* 25: 2623–2629, 2005.
14. Dickhout JG and Krepinsky JC. Endoplasmic reticulum stress and renal disease. *Antioxid Redox Signal* 11: 2341–2352, 2009.
15. Dong D, Ko B, Baumeiste P, Swenson S, Costa F, Markland F, Stiles C, Paterson JB, Bates SE, and Lee AS. Vascular targeting and antiangiogenesis agents induce drug resistance effector GRP78 within the tumor microenvironment. *Cancer Res* 65: 5785–5791, 2005.
16. Dorner AJ, Bole DG, and Kaufman RJ. The relationship of N-linked glycosylation and heavy chain-binding protein association with the secretion of glycoproteins. *J Cell Biol* 105: 2665–2674, 1987.
17. Dorner AJ, Wasley LC, and Kaufman RJ. Overexpression of GRP78 mitigates stress induction of glucose regulated proteins and blocks secretion of selective proteins in Chinese hamster ovary cells. *EMBO J* 11: 1563–1571, 1992.
18. Feng B, Yao PM, Li Y, Devlin CM, Zhang D, Harding HP, Sweeney M, Rong JX, Kuriakose G, Fisher EA, Marks AR, Ron D, and Tabas I. The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages. *Nat Cell Biol* 5: 781–792, 2003.
19. Flynn GC, Pohl J, Flocco MT, and Rothman JE. Peptide-binding specificity of the molecular chaperone BiP. *Nature* 353: 726–730, 1991.
20. Gething M-J. Role and regulation of the ER chaperone BiP. *Semin Cell Dev Biol* 10: 465–472, 1999.
21. Gonzalez-Gronow M, Cuchacovich M, Llanos C, Urzua C, Gawdi G, and Pizzo SV. Prostate cancer cell proliferation in vitro is modulated by antibodies against glucose-regulated protein 78 isolated from patient serum. *Cancer Res* 66: 11424–11431, 2006.
22. Gonzalez-Gronow M, Selim MA, Papalas J, and Pizzo SV. GRP78: a multifunctional receptor on the cell surface. *Antioxid Redox Signal* 11: 2299–2306, 2009.
23. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, and Ron D. Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol Cell* 6: 1099–1108, 2000.
24. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calton M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, and Ron D. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* 11: 619–633, 2003.
25. Hayashi T, Saito A, Okuno S, Ferrand-Drake M, and Chan PH. Induction of GRP78 by ischemic preconditioning reduces endoplasmic reticulum stress and prevents delayed neuronal cell death. *J Cereb Blood Flow Metab* 23: 949–961, 2003.
26. Haze K, Yoshida H, Yanagi H, Yura T, and Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol Biol Cell* 10: 3787–3799, 1999.
27. Hossain GS, van Thienen JV, Werstuck GH, Zhou J, Sood SK, Dickhout JG, de Koning AB, Tang D, Wu D, Falk E, Poddar R, Jacobsen DW, Zhang K, Kaufman RJ, and Austin RC. TDAG51 is induced by homocysteine, promotes detachment-mediated programmed cell death, and contributes to the development of atherosclerosis in hyperhomocysteinemia. *J Biol Chem* 278: 30317–30327, 2003.
28. Hung C-C, Ichimura T, Stevens JL, and Bonventre JV. Protection of renal epithelial cells against oxidative stress injury by endoplasmic reticulum stress preconditioning is mediated by ERK1/2 activation. *J Biol Chem* 278: 29317–29326, 2003.
29. Inagi R, Kumagai T, Nishi H, Kawakami T, Miyata T, Fujita T, Nangaku M, Inagi R, Kumagai T, Nishi H, Kawakami T, Miyata T, Fujita T, and Nangaku M. Preconditioning with endoplasmic reticulum stress ameliorates mesangioproliferative glomerulonephritis. *J Am Soc Nephrol* 19: 915–922, 2008.
30. Iwakoshi NN, Lee AH, Vallabhajosyula P, Otipoby KL, Rajewsky K, and Glimcher LH. Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. *Nature Immunol* 4: 321–329, 2003.
31. Khan MI, Pichna BA, Shi Y, Bowes AJ, and Werstuck GH. Evidence supporting a role for endoplasmic reticulum stress in the development of atherosclerosis in a hyperglycaemic mouse model. *Antioxid Redox Signal* 11: 2289–2298, 2009.
32. Kitamura M. Biphasic, bidirectional regulation of NF- κ B by endoplasmic reticulum stress. *Antioxid Redox Signal* 11: 2353–2364, 2009.
