

Endoplasmic Reticulum Stress in the Heart

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Abstract—Over the last decade, it has become clear that the accumulation of misfolded proteins contributes to a number of neurodegenerative, immune, and endocrine pathologies, as well as other age-related illnesses. Recent interest has focused on the possibility that the accumulation of misfolded proteins can also contribute to vascular and cardiac diseases. In large part, the misfolding of proteins takes place during synthesis on free ribosomes in the cytoplasm or on endoplasmic reticulum ribosomes. In fact, even under optimal conditions, $\approx 30\%$ of all newly synthesized proteins are rapidly degraded, most likely because of improper folding. Accordingly, stresses that perturb the folding of proteins during or soon after synthesis can lead to the accumulation of misfolded proteins and to potential cellular dysfunction and pathological consequences. To avert such outcomes, cells have developed elaborate protein quality-control systems for detecting misfolded proteins and making appropriate adjustments to the machinery responsible for protein synthesis and/or degradation. Important contributors to protein quality control include cytosolic and organelle-targeted molecular chaperones, which help fold and stabilize proteins from unfolding, and the ubiquitin proteasome system, which degrades terminally misfolded proteins. Both of these systems play important roles in cardiovascular biology. The focus of this review is the endoplasmic reticulum stress response, a protein quality-control and signal-transduction system that has not been well studied in the context of cardiovascular biology but that could be important for vascular and cardiac health and disease. (*Circ Res.* 2007;101:975-984.)

Key Words: ATF6 ■ ER stress ■ ischemia ■ unfolded protein response ■ XBP1

Misfolded proteins contribute to a number of neurodegenerative, immune, and endocrine pathologies, as well as other age-related illnesses. Stresses that perturb the folding of proteins during, or soon after, synthesis can lead to the accumulation of misfolded proteins and to potential cell and organ dysfunction. Cells have developed elaborate protein quality control systems for detecting and degrading dysfunctional misfolded proteins. Protein quality control components include cytosolic and organelle-targeted molecular chaperones, which help fold and stabilize proteins from unfolding, and the ubiquitin proteasome system, which degrades terminally misfolded proteins. One of the organelle-specific protein quality control systems resides in the endoplasmic reticulum (ER), and is responsive to stresses that lead to the accumulation of misfolded proteins in the lumen of the rough ER. It is becoming apparent that the accumulation of misfolded proteins in the ER can contribute to vascular and cardiac diseases; accordingly, the focus of this review is the ER stress response, a protein quality control system that resides in the ER.

General Aspects of the ER Stress Response

The rough ER serves an important role in protein biosynthesis; the demands placed on the ER protein biosynthesis machinery vary in different cell types, depending on the need for the synthesis of secreted proteins, as well as transmem-

brane proteins and many organelle-targeted proteins. The environment of the rough ER lumen is critical for ensuring the structural fidelity of proteins synthesized in this organelle. The levels of calcium, molecular chaperones, protein glycosylation machinery, and the redox status in the ER lumen must be maintained in the correct balance to ensure optimal ER protein folding (Figure 1, step 1, blue). If this balance is perturbed, ER stress ensues and an intracellular signal-transduction system, the ER stress response, also called the unfolded protein response, is activated.

The ER stress response is a highly conserved signaling system that has been studied in many different cell and tissue types.⁶⁻⁸ The ER stress response is designed to transmit information about the status of protein folding from the rough ER to other cellular locations. Soon after cells are subjected to environmental conditions that perturb the ER environment, including ischemia, the ER signals to other cellular systems in a collaborative effort to resolve the ER stress and to survive (Figure 1, steps 2 to 4, blue). The interventions necessary to resolve the ER stress depend on the strength and duration of the stress but usually include transiently reducing the quantity of protein synthesis in the ER, degrading dysfunctional misfolded ER proteins, and reestablishing an ER environment that is suitable for optimal folding of nascent and newly synthesized proteins (Figure 1, step 5, blue). Each of these responses, which take place very soon after the onset of ER

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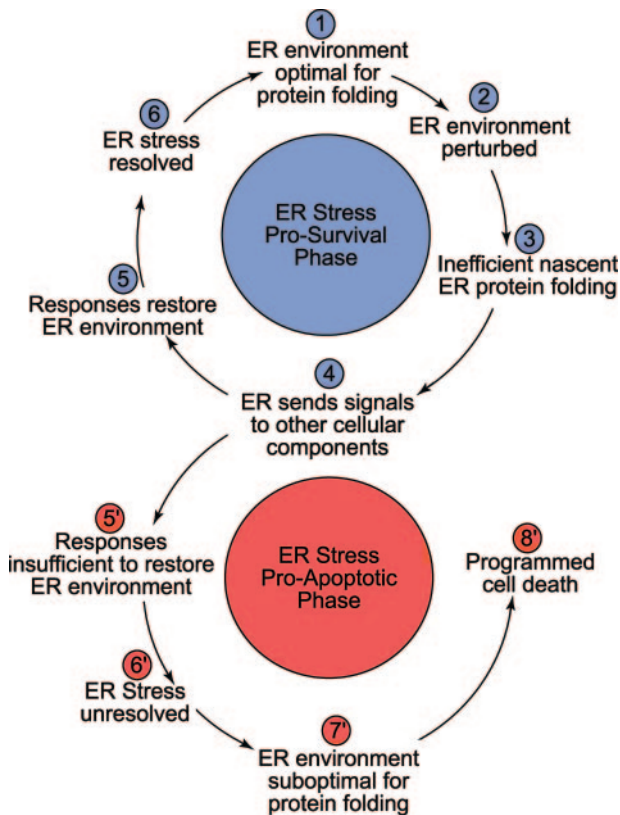


