Review Article

Endoplasmic reticulum stress and cardiovascular diseases

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Abstract The endoplasmic reticulum (ER) serves several important functions, mainly post-translational modification, folding and assembly of newly synthesized secretary proteins, synthesizing lipids and cellular calcium storage. Various factors can disrupt ER homeostasis and disturb its functions, which leads to the accumulation of unfolded and misfolded proteins and to potential cellular dysfunction and pathological consequences, collectively termed ER stress. Recent progress suggests that ER stress plays a key role in the immune response, diabetes, tumor growth, and some neurodegenerative diseases. In particular, ER stress is involved in several processes of cardiovascular diseases, such as ischemia/reperfusion injury, cardiomyopathy, cardiac hypertrophy, heart failure, and atherosclerosis. Further research on the relation of ER stress to cardiovascular diseases will greatly enhance the understanding of these pathological processes and provide novel avenues to potential therapies. **(*J Geriatr Cardiol* 2009; 6:49-55)**

Key words endoplasmic reticulum; stress; cardiovascular diseases

Introduction

The endoplasmic reticulum (ER) is the primary site for secretary protein synthesis and maturation, calcium (Ca^{2+}) storage and biosynthesis of steroids, cholesterol and other lipids. Various factors such as aging, genetic mutations or environmental factors can disrupt ER homeostasis and disturb its functions, which results in the accumulation of unfolded and misfolded proteins and leads to potential cellular dysfunction and pathological consequences, namely ER stress. Prolonged and/or severe stress leads to apoptosis and may be an important factor in the pathogenesis of many diseases, such as diabetes,[1] viral infection,[2] tumor growth,[3] inflammation, and neurodegenerative disorders including Alzheimer disease, Parkinson disease, Huntington disease and bipolar disorder,[4] collectively known as "conformational diseases."[5] Especially, ER stress is involved in several processes of cardiovascular diseases, such as ischemia/reperfusion (I/R) injury, cardiomyopathy, cardiac hypertrophy, heart failure, and atherosclerosis. Here, we focus on the relation of ER stress to cardiovascular diseases to enhance the understanding of these pathological processes and to suggest new avenues for potential therapies.

Overview of ER stress

Some chemicals such as streptovirudin (tunicamycin), dithiothreitol and thapsigargin are commonly used to evoke ER stress through inhibiting protein glycosylation, reducing formation of disulfide bonds and deleting calcium from the ER lumen, respectively. Hypoxia, nutrient deprivation, pathogen infection and overexpression of folding-defective proteins are natural inducers as well.[5] As a result, overexpressed unfolded and misfolded proteins are accumulated in ER lumen, which results in potential cellular dysfunction and pathological consequences. However, cells have developed elaborative defense systems for detecting unfolded and misfolded proteins and make appropriate adjustments to maintain ER homeostasis. Four pathways of maintaining ER homeostasis are 1) attenuation of protein synthesis, which prevents further accumulation of unfolded and misfolded proteins; 2) the transcriptional induction of ER chaperone genes to increase folding capacity; the chaperones include glucose-regulated protein 78 (GRP78)/binding protein (BiP), glucose-regulated protein 94 (GRP94) and some folding enzymes, which slow protein-folding reactions and prevent aberrant interactions and aggregation; 3) the transcriptional induction of ER-associated degradation (ERAD) component genes to increase the level of ERAD proteins by the ubiquitin proteasome system; and 4) the induction of apoptosis to safely dispose of cells injured by ER stress to ensure the survival of the whole organism.[5]

ER stress-induced survival pathways

The extent of the ER stress depends on the strength and duration of the stress. Usually, the stress is suitable for optimal folding of nascent synthesized proteins, thus enhancing the chance for survival.[6] A complex homeostatic signaling pathway, the unfolded protein response (UPR), has evolved to coordinate ER protein-folding demand and protein-folding capacity and is essential to adapt to homeostatic alterations that cause protein misfolding.[7] The canonical UPR pathway has three branches, with transmembrane