33. Lee K, Tirasophon W, Shen X, Michalak M, Prywes R, Okada T, Yoshida H, Mori K, and Kaufman RJ. IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. *Genes Dev* 16: 452–466, 2002.
34. Lievreumont J-P, Rizzuto R, Hendershot L, and Meldolesi J. BiP, a major chaperone protein of the endoplasmic reticulum lumen, plays a direct and important role in the storage of the rapidly exchanging pool of Ca^{2+} . *J Biol Chem* 272: 30873–30879, 1997.
35. Lin JH, Li H, Zhang Y, Ron D, and Walter P. Divergent effects of PERK and IRE1 signaling on cell viability. *PLoS ONE* 4: e4170, 2009.
36. Liu H, Miller E, van de Water B, and Stevens JL. Endoplasmic reticulum chaperones GRP78 and calreticulin prevent oxidative stress, Ca^{2+} disturbances, and cell death in renal epithelial cells. *J Biol Chem* 273: 12858–12862, 1998.
37. Liu XH, Zhang ZY, Sun S, and Wu XD. Ischemic preconditioning protects myocardium from ischemia/reperfusion injury through attenuating endoplasmic reticulum stress. *Shock* 30: 422–427, 2008.
38. Lodish HF and Kong N. Perturbation of cellular calcium blocks exit of secretory proteins from the rough endoplasmic reticulum. *J Biol Chem* 265: 10893–10899, 1990.
39. Lodish HF and Kong N. The secretory pathway is normal in dithiothreitol-treated cells, but disulfide-bonded proteins are reduced and reversibly retained in the endoplasmic reticulum. *J Biol Chem* 268: 20598–20695, 1993.

40. Lucero HA, Lebeche D, and Kaminer B. ERcalcistorin/protein disulfide isomerase (PDI): sequence determination and expression of a cDNA clone encoding a calcium storage protein with PDI activity from endoplasmic reticulum of the sea urchin egg. *J Biol Chem* 269: 23112–23119, 1994.
41. Ma Y and Hendershot LM. The role of the unfolded protein response in tumour development: friends or foe? *Nature* 4: 966–977, 2004.
42. Malhotra JD and Kaufman RJ. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid Redox Signal* 9: 2277–2293, 2007.
43. Malhotra JD, Miao H, Zhang K, Wolfson A, Pennathur S, Pipe SW, and Kaufman RJ. Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. *Proc Natl Acad Sci U S A* 105: 18525–18530, 2008.
44. Meerovitch K, Wing S, and Goltzman D. Parathyroid hormone-related protein is associated with the chaperone protein BiP and undergoes proteasome-mediated degradation. *J Biol Chem* 273: 21025–21030, 1998.
45. Meunier L, Usherwood YK, Chung KT, and Hendershot LM. A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. *Mol Biol Cell* 13: 4456–4469, 2002.
46. Michalak M, Corbett EF, Mesaeli N, Nakamura K, and Opas M. Calreticulin: one protein, one gene, many functions. *Biochem J* 344: 281–292, 1999.
47. Misra UK, Gonzalez-Gronow M, Gawdi G, Hart JP, Johnson CE, and Pizzo SV. The role of Grp78 in α_2 -macroglobulin-induced signal transduction. *J Biol Chem* 277: 42082–42087, 2002.
48. Misra UK, Gonzalez-Gronow M, Gawdi G, Wang F, and Pizzo SV. A novel receptor function for the heat shock protein Grp78: silencing of Grp78 gene expression attenuates α_2 M*-induced signaling. *Cell Signal* 16: 929–938, 2004.
49. Misra UK, Sharma T, and Pizzo SV. Ligation of cell surface-associated glucose-regulated protein 78 by receptor-recognized forms of α_2 -macroglobulin: activation of p21-activated protein kinase-2-dependent signaling in murine peritoneal macrophages. *J Immunol* 175: 2525–2533, 2005.
50. Myoishi M, Hao H, Minamino T, Watanabe K, Nishihira K, Hatakeyama K, Asada Y, Okada K, Ishibashi-Ueda H, Gabbiani G, Bochaton-Piallat ML, Mochizuki N, and Kitakaze M. Increased endoplasmic reticulum stress in atherosclerotic plaques associated with acute coronary syndrome. *Circulation* 116: 1226–1233, 2007.
51. Nakagawa T and Yuan J. Cross-talk between two cysteine protease families: activation of caspase-12 by calpain in apoptosis. *J Cell Biol* 150: 887–894, 2000.
52. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, and Hotamisligil GS. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* 313: 1137–1140, 2006.
