Mechanisms of ER Stress-Induced Apoptosis in Atherosclerosis

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Abstract—Endoplasmic reticulum (ER) stress is triggered by perturbations in ER function such as those caused by protein misfolding or by increases in protein secretion. Eukaryotic cells respond to ER stress by activating 3 ER-resident proteins, activating transcription factor-6, inositol requiring protein-1, and protein kinase RNA-like ER kinase (PERK). These proteins direct signaling pathways that relieve ER stress in a process known as the unfolded protein response (UPR). In pathological settings, however, prolonged UPR activation can promote cell death, and this process has recently emerged as an important concept in atherosclerosis. We review here the evidence for UPR activation and cell death in macrophages, smooth muscle cells, and endothelial cells in the context of advanced atherosclerosis as well as the existing literature regarding mechanisms of UPR-induced cell death. Knowledge in this area may suggest new therapeutic targets relevant to the formation of clinically dangerous atherosclerotic plaques. (Arterioscler Thromb Vasc Biol. 2011;31:2792-2797.)

Key Words: apoptosis ■ atherosclerosis ■ macrophages ■ ER stress ■ unfolded protein response

Introduction: The Unfolded Protein Response
All eukaryotic cells must properly fold secretory proteins in the endoplasmic reticulum (ER), and cells have developed the ability to respond to perturbations in ER function such as an increase in demand for secretory proteins, an increase in misfolded proteins, or decreased folding capacity. These perturbations trigger ER stress, which is sensed by 3 known ER resident proteins: inositol requiring protein-1 (IRE1), PERK, and activating transcription factor-6.1 Activation of each of these stress sensors leads to a series of corrective actions known as the unfolded protein response (UPR), which relieves ER stress by several mechanisms, including inhibition of protein translation, which decreases the load of proteins entering the ER, and induction of chaperone molecules, which promotes proper protein folding.1 The ER can also increase folding capacity through ER biogenesis.2 However, despite the corrective actions of a transient UPR, prolonged ER stress can trigger apoptosis, either through the functions of IRE1 at the ER or through downstream effectors such as CHOP (C/EBP-homologous protein).3 The mechanisms by which UPR signaling proteins switch from a survival function to the induction of apoptosis is a topic of intense study.

ER Stress and Apoptosis in Atherosclerotic Lesional Cells
Animal models of atherosclerosis and, most importantly, analysis of human atherosclerotic lesions have shown clear evidence that ER stress occurs in atherosclerotic plaques, particularly in the advanced stages of the disease.4 As summarized briefly here, significant progress has been made on elucidating the roles of ER stress in the three major cell types in atherosclerosis: macrophages, smooth muscle cells (SMCs), and endothelial cells. For a more comprehensive discussion of this topic, the reader is referred to recent reviews.5–6

Under normal conditions, macrophages ingest apo-B-containing lipoproteins and transport ingested lipoprotein-cholesterol from late endosomes to the ER. Although the ER usually esterifies the cholesterol to form inert lipid droplets, advanced lesional macrophages show evidence of massive unesterified, or “free,” cholesterol accumulation, which may be due to eventual failure of the cholesterol re-esterification process.7–8 In addition, lesional macrophages are exposed to oxysterols, the most abundant of which is 7-ketocholesterol. Both free cholesterol accumulation and 7-ketocholesterol induce ER stress-induced macrophage apoptosis.8–10 In one pathway detailed in the following section, prolonged ER stress leads to CHOP-mediated macrophage apoptosis through a pathway involving release of ER calcium, mitochondrial release of apoptogens, and activation of the death receptor Fas.11–13 Genetic silencing of CHOP leads to a decrease in macrophage death both in vitro and in vivo and decreased plaque rupture in mice.14–15 CHOP expression in macrophages also correlates with a decrease in the antiapo-
ptotic protein BCL-2, which correlates with macrophage apoptosis in vivo. A recent in vitro study also showed that free cholesterol-induced apoptosis in macrophages is decreased with IRE1 silencing.