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protein protein kinase R-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) serving as proximal sensors. Under non-stress conditions, the luminal domains of the monomeric and inactive forms of these sensors are occupied by GRP78 in a steady state. Upon ER stress, sequestration of GRP78 by unfolded proteins activates these sensors, inducing phosphorylation and oligomerization of IRE1 and PERK and relocalizating ATF6 to the Golgi apparatus, where it is cleaved by site 1 and site 2 proteases (S1P and S2P).[8]

PERK

PERK is a type 1 ER transmembrane protein kinase that is a monomer and associates with GRP78 under non-stress conditions. During ER stress, GRP78 relocates from PERK to misfolded proteins, which leads to PERK dimerization and autophosphorylation. Then PERK phosphorylates the alpha-subunit of the ribosomal elongation factor eukaryotic initiation factor a (eIF2-a) on Ser 51, which limits the availability of the translation initiation complex and thereby attenuates general (cap-dependent) protein translation.[9] Additionally, eIF2-a phosphorylation induces expression of the transcription factor ATF4, which induces many genes involved in relief of ER stress, such as genes that encode amino acid transporters and ER resident chaperones, during the pro-survival phase of ER stress.[10] Continued activation of PERK also leads to activation of some apoptosis-related proteins, such as CCAAT/enhancer-binding homologous protein (CHOP), c-Jun N-terminal kinase (JNK) and caspase-12 (see following for details).

IRE1

IRE1 is a type 1 ER transmembrane protein that functions as a Ser/Thr protein kinase and an endoribonuclease. Similar to PERK, during ER stress, GRP78 relocates from IRE-1 to misfolded proteins, which allows IRE1 to dimerize, thus facilitating transautophosphorylation.[11] Interestingly, the substrate for IRE-1 is the mRNA for X-box binding protein 1 (XBP1). Once activated, IRE1 splices XBP1 mRNA between nucleotides 506-579, removing a 26-nt intron sequence and enhancing IRE1-dependent translation of XBP1 mRNA,[12] which encodes an active transcription factor that transactivates several UPR target proteins, including XBP1 itself, chaperone proteins, CHOP, the PERK inhibitory protein P58IPK, ER degradation-enhancing a-mannosidase-like protein, ER oxidoreductin 1 (ERO1), ER dnaJ 4 and human ER-associated dnaJ, the last two being accessory proteins that stimulate the ATPase activity of GRP78.[13] Because XBP1 mRNA expression requires ATF6 activation, the IRE1's effect is considered to follow ATF6 activation.

ATF6

ATF6 is a 670-aa ER transmembrane protein. ATF6 exists in the ER as a dimer linked by intermolecular disulfide bonds in the luminal domain. Even though ER stress releases GRP78 from ATF6, unlike PERK and IRE-1, it is not thought to be attributable to competitive binding of GRP78 to other proteins. GRP78 dissociation and disulfide bond cleavage with ER stress facilitate the translocation of about 90-kDa ATF6 monomers to the Golgi lumen.[14] There, 2 proteases, S1P and S2P, cleave ATF6 near the ER transmembrane region, thus releasing the N-terminal cytosolic N-ATF6, about a 50-kDa fragment, which translocates to the nucleus. Then, it combines with several other proteins and binds to ER stress response elements in certain ER stress response genes and activates transcription of ATF6-inducible ER stress response-element genes.[15] Major ATF6 targets include chaperone proteins, the transcription factor XBP1, P58IPK, and CHOP.[13]

ER stress-induced apoptosis pathways

Disruption of ER homeostasis causes the ER stress response, which can eventually lead to apoptosis with severe or prolonged ER dysfunction.