53. Peyrou M and Cribb AE. Effect of endoplasmic reticulum stress preconditioning on cytotoxicity of clinically relevant nephrotoxins in renal cell lines. *Toxicol In Vitro* 21: 878–886, 2007.
54. Rao RV, Peel A, Logvinova A, del Rio G, Hermel E, Yokota T, Goldsmith PC, Ellerby LM, Ellerby HM, and Bredesen DE. Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. *FEBS Lett* 514: 122–128, 2002.
55. Reddy RK, Mao C, Baumeister P, Austin RC, Kaufman RJ, and Lee AS. Endoplasmic reticulum chaperone protein GRP78 protects cells from apoptosis induced by topoisomerase inhibitors: role of ATP binding site in suppression of caspase-7 activation. *J Biol Chem* 278: 20915–20924, 2003.
56. Ricobaraza A, Cuadrado-Teledor M, Perez-Mediavilla A, Frechilla D, Del Rio J, and Garcia-Osta A. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in Alzheimer's disease mouse model. *Neuropsychopharmacology* 34: 1721–1732, 2009.
57. Rizzuto R, Pinton P, Ferrari D, Chami M, Szabadkai G, Magalhaes PJ, Di Virgilio F, and Pozzan T. Calcium and apoptosis: facts and hypothesis. *Oncogene* 22: 8619–8627, 2003.
58. Ron D and Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 8: 519–529, 2007.
59. Ruddon RW and Bedows E. Assisted protein folding. *J Biol Chem* 272: 3125–3128, 1997.
60. Scheuner D, Song B, McEwen E, Liu C, Laybutt R, Gillespie P, Saunders T, Bonner-Weir S, and Kaufman RJ. Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Mol Cell* 7: 1165–1176, 2001.
61. Shen Y and Hendershot LM. ERdj3, a stress-inducible endoplasmic reticulum DnaJ homologue, serves as a cofactor for BiP's interactions with unfolded substrates. *Mol Biol Cell* 16: 40–50, 2005.
62. Shen Y, Meunier L, and Hendershot LM. Identification and characterization of a novel endoplasmic reticulum (ER) DnaJ homologue, which stimulates ATPase activity of BiP *in vitro* and is induced by ER stress. *J Biol Chem* 277: 15947–15956, 2002.
63. Shimizu Y and Hendershot LM. Oxidative folding: cellular strategies for dealing with the resultant equimolar production of reactive oxygen species. *Antioxid Redox Signal* 11: 2317–2331, 2009.
64. Sitia R and Braakman I. Quality control in the endoplasmic reticulum protein factory. *Nature* 426: 891–894, 2003.
65. Spiro RG, Zhu Q, Bhoyroo V, and Soling HD. Definition of the lectin-like properties of the molecular chaperone, calreticulin, and demonstration of its copurification with endomannosidase from rat liver Golgi. *J Biol Chem* 271: 11588–11594, 1996.
66. Su HL, Liao CL, and Lin YL. Japanese encephalitis virus infection initiated endoplasmic reticulum stress and an unfolded protein response. *J Virol* 76: 4162–4171, 2002.
67. Tabas I. Macrophage apoptosis in atherosclerosis: consequences of plaque progression and the role of endoplasmic reticulum stress. *Antioxid Redox Signal* 11: 2333–2339, 2009.
68. Thorp E, Li G, Seimon TA, Kuriakose G, Ron D, and Tabas I. Reduced apoptosis and plaque necrosis in advanced atherosclerotic lesions of Apo^{-/-} and Ldlr^{-/-} mice lacking CHOP. *Cell Metab* 9: 474–481, 2009.
69. Triantafilou M, Fradelizi D, and Triantafilou K. Major histocompatibility class one molecule associates with glucose regulated protein (GRP) 78 on the cell surface. *Human Immunol* 62: 764–770, 2001.
70. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, and Ron D. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287: 664–666, 2000.
71. Vassilakos A, Michalak M, Lehrman MA, and Williams DB. Oligosaccharide binding characteristics of the molecular chaperones calnexin and calreticulin. *Biochemistry* 37: 3480–3490, 1998.

72. Wang M, Wey S, Zhang Y, Ye R, and Lee AS. Role of the unfolded protein response regulator GRP78/BiP in development, cancer and neurological disorders. *Antioxid Redox Signal* 11: 2307–2316, 2009.
73. Watson LM, Chan AKC, Berry LR, Li J, Sood SK, Dickhout JG, Xu L, Werstuck GH, Bajzar L, Klamut HJ, and Austin RC. Overexpression of the 78-kDa glucose-regulated protein/immunoglobulin-binding protein (GRP78/BiP) inhibits tissue factor procoagulant activity. *J Biol Chem* 278: 17438–17447, 2003.