Figure 1. The prosurvival and proapoptotic phases of the ER stress response. The steps involved in the prosurvival (blue steps 1 to 6) and proapoptotic phases (red steps 5' to 8') of the ER stress response are shown. In general, during the prosurvival phase, components of the rapidly activated ER stress response contribute to restoring the ER luminal environment and resolving the ER stress, thus promoting survival. However, if the ER stress is not resolved during the prosurvival phase, components of the ER stress response activated at later stages, during the proapoptotic phase, initiate the programmed cell death pathway.

stress, is designed to contribute to resolving the stress and reducing the protein folding load on the ER, fostering the recovery of ER protein synthesis and, ultimately, enhancing the chances for survival. Accordingly, this aspect of ER stress is often considered the prosurvival phase (Figure 1, step 6, blue).⁹ However, if the ER stress is not resolved during the prosurvival phase, prolonged stress leads to activation of pathways that mediate programmed cell death. Accordingly, this aspect of ER stress is often considered the proapoptotic phase (Figure 1 steps 5' to 8', red). Thus, like many cell signaling pathways, the ultimate outcome of the ER stress response is context dependent, providing this complex signaling process with the conditional ability to facilitate survival or death. The molecular aspects of the ER stress response and the current understanding of the mechanisms by which prosurvival or proapoptotic ER stress can lead to survival or cell death are discussed below.

Molecular Aspects of the ER Stress Response

The ER stress response was first studied in mammalian cells, where it was shown that toxins that alter ER calcium, ER redox status, or the glycosylation of nascent ER proteins, such as A23187, dithiothreitol, or tunicamycin, respectively,

resulted in the accumulation of unfolded ER proteins and induction of ER resident proteins in the heat shock family of chaperones. Included among the chaperones first shown to be induced by ER stress are glucose-regulated proteins 78 and 94 (GRP78 and GRP94), as well as the lectin-like chaperones calnexin and calreticulin and ER degradation enhancer mannosidase α -like 1, or EDEM.^{10–13} All of these chaperones are involved in the recognition of and refolding of misfolded proteins, as well as the targeting of terminally misfolded proteins for eventual degradation by proteasomes. Initially, ER stress causes the accumulation of misfolded proteins in the ER lumen, which is attributable to inefficient disulfide bond formation, impaired glycosylation, and/or reduced chaperone capacity (Figure 2). Such misfolded proteins attract chaperones that serve a refolding function, as well as an important role in activating 3 ER transmembrane proteins, PERK (protein kinase R [PKR]-like ER kinase), inositol-requiring enzyme (IRE)-1, and activating transcription factor (ATF)6, which serve as the proximal effectors of the endoplasmic reticulum stress response (ERSR).

Protein Kinase R–Like ER Kinase

PERK is a type 1 ER transmembrane protein kinase that is a monomer under unstressed conditions.¹⁴ In the absence of ER stress, the luminal domain of monomeric PERK associates with the ER chaperone GRP78 (Figure 2A, PERK). However, after ER stress, in efforts to assist folding, GRP78 relocates from PERK to misfolded ER proteins. GRP78 relocation allows PERK to dimerize, which facilitates transautophosphorylation in a mechanism similar to growth factor receptor activation^{15,16} (Figure 2A, PERK active). After dimerization and autophosphorylation, PERK is activated and it phosphorylates the α -subunit of the ribosomal elongation factor eIF2 α on serine-51; this phosphorylation event decreases cap- or eIF2 α -dependent translation, resulting in a global translational inhibition (Figure 3A).¹⁵ Global translational inhibition reduces the protein-folding load on the ER and allows the cell to focus resources on resolving the ER stress, thus facilitating survival. Even though PERK mediates global translational arrest, many of the mRNAs encoded by ER stress response genes possess structural features that allow them to escape PERK-mediated translational inhibition.¹⁷ For example, the GRP78 mRNA has an internal ribosomal entry sequence that allows continued ribosomal initiation and translation, even when eIF2 α is phosphorylated.¹⁸

During the prosurvival phase of the ER stress response, PERK-mediated eIF2 α phosphorylation is transient,¹⁷ suggesting that it provides cells with a brief respite from the rigors of protein synthesis but then allows resumption of translation necessary to recover from the ER stress. In addition to mediating translational arrest, eIF2 α phosphorylation also induces expression of the transcription factor ATF4. During the prosurvival phase of ER stress, ATF4 induces numerous genes involved in resolution of the ER stress, such as genes that encode amino acid transporters and ER resident chaperones.¹⁹ However, after prolonged ER stress, continued ATF4 expression mediates the upregulation of genes that contribute to programmed cell death. For example, ATF4 induces the transcription factor C/EBP ho-