CHOP-mediated ER stress-induced cell death

Probably the most significant ER stress-induced apoptotic pathway is mediated through the factors CHOP/growth arrest and DNA damage 153 (GADD153). CHOP is a bZiP transcription factor induced through PERK and ATF6 unfolded-protein response pathways. CHOP activates the transcription of several genes that may potentiate apoptosis, which induces numerous proapoptotic proteins, including GADD34, ERO1, death receptor 5, and carbonic anhydrase VI.[16] CHOP has also been implicated in repressing transcription of the antiapoptotic B cell leukemia 2 (BCL2) protein, which leads to enhanced oxidant injury and apoptosis. However, the capacity of CHOP-mediated ER stress-induced apoptosis depends on the duration of the stress state. Long-term exposure to mild stress can lead to adaptation by selective attenuation of CHOP expression mediated by degradation of CHOP mRNA and CHOP protein, whereas expression of downstream targets encoding adaptive functions, such as ER chaperones GRP78 and GRP94, is persistent because of long-lived mRNAs and proteins.[17]

JNK-mediated ER stress-induced cell death

In addition to mediating splicing of XBP1 mRNA, the cytoplasmic-domain IRE1 recruits the adaptor molecule TNF-receptor-associated factor 2 (TRAF2). The IRE1–TRAF2 complex can recruit apoptosis-signal-regulating kinase, a mitogen-activated protein kinase (MAPK) kinase kinase shown to relay various ER stress-induced apoptosis signals to the downstream MAPKs JNK and p38.[17] IRE1–TRAF2 activates the transcriptional repressor ATF3 as well, leading to apoptosis.[16] TRAF2 also associates with and regulates caspase-12 activity.[16]

Caspase 12-mediated ER stress-induced cell death

Caspases are well-known proapoptotic compo nents, and caspases 2, 3, 4, 7, 9, and 12 are believed to be involved in ER stress-induced cell death. Like caspase-12, members of the proapoptotic BCL2 family, BCL2-associated X protein (BAX) and BCL2 homologous antagonist/killer (BAK), also colocalize to the ER membrane and function to activate apoptosis through caspase-12.[16] Then, caspase-12 activates caspase-9, which in turn activates caspase-3, leading to cell death.[16] There are three models for the activation of caspase-12: (1) Ca^{2+} released from the ER activates m-calpain, which would translocate from the cytosol to the ER and cleave the caspase recruitment pro-domain of caspase-12; (2) pro-caspase-12 is released from TNF receptor-associated factor 2, then homodimerizes and undergoes auto-processing; or (3) caspase-7 cleaves pro-caspase-12 in the middle of the pro-domain, which triggers auto-processing of caspase-12 itself.[18]

BCL2-regulated ER stress-induced cell death

During ER stress, proapoptotic members of the BCL2 family are recruited to the ER surface and activate caspase-12. BCL2-interacting mediator of cell death (BIM) is translocated from the dynein-rich compartment to the ER membrane and activates caspase-12 in response to ER stress, whereas an antiapoptotic factor, BCL2-like 1, binds to BIM and inhibits its translocation.[19] Meanwhile, BAX and BAK oligomerize to permit Ca^{2+} efflux to the cytoplasm. Increased cytosolic Ca^{2+} level can activate both mitochondria-dependent and -independent caspase cascades.[20] Ca^{2+} released from the ER enters mitochondria for depolarizing the inner membrane of mitochondria, thus promoting cytochrome c release and activating apoptosis protease-activating factor 1/procaspase-9-regulated apoptosis. Therefore, BCL2 family members regulate ER stress-induced apoptosis, possibly through Ca^{2+} signaling.

ER stress and heart diseases

ER stress in ischemic heart diseases

Harpster and colleagues[21] reported that numerous ER stress response genes, such as GRP78, ATF4, XBP1, and KDEL (Lys-Asp-Glu-Leu) ER protein retention receptor 3, were induced within 24 h of in vivo myocardial infarction in mice hearts. Thuerauf et al.[22] found that cultured neonatal rat ventricular myocytes subjected to hypoxia (16 h) exhibited increased XBP1 mRNA splicing, XBP1 protein expression, GRP78 promoter activation, and GRP78 protein levels; the phenomenon appeared in surviving cardiomyocytes bordering the infarct zone in mouse with in vivo myocardial infarction. Meanwhile, dominant-negative XBP1 increased apoptosis in isolated cardiomyocytes in response to simulated I/R, which suggests that in this context, ER stress may be cardioprotective.