74. Wiertz EJ, Tortorella D, Bogoy M, Yu J, Mothes W, Jones TR, Rapoport TA, and Ploegh HL. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. *Nature* 384: 432–438, 1996.
75. Wilson KM, McCaw RB, Leo L, Arning E, Lhotak S, Bottiglieri T, Austin RC, and Lentz SR. Prothrombotic effects of hyperhomocysteinemia and hypercholesterolemia in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 27: 233–240, 2007.
76. Ye J, Rawson RB, Komuro R, Chen X, Dave UP, Prywes R, Brown MS, and Goldstein JL. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol Cell* 6: 1355–1364, 2000.
77. Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, and Tohyama M. Activation of caspase-12, an endoplasmic reticulum (ER) resident caspase, through tumor necrosis factor-associated factor 2-dependent mechanism in response to the ER stress. *J Biol Chem* 276: 13935–13940, 2001.
78. Yoshida H, Matsui T, Yamamoto A, Okada T, and Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107: 881–891, 2001.
79. Yu Z, Luo H, Fu W, and Mattson MP. The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: suppression of oxidative stress and stabilization of calcium homeostasis. *Exp Neurol* 155: 302–314, 1999.
80. Zhang PL, Lun M, Teng J, Huang J, Blasick TM, Yin L, Herrera GA, and Cheung JY. Preinduced molecular chaperones in the endoplasmic reticulum protect cardiomyocytes from lethal injury. *Ann Clin Lab Sci* 34: 449–457, 2004.
81. Zhao L, Ackerman SL. Endoplasmic reticulum stress in health and disease. *Curr Opin Cell Biol* 18: 444–452, 2006.
82. Zhou J, Lhotak S, Hilditch BA, and Austin RC. Activation of the unfolded protein response (UPR) is an early event in atherosclerotic lesion development in apoE-deficient mice. *Circulation* 111: 1814–1821, 2005.

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Abbreviations Used

α_2 M	= α_2 -macroglobulin
ASK-1	= apoptosis-signaling kinase 1
ATF	= activated transcription factor
ATP	= adenosine triphosphate
BAP	= BiP-associated protein
CGL	= cystathionine γ -lyase
CHOP	= C/EBP homologous protein
COP	= coatomer protein
DTT	= dithiothreitol
eIF2 α	= eukaryotic translation-initiation factor 2 alpha
ER	= endoplasmic reticulum
ERAD	= ER-associated degradation
ERO1	= ER oxidoreductase 1
GRP78	= glucose-regulated protein-78
GRP94	= glucose-regulated protein-94
GSH	= reduced glutathione
H ₂ O ₂	= hydrogen peroxide
IgG	= immunoglobulin
IRE1	= inositol-requiring protein 1
MEFs	= mouse embryonic fibroblasts
NF- κ B	= nuclear factor- κ B
NO	= nitric oxide
PAK-2	= protein kinase 2
4-PBA	= 4-phenylbutyrate
PDI	= protein disulfide isomerase
PERK	= PKR-like ER kinase
ROS	= reactive oxygen species
TDAG51	= T-cell death-associated gene 51
TUDCA	= tauroursodeoxycholic acid
UPR	= unfolded protein response
VPA	= valproic acid
XBP1	= X-box-binding protein

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1. Bhagwat V. Alapure, Jaime K. Stull, Zeynep Firtina, Melinda K. Duncan. 2012. The unfolded protein response is activated in connexin 50 mutant mouse lenses. *Experimental Eye Research* **102**, 28-37. [[CrossRef](#)]
2. Peter J. Belmont, Wenqiong J. Chen, Donna J. Thuerauf, Christopher C. Glembotski. 2012. Regulation of microRNA expression in the heart by the ATF6 branch of the ER stress response. *Journal of Molecular and Cellular Cardiology* **52**:5, 1176-1182. [[CrossRef](#)]
3. Martin Lotz, Richard F. Loeser. 2012. Effects of aging on articular cartilage homeostasis. *Bone* . [[CrossRef](#)]
4. Sana Basseri, Richard C. Austin. 2012. Endoplasmic Reticulum Stress and Lipid Metabolism: Mechanisms and Therapeutic Potential. *Biochemistry Research International* **2012**, 1-13. [[CrossRef](#)]