Proximal Effectors of the ER Stress Response

altered ER Ca, redox status or glycosylation
by treatment with TG, DTT, TM, I, I/R

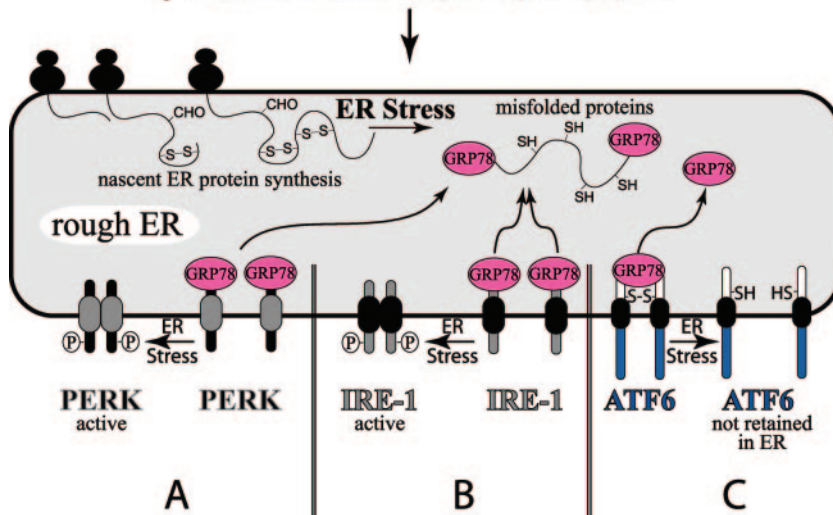


Figure 2. Proximal effectors of the ER stress response. Compounds such as thapsigargin (TG), dithiothreitol (DTT), and tunicamycin (TM) can decrease ER calcium, impair ER redox status, and reduce ER protein glycosylation, respectively, all of which decrease the efficiency of protein folding in the ER lumen. Ischemia (I) and I/R can also affect the ER luminal environment in ways that are predicted to impair ER protein folding and activate the ER stress response. The 3 primary effectors that sense ER stress are PERK (A), IRE-1 (B), and ATF6 (C). The relocalization of GRP78 from the luminal domains of PERK, IRE-1, and ATF6 contributes to the activation of these 3 effectors of the ER stress response. In the case of ATF6, intermolecular disulfide bond cleavage during ER stress results in monomer formation, which decreases ER retention, facilitating relocalization of ATF6 to the Golgi.

mologous protein,^{20,21} which induces numerous proapoptotic proteins, including GADD34,²² and Tribbles-related protein 3.²³ Moreover, CHOP regulates expression of several Bcl₂ family members; for example, CHOP reduces expression of antiapoptotic Bcl₂²¹ but increases expression of the proapoptotic Bim,²⁴ thus contributing to cell death. During the proapoptotic phase of ER stress, continued activation of PERK leads to its association with TNF receptor-associated factor 2,²⁵ which recruits and activates apoptosis signaling kinase 1, leading to c-Jun N-terminal kinase activation and programmed cell death.²⁶ PERK can also activate caspase-12, which can activate programmed cell death during the proapoptotic phase of the ER stress response.

Inositol-Requiring Protein-1

In the context of the ER stress response, IRE-1 was first identified in yeast as a gene that encodes a type 1 ER transmembrane protein that activates the transcription factor, Hac1 (homologous to ATF/CREB1).²⁷ The IRE-1 gene was discovered later the same year in mammalian cells.²⁸ IRE-1 is a type 1 ER transmembrane protein that functions as a kinase and as an endoribonuclease. Like PERK, in the absence of stress, GRP78 binds to the luminal domain of IRE-1 monomers (Figure 2B, IRE-1). Also in comparison with PERK, during the prosurvival phase of the ER stress response, GRP78 relocates to misfolded proteins, which allows IRE-1 to dimerize, thus facilitating transautophosphorylation.²⁹ However, in contrast to PERK, transautophosphorylation of IRE-1 activates a novel endoribonuclease activity³⁰ (Figure 2B, IRE-1 active). In mammalian cells, the substrate for the IRE-1 endoribonuclease is the mRNA for X-box-binding protein-1 (XBP1), the mammalian homolog to Hac1 in yeast. In the absence of stress, the XBP1 mRNA encodes a rapidly degraded, 288-aa protein that has b-Zip dimerization, DNA-binding, and nuclear localization domains but does not have a transcriptional activation domain, and so it cannot induce

transcription (Figure 3B, XBP1_{inactive}). After activation, the IRE-1 endoribonuclease splices the XBP1 mRNA in the cytoplasm, in juxtaposition to the ER membrane, generating an mRNA featuring a new open reading frame that encodes a 376-aa protein. This version of XBP1 is composed of the same b-Zip, DNA-binding, and nuclear localization domains as XBP1_{inactive} but also has a functional C-terminal transcriptional activation domain that confers its ability to transcriptionally induce numerous ER stress response genes (Figure 3B, XBP1_{active}). XBP1_{active}, which is more stable than XBP1_{inactive}, binds primarily to ER stress response elements in ER stress response genes to induce expression.³¹ One role recently delineated for XBP1_{inactive} involves a surveillance mechanism. In the absence of ER stress, XBP1_{inactive} binds to any XBP1_{active} that might be generated because of leakiness of the system. Then XBP1_{inactive} escorts XBP1_{active} out of the nucleus and facilitates its degradation.³² This is thought to ensure the quiescence of XBP1-inducible ER stress response gene transcription in the absence of ER stress.