Terai et al.[23] found that pharmacological activation of AMP-activated protein kinase (AMPK) inhibited hypoxia-associated ER stress in cardiomyocytes, which was abolished by small interfering RNA (siRNA) of elongation factor 2 (eEF2) kinase. AMPK activation is associated with the inhibition of protein synthesis via phosphorylation of eEF2 in cardiomyocytes. Furthermore, siRNA-mediated suppression of caspase-12 or CHOP expression mitigated the ischemia-induced cardiomyocyte apoptosis. These results suggest that AMPK protects cardiomyocytes against hypoxic injury by attenuating ER stress. Zhu et al.[24] reported on activated calreticulin (an ER chaperone specifically involved in the folding of glycoprotein) and caspase-12 expression during hypoxic preconditioning (HPC) and hypoxia/reoxygenation (H/R) in primary cultured neonatal cardiomyocytes, which was almost eliminated when the inhibitor of p38 MAPK but not JNK was used before HPC. These results suggest that HPC protects neonatal cardiomyocytes against severe ER stress-induced apoptosis by pre-invoking a proper ER stress response. In addition, ischemic postconditioning could suppress I/R-induced ER stress, as shown by a decrease in calreticulin expression and caspase-12 activation. Meanwhile, hypoxic postconditioning up-regulated p38 MAPK phosphorylation and down-regulated JNK phosphorylation in cardiomyocytes subjected to H/R in vitro.[25]

Martindale et al.[26] showed GRP78 and GRP94 upregulated in Langendorff-perfused mouse hearts subjected to I/R injury, and overexpression of activated ATF6 in transgenic mouse hearts decreased ischemic damage and increased ventricular pump function in an ex vivo I/R model. Overexpression of GRP94 protected cardiac myocytes against simulated ischemia and Ca^{2+} overload, both inducers of ER stress.[27] In contrast, suppression of GRP78 expression by gene silencing abrogated the protective effect of endothelin-1 preconditioning in hypoxic cardiomyocytes.[28] I/R also causes Ca^{2+} imbalance between cytosolic and the ER, which perturbs ER chaperone functions and myocardial contractility.[29,30]

Azfer and colleagues[31] reported that a cluster of ER stress-related genes, such as GRP78, GRP94, ATF6, ERO1, CHOP and members of the protein disulfide isomerase (PDI) group, were transcriptionally activated in the hearts of monocyte chemoattractant protein 1 (MCP-1)-overexpressing mice. MCP-1 recruits inflammatory cells to the heart, which leads to ischemic heart disease. At the same time, ERAD was also activated, especially in degenerating cardiomyocytes.

In a word, I/R generates ER stress in cardiac myocytes, and ER stress is initially a defensive mechanism but in excess, triggers cell death.

ER stress and cardiomyopathy

Hamada et al.[32] found that dialated cardiomyopathy development in transgenic mice expressing a mutant KDEL

