A recent coronary syndrome (ACS) represents a series of indications of sudden cardiac ischemia that involve a variety of causes, including acute myocardial infarction and unstable angina. ACS usually is associated with thrombus formation in the coronary artery or coronary artery vasospasm, resulting in myocardial ischemia. Thrombus formation may involve platelet adhesion and degranulation on damaged or dysfunctional endothelium overlaying the intact or ruptured atherosclerotic plaque, as well as microthrombi. Atherosclerotic plaque rupture represents a series of deleterious events linked to the breakdown of the fibrous cap. This process may occur as a result of the increased secretion of matrix metalloproteinases by lesion resident macrophages or enhanced apoptotic cell death within the fibrous cap. However, viable smooth muscle cells (SMCs) within the fibrous cap synthesize interstitial collagen fibers that enhance the structural integrity of the plaque and prevent its rupture.

Protein folding in the ER is aided by molecular chaperones that prevent the aggregation of misfolded proteins and assist proteins in overcoming the thermodynamic barriers that occur during the intermediate misfolded conformation to a final correctly folded and functional molecule. These ER resident molecular chaperones, including the 78-kDa glucose-regulated protein (GRP78) and protein disulfide isomerase, are induced by agents and/or conditions that cause protein misfolding in the lumen of the ER. Recent studies have identified several ER stress–inducing agents that are relevant to ACS, including homocysteine, peroxynitrite, and oxysterols. If an increase in molecular chaperones is unable to assist in the folding and/or clearance of misfolded proteins after exposure to these ER stress–inducing agents, activation of the UPR may trigger cellular apoptosis. It is well documented that the UPR signaling cascade contains late-phase proteins that are proapoptotic, including CHOP and TDAG51, whereas GRP78 has been shown to protect cells from apoptosis.

In this issue of Circulation, Myoishi and colleagues provide convincing evidence for the importance of ER stress in ACS. Coronary artery segments were obtained at autopsy (152 specimens from 71 patients) and tissue obtained by directional coronary atherectomy (40 patients). The autopsy specimens were classified into 5 groups: diffuse intimal thickening (normal), fibrous plaques (fibrous), thick-cap atheroma (thick), thin-cap atheroma (thin), and ruptured plaques (ruptured). The directional coronary atherectomy specimens were classified according to clinical manifestation as stable angina pectoris and unstable angina pectoris. Specifically, the authors demonstrated in the cap region the association between ER stress markers, including GRP78 and CHOP, and thin-walled or ruptured atherosclerotic plaques and unstable angina in coronary artery disease. Earlier studies performed in animal models of atherosclerosis showed that the ER stress markers GRP78 and CHOP are indeed upregulated in atherosclerotic lesions. However, Myoishi and colleagues have demonstrated for the first time in human coronary artery disease that thin-walled plaques prone to
rupture and ruptured plaques show increased expression of ER stress markers, compared with stable plaques. Immunohistochemical studies showed increased staining with antibodies directed against the C-terminal KDEL retention sequence (known to recognize GRP78 and GRP94) or the proapoptotic gene CHOP were upregulated. KDEL upregulation was likely due to an increase in the ER resident molecular chaperone GRP78 as detected by increased mRNA expression with in situ hybridization in thin-walled plaques. In addition, in the atherec-tomy specimens, the number of KDEL- and CHOP-positive cells was significantly higher in unstable than in stable angina pectoris. From these findings, the authors propose a positive correlation between ER stress marker expression and plaque vulnerability.

Thin-cap fibroatheroma is associated with a high risk of ACS and is postulated to be a precursor to plaque rupture. Thin-cap fibroatheromas and ruptured lesions are characterized by a thin (<65 μm) cap and, unlike stable thick-cap and fibrous lesions, contain a large number of macrophages in the cap region.20,21 This was indeed illustrated (but not commented on by the authors) in this study; the representative pictures of the normal, fibrous, thick, and stable angina pectoris groups showed almost no macrophages by CD68 immunostaining. However, in the thin, ruptured, and unstable angina pectoris groups, a large number of macrophages were identified by CD68 immunostaining. This indicates an active disease process in which macrophages/foam cells produce cytokines and matrix metalloproteinases and stimulate SMCs. Both macrophages and SMCs in these active lesions expressed KDEL and CHOP. These markers, however, were not observed in the normal, thick, and fibrous plaques or in directional coronary atherectomy specimens representing stable angina. This suggests that stable lesions were not undergoing ER stress.