Activating Transcription Factor 6

ATF6 is a 670-aa ER transmembrane protein.^{33,34} In comparison to PERK and IRE-1, in the absence of ER stress, the luminal domain of ATF6 is associated with GRP78. And even though ER stress releases GRP78 from ATF6, in contrast to PERK and IRE-1, this is not thought to be attributable to competitive binding of GRP78 to other proteins.³⁵ Moreover, ATF6 exists in the ER as a dimer linked by intermolecular disulfide bonds in the luminal domain (Figure 2C, ATF6). GRP78 dissociation and disulfide bond cleavage on ER stress facilitate the translocation of ≈90-kDa ATF6 monomers, so called p90,³⁶ to the Golgi lumen,³⁷ where 2 proteases, S1P and S2P, cleave it near the ER transmembrane region, thus releasing the N-terminal cytosolic, ≈400-aa 50-kDa fragment N-ATF6, sometimes called p50³⁸ (Figure 3C, ATF6). N-ATF6, which possesses a transcriptional activation domain, nuclear

Distal Effectors of the ER Stress Response

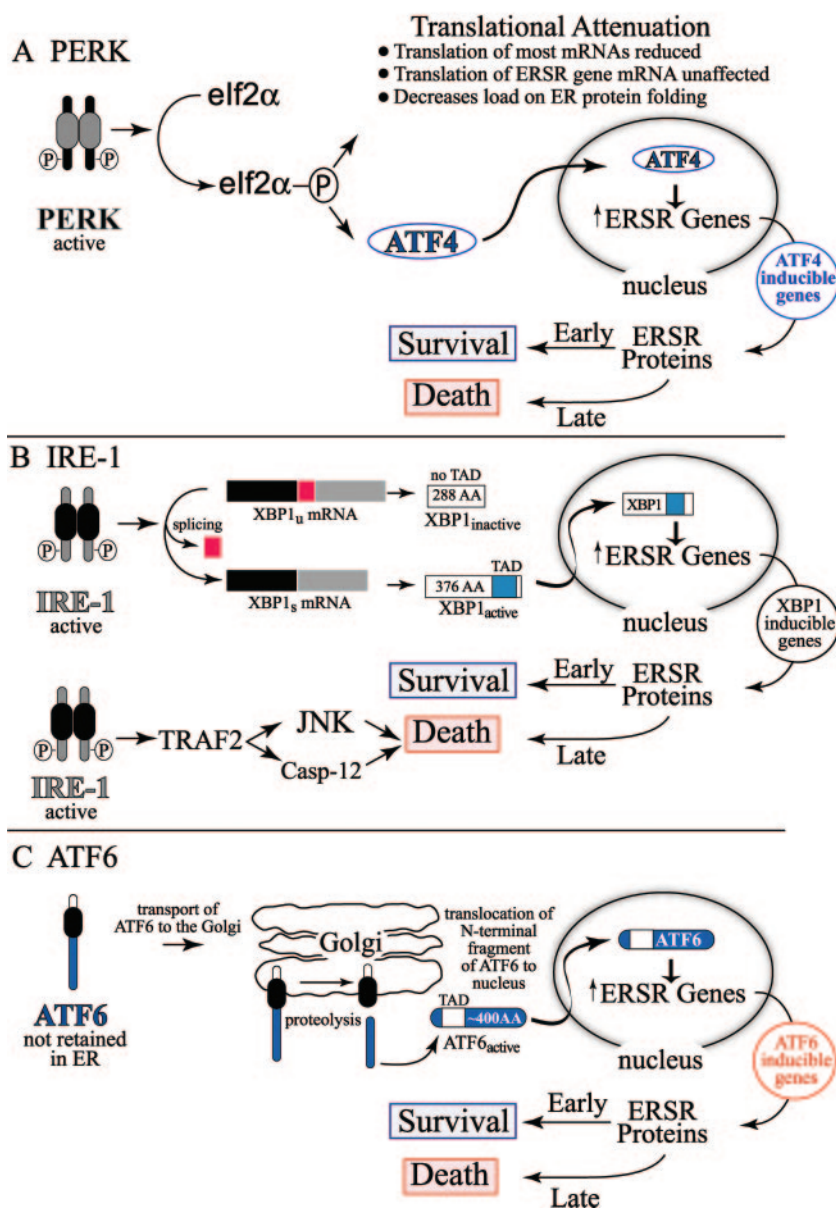


Figure 3. Distal effectors of the ER stress response. The effects of the PERK, IRE-1, and ATF6 branches of the ER stress on downstream, distal targets are shown. Each branch mediates the induction of genes during the early, prosurvival phase of ER stress that contribute to enhancing ER protein folding, thus promoting survival. However, should these efforts be insufficient to resolve the stress, each of the branches also mediates the induction of genes later, during the proapoptotic phase, that contribute to programmed cell death. A, Effect of the PERK branch of the ER stress response. Activated PERK phosphorylates the ribosomal subunit $elf2\alpha$. Phosphorylation of $elf2\alpha$ leads to a global inhibition of protein synthesis, reducing the load on the ER protein folding machinery until the ER stress is resolved. Paradoxically, phosphorylated $elf2\alpha$ increases the translation of the mRNA that encodes the transcription factor ATF4. During the prosurvival and proapoptotic phases of the ER stress response, ATF4 transcriptionally induces numerous genes that lead to resolution of the ER stress (and, therefore, survival) or to programmed cell death, respectively. B, Effect of the IRE-1 branch of the ER stress response induces the transcription of ER stress response genes, many of which are designed to help resolve the ER stress during the early, prosurvival phase, but some of which induce genes that trigger programmed cell death during late, or long-term, activation of the ER stress response. C, The effect of the ATF6 branch of the ER stress response. Similar to the IRE-1 branch, on activation, the ATF6 branch of the ER stress response induces the transcription of ER stress response genes, many of which are designed to help resolve the ER stress during the early, prosurvival phase, but some of which induce genes that trigger programmed cell death during later, long-term activation of the ER stress response. Although many ER stress response genes are activated by ATF4, ATF6, or XBP1, many genes are activated preferentially or exclusively by only 1 of these 3 transcription factors.

localization signal, and DNA-binding domain, translocates to the nucleus, where it combines with several other proteins (eg, NFY-A, -B, and -C) and, like XBP1, binds to ER stress response elements in certain ER stress response genes and activates transcription of ATF6-inducible ERSR genes.^{34,39–41} Like XBP1, in general, genes induced by ATF6 during the prosurvival phase of ER stress foster resolution of the stress and, thus, survival, whereas those genes induced on the proapoptotic phase of ER stress contribute to programmed cell death.