receptor in the early secretory pathway; these are proteins destined to the extracellular space or to organelles of the secretory route that are synthesized by ER-bound ribosomes and enter into the ER. Ultrastructural analysis revealed expanded sarcoplasmic reticulum and aggregated proteins that obstructed the adjacent transverse tubules of the mutant cardiomyocytes. The authors also observed enhanced expression of CHOP and apoptosis in mutant hearts. These results suggest that the dilated cardiomyopathy in the mutant KDEL-receptor transgenic mice is associated with ER stress. Mao et al.[33] reported that autoimmune cardiomyopathy could be produced by beta(1)-adrenergic receptor [beta (1)-EC(II)]-mediated activation of the CaMKII/p38 MAPK/ ATF6 signaling pathway in rabbits subjected to beta(1)-EC (II) immunization for 6 months. Beta(1)-EC(II) immunization produced progressive left-ventricular dilation, systolic dysfunction, and myocyte apoptosis, which was associated with activation of GRP78 and CHOP and increased cleavage of caspase-12, as well as increased CaMKII activity, p38 MAPK phosphorylation and nucleus translocation of cleaved ATF6. A related paper showed that adoptive passive transfer of beta(1)-EC(II) immunoglobulin from rabbit autoimmune cardiomyopathy produced early cardiomyopathy and myocyte apoptosis in severe recombination activating gene 2 knock-out ($Rag2^{-/-}$) immunodeficient mice(the mice fail to generate mature T and B lymphocytes and are used for adoptive transfer experiments), which was associated with increased ER stress, as evidenced by upregulation of GRP78 and CHOP and cleavage of ATF6.[34] In addition, cardiac-specific expression of MCP-1 in mice caused ischemic cardiomyopathy associated with activation of ER stress, with increased expression of GRP78, PDI, and heat shock protein 25 (HSP25), HSP40, and HSP70.[35]

ER stress in cardiac hypertrophy and heart failure

Heart failure is characterized by cardiomyocyte contractile dysfunction and cell loss that diminish contractility and facilitate slippage of muscle bundles, ventricular wall thinning and chamber dilatation. Brostrom et al.[36] applied arginine vasopressin to cultured H9c2 cardiomyocytes to induce cellular hypertrophy. GRP78 and GRP94 increased preferentially within 8 h after vasopressin treatment. Cotreatment with phorbol myristate acetate (a protein kinase C agonist) decreased vasopressin-dependent Ca^{2+} mobilization and slowed the appearance of new GRP78 molecules, whereas 24-h pretreatment with phorbol ester prolonged vasopressin-dependent Ca^{2+} mobilization and further increased rates of GRP78 synthesis in response to the hormone. Additionally, pressure overload by transverse aortic constriction in a mouse model induced prolonged ER stress (GRP78, GRP94 and calreticulin overexpression) after 1 week (the phase of hypertrophy) and apoptosis after 4 weeks (the phase of heart failure). ER stress-initiated apoptosis seemed to depend on CHOP expression but not JNK phosphorylation or caspase-12 cleavage. Most importantly, the ER was expanded and ER chaperones GRP78, GRP94 and calreticulin were upregulated in human heart failure in parallel with elevated atrial and brain natriuretic peptide levels.[37] These findings are underpinned by other reports that pressure overload induced by abdominal aortic banding in rats upregulated GRP78 and CHOP expression and evoked XBP1 mRNA splicing after 24 h.[32] Moreover, the ER stress response can be activated in hearts of transgenic mice that overexpressing MCP-1developed heart failure.[38]

All these reports imply that ER stress is an important essential mechanism in cardiac hypertrophy and heart failure.

ER stress and diabetic cardiomyopathy

In all kinds of diabetes, diabetic cardiomyopathy, a heart muscle-specific disease without any vascular pathology, has been described. Ultrastructural analysis revealed a swelled ER in the diabetic rat myocardium.[39] Li et al.[40] found overexpressed protein and mRNA levels of myocardial GRP78 and caspase-12 and activated caspase-12 in the heart of rats with type 1 diabetes induced by intraperitoneal injection of streptozocin. These results suggest that ER stress is induced in the diabetic rat myocardium, and ER stress-associated apoptosis takes part in the pathophysiology of diabetic cardiomyopathy.