ER stress is necessary but sometimes not sufficient to induce apoptosis in macrophages. In particular, in vitro and in vivo studies have shown that macrophage apoptosis often requires a “second hit” via pattern recognition receptors, notably scavenger and toll-like receptors. Among the pattern recognition receptor ligands that can trigger apoptosis in ER-stressed macrophages are oxidized phospholipids, which are present in atherosclerotic lesions. Oxidized phospholipids triggers apoptosis in ER-stressed macrophages by activating a CD36-TLR2 pathway that initiates an oxidative burst. This oxidative burst, mediated primarily by NADPH oxidase, is sustained in the presence of prolonged ER stress, and, interestingly, the resulting prolonged oxidative stress further amplifies the CHOP pathway. Genetic silencing of the NADPH oxidase subunit Nox2 leads to a decrease in ER stress-mediated macrophage death.

Apoptosis of SMCs within the plaque may destabilize the lesion due to decreased collagen production and consequent thinning of the protective fibrous cap. Several inducers of ER stress have been identified in SMCs. Cell culture studies have identified increased CHOP expression in SMCs following mechanical stretch, or treatment with 7-ketocholesterol, unesterified cholesterol, homocysteine, or glucosamine. Activation of the IRE1 branch of the UPR has also been identified in the studies using 7-ketocholesterol, homocysteine, and glucosamine. The increase of these ER stress markers in SMCs in vivo has been identified in mouse models of hyperhomocysteinemia and hyperglycemia and in human lesions, yet studies demonstrating a causal relationship between activation of the UPR and SMC apoptosis are lacking. One study used the proteasome inhibitor bortezomib to induce ER stress and SMC apoptosis in vivo, but whether it was the ER stress that actually caused the observed apoptosis is not known. The mechanism of ER stress-induced apoptosis in SMCs is not clear, although it may involve release of ER calcium. Thus, much more work is needed to understand the mechanisms and consequences of ER stress-induced SMC apoptosis in atherosclerosis.

Activation of the UPR has also been identified in endothelial cells both in vitro and in swine. Atherosclerotic-relevant inducers of ER stress in endothelial cells include shear stress from disturbed blood flow, which is associated with activation of the IRE1 branch of the UPR, as well as homocysteine and modified forms of LDL, which are associated with both the IRE1 and CHOP branches of the UPR. Most of these studies have focused on the activation state of endothelial cells related to their role in lipoprotein permeability and recruitment of inflammatory cells rather than on apoptosis. However, an intact endothelium plays a critical role in advanced atherosclerosis because it forms the final barrier of separation between the atherosclerotic plaque and the vessel lumen. Moreover, apoptotic endothelial cells become procoagulant and increase adhesiveness of platelets, which are important processes that occur as a consequence of plaque erosion or rupture. Causal relationships between the UPR and endothelial cell apoptosis in vivo have yet to be established and therefore represent a potentially important opportunity for new research in this area.

Mechanisms of ER Stress-Induced Apoptosis

Prolonged or chronic activation of the 3 UPR pathways can trigger 1 or more proapoptotic signaling pathways (Figure). Studies have traditionally divided UPR-mediated proapoptotic signaling between that mediated by IRE1 and that mediated by CHOP. Although CHOP can be induced by all 3 ER stress sensors, it is most strongly induced by activation of PERK. We review below the general mechanistic principles elucidated for each pathway, though increasing evidence suggests that the precise mechanisms of ER stress-induced apoptosis are cell type- and stimulus-specific.

IRE1-Mediated Apoptosis

IRE1 exists as two isoforms, IRE1-α and IRE1-β, which are differentially expressed by cell type. IRE1-α is the isoform expressed by the cell types described above and hereafter referred to simply as IRE1. IRE1 contains a kinase domain and an endoribonuclease domain. During ER stress, IRE1 is activated by auto-phosphorylation. The nuclease activity of activated IRE1 triggers the splicing and subsequent translation of a specific mRNA transcript for X-box binding protein-1, a transcription factor that induces several chaperones and other mediators that help reduce ER stress. At higher levels of stress signaling, however, IRE1 activation may trigger the generalized splicing of essential mRNAs rather than X-box binding protein-1 specifically. The degradation of essential membrane-associated mRNAs, a process known as regulated ire1 dependent decay (RIDD), may lead to apoptosis. Excessive IRE1 autophosphorylation and RIDD have been closely linked with the kinase function of IRE1, as kinase-inhibited IRE1 maintains a preference for prosurvival X-box binding protein-1-splicing over RIDD-mediated apoptosis. Although RIDD has been demonstrated in a pancreatic beta cell culture model of ER stress and apoptosis, no study has demonstrated RIDD in cells of the cardiovascular system, and in vivo evidence for RIDD-induced apoptosis has not yet been demonstrated.