ER Stress Response Gene Induction

At least 3 different elements have been identified in the regulatory regions of ATF6- and XBP1-inducible genes; via these elements, ER stress response genes can be induced by either XBP1 or ATF6, whereas others exhibit specificity for 1 of these transcription factors.^{42,43} Numerous ER stress-

inducible genes have been identified, either by partial genome microarray analyses or other approaches. Among the genes most commonly identified in these studies are those involved in protein folding, ER-associated protein degradation (ERAD), transcriptional control, translational control, and protein glycosylation (Table). Many of these gene products augment ER protein biosynthesis and are therefore prosurvival oriented, whereas others contribute to apoptosis (eg, CHOP, GADD45, PUMA). Accordingly, it is probable that the balance in the expression of ER stress response genes dictates how long the prosurvival phase of the response lasts before the proapoptotic phase predominates. This concept was examined in neuroblastoma cells, where it has been shown that the genes activated at early times of ER stress, within 4 hours of tunicamycin treatment, encode proteins that enhance ER protein folding, whereas some genes induced at

Table. Partial List of Mammalian ER Stress Response Genes

No.	Alias or Protein Name	Symbol	Refs	Function	Notes
1	PDI associated 4	Pdia4	97	A	3
2	calreticulin	Calr	97,98	A	2
3	Calnexin	CANX	98	A	
4	Oxidoreductin-1-Laspha	Ero1L	48	A	1,3
5	Glutaredoxin (thioltransferase)	GLRX	98	A	
6	PDI; ERp61; Pdia3	GRP58	97,98	A	2,3
7	GRP78	Hspa5	48,97,98	A	1,2,3
8	PDI associated 1; ERp59; Thbp	P4hB	60,98	A	2
9	P5 protein disulfide isomerase	Pdia6	98	A	
10	Stanniocalcin 1	STC1	107	A	
11	Stromal cell-derived factor 2-like 1	SDF2L1	98	A	
12	Stress-associated ER protein 1	SERP1	98	A	3
13	stress 70 chaperone, microsome-associated	STCH	98	A	
14	GRP94	Tra1	48,60,97,98	A	1,2
15	ERdj3; HEDJ; HSP40	DNAJB11	60	A,B	1,3
16	ERdj4	DNAJB9	48,60	A,B	1,3
17	P58; P58IPK	DNAJC3	48,98,60	A,B	1
18	Derlin-2, Der1-like domain family, member 2	DERL2	106	B	
19	Derlin-3, Der1-like domain family, member 2	DERL3	106	B	
20	EDEM	Edem1	98	B	
21	HERP; Mif1; SUP	Herpud1	48,60,98	B	1,2
22	Sec61 alpha 1 subunit	SEC61A1	98	B	1,3
23	SEC63-like; ERdj2; translocating protein SEC63	SEC63-like	98	B	
24	Activating transcription factor 3	ATF3	98	C	1
25	Activating transcription factor 4	ATF4	48	C	1
26	Activating transcription factor 6	ATF6	98	C	3
27	BBF2H7, CREB protein 3-like 2	CREB3I2	105	C	
28	CHOP; DNA damage inducible transcript 3	DDIT3	48,97,98	C	1,2,3
29	Growth arrest DA damage inducible gamma	GADD45g	48,98	C	2
30	Luman/CREB3	CREB3	110	C	
31	c-Myc; Myc proto-oncogene protein	MYC	60	C	2
32	PUMA; p58-upregulated mediator of apoptosis	BBC3	76	C	
33	STAT Induced STAT inhibitor	Socs3	100	C	2
34	Tisp40, CREB protein 3-like 4	CREB3I4	111	C	
35	Nip kinase; Ifid2	Trb3	101	C	
36	Nucleobindin	NUCB2	102	C	
37	OASIS, CREB protein 3-like 1	Creb3I1	104	C	
38	X-box binding protein 1	XBP1	48,98	C	1,2,3
39	asparagine synthetase	Asns	97	D	1,3
40	Growth arrest-specific 5	Gas5	48,60	D	
41	Asn-linked glycosylation 12 homologue	ALG12	98	E	
42	Oligosaccharide transferase 48 kDa subunit	DDOST	98	E	
43	Phosphatidylinositol glycan; GPI3	PIGA	98	E	
44	Phosphatidylinositol glycan, class B	PIGB	98	E	
45	Ribophorin 1; OST1	RPN1	98	E	3
46	Subunit of oligosaccharidetransferase complex	STT3A	98	E	3
47	UDP-glucose ceramide glycosyltransferase-like 1	UGCGL1	98	E	
48	arginine-rich, mutated early stage tumors	Armet	48	U	1,2
49	Chemokine (CXC motif) ligand 3	CXCL3	113	U	