ER stress and vascular diseases

ER stress and atherosclerosis

Atherosclerosis is a disease in which arteries harden and narrow because of the accumulation of fatty substances, cholesterol, cellular waste, Ca^{2+}, and other substances in the arterial wall. One of the risk factors for atherosclerosis is the accumulation of homocysteine, which is an intermediate factor produced during the metabolism of sulfur amino acids.[41] Homocysteine is converted to cysteine and remethylated in part to methionine by vitamin B_{12} and folate under the normal condition. When normal metabolism is disturbed because of deficiency of cystathionine-β-synthase (CBS), which condenses homocysteine and serine to form cystathionine and requires vitamin B_6 for activation, homocysteine accumulates in blood and results in severe hyperhomocysteinemia. In cultured vascular endothelial cells, homocysteine induces protein misfolding in the ER by interfering with disulfide bond formation[42] and activates the UPR to induce expression of several ER stress response proteins such as GRP78, GRP94, CHOP, HERP and T-cell death-associated gene 51 (a member of the pleckstrin homology-related domain family).[41,44-46] Homocysteine could also trigger apoptosis by a signaling pathway that requires intact IRE1.[45] These studies support that hyperhomocysteinemia can disrupt ER homeostasis to cause UPR induction in vasculature. This finding is consistent

with the observed activation of UPR markers in livers of normal or $CBS^{-/-}$ mice in response to hyperhomocysteinemia.[47]

Free cholesterol loading of macrophages elevates levels of cell-surface Fas ligand and activates proapoptotic Bax protein and increases mitochondrial-dependent apoptosis. Recently, some evidence showed that depletion of calcium stores in the ER and subsequent activation of the UPR is the dominant driving force in cholesterol-induced macrophage death.[48] The ER stress response is activated when macrophages accumulate cholesterol. Feng et al.[49] reported that selectively blocking the accumulation of cholesterol in the ER membrane protected macrophages against cholesterol-induced apoptosis. Furthermore, recent findings suggest that defective insulin signaling and reduced Akt activity impair the ability of macrophages to deal with ER stress-induced apoptosis within atherosclerotic plaques.[50] These findings suggest that the UPR plays a key role in the progression of the atherogenic disease process.

ER stress and thrombotic disease

Tissue factor (TF) is an integral membrane glycoprotein essential for the initiation of the extrinsic coagulation cascade. Diet-induced hyperhomocysteimia in apoE-deficient mice increases the expression of TF.[47] Consistent with this finding, in vitro studies have shown that homocysteine can induce TF procoagulant activity.[51] Toschi et al.[52,53] reported that an increase in TF expression or activity, or both, could enhance thrombin generation, thereby increasing lesion thrombogenicity and the risk of thrombotic complications. Watson et al.[54] found that overexpression of GRP78 inhibits thrombin generation by inhibiting TF procoagulant activity, which suggests that inhibition of ER stress could suppress blood coagulation.

ER stress and diabetic retinopathy

Diabetic retinopathy is one of the major microvascular complications associated with diabetes mellitus, and the selective degeneration of retinal capillary pericytes is considered a hallmark of early retinopathy.[55] Rat retinal capillary pericytes treated with hypoglycemia but not hyperglycemia were found with activated UPR-specific enzymes. Strong UPR activation leads to apoptosis in pericytes cultured in glucose concentrations ranging from high to low or without glucose. Thus, induction of UPR is related to not only absolute concentrations but also a shift from high to low concentrations of glucose.[56] So, glucose fluctuations commonly occur in patients with diabetes and may be a reason for the pathogenesis of diabetic retinopathy.

Conclusions and perspectives

As an initial stress response in human cells, ER stress is involved in several pathological processes of cardiovascular diseases. The initial effect of ER stress is a defensive mechanism for detecting unfolded and misfolded proteins, in providing appropriate adjustments to maintain ER homeostasis, which might provide cardiovascular protection. However, if the stress is severe or prolonged, cardiomyocytes and vascular cells may become dysfunctional and undergo apoptosis, which leads to cardiovascular diseases. However, whether the ER stress observed in these conditions is a primary pathogenetic cause of diseases (as a player) or only a secondary phenomenon (as a partner) is unclear. Further studies should focus on the integration of the ER stress response with other stress responses in cells and the molecular pathways underlying ER stress-induced cell death. In addition, some translational research is warranted to identify the therapeutic agents specifically targeted for diseases associated with ER pathologies to address cardiovascular and other diseases.

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