5. James J. Galligan, Rebecca L. Smathers, Colin T. Shearn, Kristofer S. Fritz, Donald S. Backos, Hua Jiang, Christopher C. Franklin, David J. Orlicky, Kenneth N. MacLean, Dennis R. Petersen. 2012. Oxidative Stress and the ER Stress Response in a Murine Model for Early-Stage Alcoholic Liver Disease. *Journal of Toxicology* **2012**, 1-12. [[CrossRef](#)]
6. I.A. Lian, M. Løset, S.B. Mundal, M.H. Fenstad, M.P. Johnson, I.P. Eide, L. Bjørge, K.A. Freed, E.K. Moses, R. Austgulen. 2011. Increased endoplasmic reticulum stress in decidual tissue from pregnancies complicated by fetal growth restriction with and without pre-eclampsia. *Placenta* . [[CrossRef](#)]
7. James W. Ramadan, Stephen R. Steiner, Christina M. O'Neill, Craig S. Nunemaker. 2011. The central role of calcium in the effects of cytokines on beta-cell function: Implications for type 1 and type 2 diabetes. *Cell Calcium* . [[CrossRef](#)]
8. Martin K. Lotz, Beatriz Caramés. 2011. Autophagy and cartilage homeostasis mechanisms in joint health, aging and OA. *Nature Reviews Rheumatology* **7**:10, 579-587. [[CrossRef](#)]
9. William L. Corwin, John M. Baust, John G. Baust, Robert G. Van Buskirk. 2011. The unfolded protein response in human corneal endothelial cells following hypothermic storage: Implications of a novel stress pathway. *Cryobiology* **63**:1, 46-55. [[CrossRef](#)]
10. Wen-Chin Weng, Wang-Tso Lee, Wen-Ming Hsu, Bei-En Chang, Hsinyu Lee. 2011. Role of Glucose-regulated Protein 78 in Embryonic Development and Neurological Disorders. *Journal of the Formosan Medical Association* **110**:7, 428-437. [[CrossRef](#)]
11. Shuang-feng Chen, Hai-ying Chen, Xian-bin Liu, Ying-xin Zhang, Wei Liu, Wei-hua Wang, Bin Zhang, Le-Xin Wang. 2011. Apoptotic effect of MG-132 on human tongue squamous cell carcinoma. *Biomedicine & Pharmacotherapy* . [[CrossRef](#)]
12. Ruofeng, Wen Long Zhai, Hai Yan Yang, Hui Jin, Qin Xian Zhang. 2011. Induction of ER stress protects gastric cancer cells against apoptosis induced by cisplatin and doxorubicin through activation of p38 MAPK. *Biochemical and Biophysical Research Communications* **406**:2, 299-304. [[CrossRef](#)]
13. Christa Hahmann , Amiee Weiser , Derek Duckett , Thomas Schroeter . 2011. A Predictive Nuclear Translocation Assay for Spliced X-Box-Binding Protein 1 Identifies Compounds with Known Organ Toxicities. *ASSAY and Drug Development Technologies* **9**:1, 79-87. [[Abstract](#)] [[Full Text HTML](#)] [[Full Text PDF](#)] [[Full Text PDF with Links](#)]
14. Catheleyne D'hondt, Raf Ponsaerts, Humbert De Smedt, Mathieu Vinken, Elke De Vuyst, Marijke De Bock, Nan Wang, Vera Rogiers, Luc Leybaert, Bernard Himpens, Geert Bultynck. 2011. Pannexin channels in ATP release and beyond: An unexpected rendezvous at the endoplasmic reticulum. *Cellular Signalling* **23**:2, 305-316. [[CrossRef](#)]
15. J.G. Baust, W.L. Corwin, J.M. BaustCell Preservation Technology 179-190. [[CrossRef](#)]
16. Šárka Lhoták, Ji Zhou, Richard C. AustinImmunohistochemical Detection of the Unfolded Protein Response in Atherosclerotic Plaques **489**, 23-46. [[CrossRef](#)]
17. Beth A. Dombroski, Renuka R. Nayak, Kathryn G. Ewens, Wendy Ankener, Vivian G. Cheung, Richard S. Spielman. 2010. Gene Expression and Genetic Variation in Response to Endoplasmic Reticulum Stress in Human Cells. *The American Journal of Human Genetics* **86**:5, 719-729. [[CrossRef](#)]
18. Eva Sammels, Jan B. Parys, Ludwig Missiaen, Humbert De Smedt, Geert Bultynck. 2010. Intracellular Ca²⁺ storage in health and disease: A dynamic equilibrium. *Cell Calcium* **47**:4, 297-314. [[CrossRef](#)]
19. Alan R. HipkissMitochondrial Dysfunction, Proteotoxicity, and Aging **50**, 123-150. [[CrossRef](#)]