(Continued)

Table. Continued.

No.	Alias or Protein Name	Symbol	Refs	Function	Notes
50	ERGIC-53, lectin, mannose-binding, 1	LMAN1	103	U	
51	Heme oxygenase-1	HMOX1	108	U	
52	Interleukin 6	IL6	113	U	2
53	Interleukin 8	IL8	113	U	
54	Monocyte chemoattractant protein 1, MCP1	CCL2	113	U	
55	Neurotrophin receptor associated death domain	NRADD	109	U	
56	Pentraxin-related gene, pentraxin-related protein	Ptx3	112	U	1,2
57	START domain containing 4, sterol regulated	STARDA4	107	U	

Shown is a partial list of genes that have been identified by microarray studies and other methods to be induced in cultured mammalian cells treated with tunicamycin or other activators of ER stress.^{42,70,97–99,100–113} The alias or protein name and the symbol for each gene are shown, as well as the reference(s) reporting the gene as ER stress responsive, the putative function in ER stress, and notes about induction of these genes by hypoxia, myocardial infarction, and heart failure. The putative functions in ER stress are keyed as follows: A indicates protein folding; B, ERAD; C, transcriptional control; D, translational control; E, protein glycosylation; U, unknown function in the context of ER stress. The notes refer to ER stress response genes induced by (1) hypoxia in HeLa cells,¹¹² (2) acute myocardial infarction in mouse hearts,⁷⁹ or (3) in an MCP1-overexpressing model of heart failure.¹¹⁴

later times (8 to 12 hours) encode proteins that contribute to degradation of terminally misfolded proteins and apoptosis.⁴⁴

Degradation of Misfolded ER Proteins

Although many aspects of the ER stress response are oriented toward refolding misfolded proteins, the ER also has a mechanism for degrading terminally misfolded, nonfunctional proteins. Terminally misfolded ER proteins are susceptible to ubiquitination and proteasomal degradation by or ERAD.⁴⁵ In contrast to the ligases that recognize and ubiquitinate misfolded proteins that accumulate in the cytoplasm, the ubiquitin ligases involved in ERAD are spatially separated from misfolded ER proteins by the ER membrane. Accordingly, ERAD substrates must first be retrotranslocated from the ER lumen to the cytosolic face of the ER, where they become targets of degradation by a dedicated ubiquitin proteasome system residing on the surface of the ER.⁴⁶

In addition to ERAD, autophagy is also involved in the degradation of terminally misfolded ER proteins. During autophagy, which, like ER stress, is stimulated by nutrient deprivation, cytoplasmic proteins or organelles are surrounded by double membrane-bound vesicles, autophagosomes, facilitating their degradation on fusion with lysosomes.⁴⁷ One mechanism by which autophagy contributes to ER protein quality control involves ERAD overload, which can result in the escape of some terminally misfolded proteins into the cytosol, where they can be degraded by autophagy.⁴⁸ Another mechanism involves the formation of autophagosomes by fragments of ER membrane during ER stress in a process called reticulophagy.⁴⁹ Thus, autophagy is an alternate to ERAD for the clearance of misfolded ER proteins.