Other downstream effectors of proapoptotic IRE1 signaling include the proapoptotic members of BCL-2 family of proteins (see below) and c-Jun N-terminal kinase (JNK). Activation of JNK, which can be proapoptotic, has been demonstrated to be partially IRE1-dependent. The functional role of JNK in apoptosis is not clear, although JNK has been shown in vitro to activate the proapoptotic protein BIM and deactivate the antiapoptotic protein BCL-2, an antiapoptotic protein that functions as an inhibitor of the proapoptotic proteins BAX and BAK. IRE1 has also been more directly linked to BAX and BAK through IRE1-mediated cytochrome c release from the mitochondria and by direct interaction with BAX and BAK at the ER. Despite many elegant in vitro studies of IRE1-mediated stress signaling and apoptosis, there is not yet any in vivo evidence for IRE1-dependent apoptosis.

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CHOP-Mediated Apoptosis

During ER stress, ER-bound PERK becomes phosphorylated, and its proapoptotic function is exerted through phosphorylation of eukaryotic translation initiation factor 2 alpha, which in turn promotes translation of activating transcription factor 4 and subsequent induction of CHOP. CHOP can also be induced by activating transcription factor-6 and XBP-1, but the PERK-eukaryotic translation initiation factor 2 alpha pathway is dominant.37

One mechanism of CHOP-induced apoptosis involves interaction with members of the BCL-2 family of proteins. One such interaction involves transcriptional downregulation of BCL-2. This mechanism has been demonstrated in vitro in a CHOP-transfected rat fibroblast cell line and in cultured cortical neurons.48–50 Genetic deletion of macrophage Bcl2 in vivo increased macrophage apoptosis in advanced atherosclerotic lesions, consistent with the proapoptotic mechanism of CHOP-mediated downregulation of Bcl2.16,51 CHOP can also induce the transcription of BIM, which plays a role in mitochondrial-mediated apoptosis.52

A second major mechanism of CHOP-induced apoptosis involves a calcium signaling pathway. CHOP-dependent activation of the ER oxidase 1α triggers ER calcium release by activating the ER calcium channel IP3R1.13 Cytoplasmic calcium activates the calcium-sensing enzyme CaMKII, which in turn triggers a number of downstream apoptosis pathways, including induction of the Fas death receptor, activation of JNK, and mitochondrial release of apoptogens.12

Potential Integration of the UPR Pathways: The BCL-2 Protein Family

Despite many studies on IRE1 and CHOP-mediated apoptosis, there are few studies in which both pathways are analyzed in the same model system. It is therefore unclear if the proapoptotic branches of the UPR are distinct pathways or if they converge to induce apoptosis by one or more common downstream effectors. The BCL-2 family of proteins is one potential point of integration between IRE1 and CHOP-mediated apoptosis (Fig. 1).53 Many mechanisms of ER stress-mediated apoptosis have been shown to involve at least 1 member of the BCL-2 family, although these studies need to be carefully interpreted in view of the myriad cell types and stressors used to investigate their roles in ER stress-induced apoptosis.

The BCL-2 family includes both antiapoptotic and proapoptotic members that regulate cross talk between the ER and mitochondria. The most well-characterized proapoptotic BCL-2 family members are BAX and BAK. During intrinsic...
stress signaling, these two proteins become activated and oligomerize in the mitochondrial membrane, which induces the release of cytochrome c and other apoptogens from the mitochondria.\(^5\) Cytochrome c then forms a complex with other apoptosome components, which activates caspase-9 and triggers apoptosis.\(^5\) Protection of the mitochondria, therefore, may be an important therapeutic target for preventing ER stress-induced apoptosis.