Although autophagy can be activated independently of ER stress, recent studies have demonstrated that autophagy can be activated by ER stress; this activation requires PERK and IRE-1.⁵⁰ In fact, it appears that even in the absence of ER stress, activation of autophagy requires PERK.⁵¹ Autophagy is generally viewed as an adaptation that promotes survival during nutrient starvation. Consistent with this view are findings that cells exhibit increased susceptibility to death from ER stress when autophagy is inhibited.⁵²

ER Stress and Disease

Several diseases associated with the misfolding of proteins, including the conformational diseases,⁵³ are thought to involve activation of the ER stress response. Overactivation of ER stress has been linked to Alzheimer's and Parkinson's diseases,⁵⁴ amyotrophic lateral sclerosis,⁵⁵ and Huntington's disease.⁵⁶ In Alzheimer's disease, an accumulation of amyloid β -peptide results in chronic activation of ER stress^{57,58}; the proapoptotic phase of ER stress would be likely to predominate on such this long-term ER stress, which would contribute to the neurodegeneration that serves as a hallmark of this disease. Malfunction of the ER stress response may also contribute to other pathologies, including diabetes. Mutations in ATF6 in a population of Pima Indians correlates with increased susceptibility to type 2 diabetes.⁵⁹ Although the effect of the mutations on ATF6 as a transcription factor are not known, if they reduce its efficacy, this would translate to decreased induction of ER chaperones and protein disulfide isomerases, which would be expected to impair insulin folding. A mutation in PERK is thought to contribute is associated with Wolcott-Rallison syndrome; this mutation increases pancreatic β -cell apoptosis and can precipitate type 1 diabetes,⁶⁰ which has also been seen in mice lacking PERK.^{61,62}

Because ischemia is associated with reduced nutrient and oxygen delivery to tissues, it is possible that ER stress is activated during ischemia. Consistent with this hypothesis are studies showing that in animal models, cerebral ischemia activates several features of the ER stress response, including PERK, eIF2 α phosphorylation, and inhibition of protein synthesis.⁶⁴ Moreover, gene array studies have shown increased expression of numerous ER stress response genes in the brains of rats subjected to transient cerebral artery occlusion.⁶⁵ The duration of cerebral ischemia could dictate whether the effect of ER stress is oriented toward survival or tissue damage. Although this possibility has yet to be examined in detail in tissues, tumor cells exhibit an interesting ability to alter the downstream effects of ischemia-activated ER stress in ways to foster protection, even after chronic ischemia.⁶⁶ This ability is thought to enhance survival in aggressively growing solid tumors, where the rate of growth

has surpassed neoangiogenesis,⁶⁷ thus forming the basis of the therapeutic strategy of inhibiting the ER stress response.⁶⁸

ER Stress in the Myocardium

Several studies have correlated ER stress with myocardial damage. For example, the ER stress response is activated in the hearts of transgenic mice that overexpress monocyte chemoattractant protein-1 and develop heart failure,³⁶ suggesting that in this model, the proapoptotic phase of ER stress contributes to the loss of myocardium associated with failure. In further support of a role for ER stress in heart failure is the finding that transgenic overexpression of a mutant KDEL receptor, an ER protein that facilitates ER protein targeting, activates the ER stress response in mouse hearts and causes dilated cardiomyopathy.⁶⁹ Also, overexpression of the ER stress response gene product p53-upregulated modulator of apoptosis (PUMA) contributes to ER stress-mediated apoptosis in cultured cardiomyocytes⁷⁰ and targeted deletion of PUMA in mouse hearts attenuates cardiomyocyte death during *ex vivo* I/R.⁷¹

In contrast to the studies cited above, other studies suggest that ER stress might protect the heart, and even foster hypertrophic growth of the myocardium. For example, the ER stress response is activated in the hearts of mice subjected to transaortic constriction-induced hypertrophy,⁷² suggesting that the pro-survival phase of ER stress might help accommodate the increased protein synthesis that takes place during overload-induced hypertrophy. Additionally, overexpression of the ER stress response gene GRP94 protects cardiac myocytes from oxidative damage,⁷³ and endothelin-1-mediated protection of cultured cardiac myocytes from hypoxic damage is abolished by antisense directed against the ER stress response gene GRP78.⁷⁴ GRP78 expression is increased in cultured cardiomyocytes that hypertrophy in response to vasopressin.⁷⁵

ER stress may also play an important role in the developing myocardium. Compared with the adult mouse heart, GRP78 expression is upregulated in the neonatal mouse heart⁷⁶; moreover, GRP78 is required for normal cardiac development.⁷⁷ Targeted disruption of the XBP1 gene in mice is embryonic lethal because of cardiac development defects,⁷⁸ indicating that the ER stress response is required for proper cardiac development.

Because ischemia activates ER stress in the brain, several recent studies have addressed whether ER stress is activated in the myocardium by ischemia or ischemia/reperfusion (I/R). A microarray study showed that numerous ER stress response genes were induced within 24 hours of *in vivo* myocardial infarction in mouse hearts (see the Table, Notes column).⁷⁹ In a corroborating study, immunoblotting and immunocytofluorescence showed that several markers of ER stress were increased in mouse hearts subjected to *ex vivo* I/R, as well as in surviving cardiac myocytes bordering the infarct zone in a mouse model of *in vivo* myocardial infarction.⁸⁰ XBP1 was activated in cultured neonatal rat ventricular myocytes subjected to simulated ischemia.^{80–82}

In studies designed to determine possible roles of ER stress in the heart, it was shown that dominant-negative XBP1 increased apoptosis in isolated cardiomyocytes in response to simulated I/R, suggesting that in this context, ER stress may be cardioprotective.⁸⁰ In further support of protective roles for ER stress was

the finding that overexpression of activated ATF6 in transgenic mouse hearts decreased ischemic damage and increased ventricular pump function in an *ex vivo* I/R model.⁸³

Thus, ER stress can be activated by ischemia and, in some studies, by I/R; however, it is not clear whether ER stress is protective or damaging in this context. Perhaps mild or brief episodes of ischemia favor activation of pro-survival aspects of ER stress, whereas severe or long episodes lead to eventual activation of proapoptotic aspects. Moreover, it is possible that the time to transition between pro-survival and proapoptotic aspects of ischemia-activated ER stress can be influenced by other factors, including the presence of additional ER stresses, or existent cardiac pathologies, both of which might decrease the time to transition. Further studies are required to delineate the circumstances under which ER stress is protective or damaging in the myocardium and what ER stress-inducible genes and pathways are important contributors to these outcomes in the heart.

ER Stress in the Vasculature

The abnormal deposition of free cholesterol in coronary arteries is toxic to many different vascular cell types, including macrophages, endothelial cells, and smooth muscle cells.⁸⁴ This toxicity leads to eventual apoptosis of these vascular cells, which is believed to promote the progression of atherosclerosis.⁸⁴ Many mechanisms have been proposed to explain cholesterol-induced apoptosis of macrophages; one hypothesis is that free cholesterol accumulation in the plasma membrane disrupts the function of transporters, enzymes and receptors in the plasma membrane, leading eventually to apoptosis.⁸⁵ A second hypothesis suggests that the ER membrane plays a central role in cholesterol-induced apoptosis of vascular cells.⁸⁶ For example, the ER stress response is activated when macrophages accumulate cholesterol, and under these conditions, inhibition of certain aspects of the ER stress response inhibits apoptosis. Moreover, selectively blocking the accumulation of cholesterol in the ER membrane protects macrophages from cholesterol-induced apoptosis.⁸⁷ But how does excess cholesterol activate the ER stress response? Although intracellular cholesterol accumulates in many membranes, including the plasma membrane, mitochondria, and in the ER, excess cholesterol in the ER membrane has been shown to inhibit sarco/endoplasmic reticulum calcium ATPase (SERCA). SERCA inhibition reduces the level of calcium in the ER lumen, which in turn activates the ERSR.⁸⁸ Moreover, because the accumulation of excess cholesterol in the ER membrane is usually chronic, long-term activation of certain aspects of the ERSR lead to macrophage apoptosis.

Another contributor to atherosclerosis is the generation of reactive oxygen species (ROS) in endothelial cells.⁸⁹ Vascular ROS, which is increased by numerous cardiovascular risk factors, is believed to contribute to many aspects of vascular inflammation, including fatty lesion development and eventual plaque rupture.⁹⁰ Recent studies have shown that paraoxonase 2 (PON2) is an ER resident enzyme expressed in all vascular cell types and that it reduces ROS generation in the ER, thus moderating ROS-activated ER stress and reducing apoptosis.⁹¹ In the same study, it was shown that the regulatory region of the PON2 gene has putative ER stress response elements, which are responsible for transcriptional induction

of PON2 during ER stress. In this regard, as an ER-localized ER stress response gene product, PON2 represents 1 of the protective aspects of the ERSR in vascular cells.

Like elevated plasma cholesterol, hyperhomocysteinemia is another risk factor for vascular diseases that may involve the ER stress response.⁹² Homocysteine is generated during the conversion of dietary methionine to cysteine. Mutations in the enzymes that carry out this conversion, as well as certain vitamin deficiencies, have been implicated in hyperhomocysteinemia. Individuals with hyperhomocysteinemia exhibit increased arterial intimal thickening and fibrous plaques in smooth muscle cells, which lead to thrombosis and infarction in a manner analogous to atherosclerosis.⁹³ By disrupting nascent ER protein disulfide formation, homocysteine activates features of the ERSR in vascular endothelial cells,⁹⁴ which causes IRE-1-dependent apoptosis,⁹⁵ as well as disrupting cholesterol and triglyceride biosynthesis pathways.⁹⁶ Accordingly, the ER stress response is likely to contribute to the myriad of mechanisms by which elevated cholesterol or homocysteine increase the risk of vascular disease.

Conclusions

Although the ER stress response has been extensively studied in tumor cells, cell lines, and endocrine and brain tissue, relatively few studies of ER stress have been performed in the cardiovascular context. However, it is now clear that in cardiomyocytes, as well as vascular cells, ER stress can be activated under a variety of conditions, some of which are associated with the development of pathologies. Analogous to the effects of ER stress activation in tumor cells, it is possible that the initial effects of ER stress, which would favor the prosurvival phase of the pathway, might provide cardiovascular protection. However, in contrast to tumor cells, which in some cases are able to remodel ER stress response pathways to provide protection, even during prolonged stress, cardiomyocytes and vascular cells may exhibit a relatively compressed time of transition from prosurvival to proapoptotic phases of ER stress. Accordingly, a better understanding of the basic biology of ER stress in the heart and vasculature is therefore required to fully appreciate the impact of this intricate signaling system to cardiovascular health and disease.

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None.

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