More than 10 BCL-2 family members have been identified as BAX/BAK activators in various cell types.\(^5\) The activators, which are termed BH3-only proteins because they share a common BH3 protein domain, are brought into play by either transcriptional upregulation or by post-translational events such as phosphorylation. On activation, they translocate to the mitochondria and bind and activate BAX/BAK or downregulate the BAX/BAK suppressors. For example, activation of BID by proteolytic cleavage or BIM by phosphorylation leads to translocation to the mitochondria, activation of BAX/BAK, and proapoptotic cytochrome c release.\(^5,5\) However, emerging evidence suggests that the BCL-2 family members that suppress and activate BAX/BAK are cell type- and stimulus-specific. Furthermore, some cell types may have multiple BH3-only members acting in a redundant fashion, as has been shown recently for BID, BIM, and PUMA.\(^5\)

There are at least 7 known BCL-2 family members that are suppressors of BAX/BAK activation. These proteins function by binding and sequestering either BAX/BAK activator proteins or BAX and BAK themselves in the mitochondria. The net result of either mechanism is the prevention of BAX/BAK activation.\(^5\) BCL-2 and BCL-xL are examples of BAX/BAK suppressors, and BCL-2 has been shown to be downregulated by both CHOP (above) and JNK.\(^5,48\)

Recent studies have demonstrated that certain BCL-2 family proteins can also be localized to the ER. At the ER, proteins such as BAX and BAK regulate calcium homeostasis, which, as mentioned above, can be disrupted during ER stress.\(^5\) Nonetheless, all of the BCL-2 signaling mechanisms in the literature have pointed toward an intrinsic, mitochondria-dependent, pathway of apoptosis. A major challenge for the field now is to identify the precise BCL-2 family members regulating ER stress-induced apoptosis specifically in cardiovascular disease, as well as the relative contribution of each upstream UPR pathway to BCL-2 family activation.

**Summary and Future Directions**

Evidence from many laboratories indicates that cells in atherosclerotic lesions, particularly advanced atherosclerotic lesions, undergo a heightened and/or prolonged state of ER stress. In the case of macrophages, prolonged ER stress is likely a significant contributor to advanced lesion macrophage death and ensuing plaque necrosis. Although not reviewed here, prolonged ER stress may also enhance the inflammatory response of plaque macrophages.\(^6\) The roles of ER stress in SMCs and endothelial cells are much less certain and thus represent important areas of future study. In terms of mechanistic insight, more work is needed to determine how the different branches of the UPR may conspire, eg through coordination of the Bcl2 family of proteins and/or JNK, to cause cell death, inflammation, and other proatherogenic effects in all 3 cell types. Moreover, our understanding of the precise molecular links between the UPR effector molecules and death signaling and inflammation is not complete, and filling in these critical gaps is an important future goal. In all cases, relevance to various stages of atherosclerosis will need to be tested using precise molecular-genetic and pharmacological causation strategies in animal models of atherosclerosis.

As our understanding of the critical role of ER stress in atherosclerosis increases, so too do the number of opportunities to therapeutically target this process in atherosclerosis. In considering potential anti-ER stress strategies, however, one should note that the UPR is also a survival mechanism, and careful consideration of the contribution of each UPR branch to the progression of atherosclerosis must be understood if any UPR pathways can be modulated for therapeutic gain. One approach to relieving ER stress therapeutically has been to use low molecular weight compounds called chaperones, which decrease ER stress by facilitating proper folding and decreasing the accumulation of misfolded proteins in the ER.\(^62\)–\(^64\) Two such compounds are 4-phenyl butyrate and taurine-conjugated deoxycholic acid. 4-phenyl butyrate was shown to decrease lesion area, and lesional ER stress and apoptosis in Western diet-fed Apoe\(^{-/-}\) mice.\(^6\) Treatment of Western diet-fed Ampka2\(^{-/-}\)/Ldrl\(^{-/-}\) mice with taurine-conjugated deoxycholic acid resulted in a decrease in lesional ER stress and atherosclerotic lesion area.\(^6\) However, the exact mechanism for these improvements remains undefined and additional studies are needed. The results of additional studies discussed in this review should help identify additional novel therapeutic approaches, particularly those focused on stabilizing the minority of dangerous plaques that cause acute cardiovascular disease.

**Disclosures**

None.

**References**