Although it is clear that ER stress is involved in the disease process leading to ACS, it is less clear how this ER stress is caused. To further explore a potential molecular mechanism for ER stress activation within the unstable lesions, coronary artery sections were immunostained with an antibody against the oxysterol, 7-ketocholesterol (7-KC). Increased staining for 7-KC was observed in the thin-cap atheromas, whereas no immunoreactivity was observed in thick-cap atheromas.

The involvement of 7-KC in coronary artery disease was further examined through in vitro experimentation. Coronary artery SMCs or THP-1 cells, a human monocytic leukemia cell line, exposed to 7-KC showed increased steady-state mRNA levels of GRP78 and CHOP. The induction of ER stress marker expression after incubation with 7-KC was prevented with the addition of the antioxidant glutathione or its precursor N-acetylcysteine. The exposure of cells to 7-KC induced the intracellular production of reactive oxygen species (ROS), as shown by DCFA fluorescence, whereas the addition of glutathione decreased the DCFA signal. The effect of 7-KC on apoptotic...
cell death was examined in coronary artery SMCs and THP-1 cells with a terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling assay. Indeed, the addition of 7-KC induced CHOP expression and increased apoptotic cell death.

It has been previously reported that 7-KC induces ROS, ER stress, and apoptosis in cultured human SMCs.13 This earlier study found that 7-KC induced apoptosis through the upregulation of the ROS-generating NAD(P)H oxidase subunit Nox-4. It was found that 7-KC induction of GRP78 and CHOP was dependent on Nox-4 expression. 7-KC–induced Nox-4 expression was found to be dependent on the ER stress sensor Ire-1. Interestingly, Nox-4 upregulation preceded ER stress because siRNA knockdown of Nox-4 inhibited CHOP and GRP78 expression and subsequent apoptosis. However, it remains unclear how Nox-4–driven ROS production results in protein misfolding in the ER. Previous studies that examined cholesterol loading in the ER membrane were able to determine an effect on ER Ca2+ depletion, leading to ER stress.10 Although it has been demonstrated that 7-KC specifically induces Ca2+ oscillation in SMCs, siRNA knockdown of Nox-4 had no effect on these Ca2+ perturbations. 7-KC–induced ROS production was shown to be detectable with DCFA, whereas polyethylene glycol/superoxide dismutase reduced this effect,13 implying that superoxide and hydrogen peroxide were generated. Determining the role of these ROS in ER stress induction, if any, might clarify the mechanism behind 7-KC–induced ER stress.

Unresolved ER stress leads to apoptosis of SMCs and likely contributes to plaque rupture. To further the hypothesis of Myoishi and colleagues that ER stress causes plaque rupture, the ability to block ER stress–induced apoptosis in animal models of atherosclerosis may provide answers. Although established animal models of plaque rupture do not exist, studies in mouse models of atherosclerosis could examine surrogate measures of rupture, including plaque and necrotic core size or rate of SMC apoptosis. Knockout of ER stress proapoptotic genes CHOP or TDAG51 would provide at least partial answers to the role of ER stress–induced apoptosis as a cause of ACS. Ultimately, therapeutic strategies that resolve ER stress such as small chemical chaperones that mimic the effect of endogenous ER resident molecular chaperones22 and thus prevent ER stress–induced apoptosis may be of tremendous value in stabilizing thin-cap atheroma and preventing ACS.

The initiation and progression of atherosclerotic disease appear to involve ER stress/UPR activation, as observed in apolipoprotein E–deficient mice fed a control chow diet (Figure).18 The resolution of this cellular stress response over time, be it increased protein synthetic capacity of lesion-resident SMCs or the induction of apoptotic cell death resulting from conditions or agents that cause ER stress, may ultimately determine the fate of the fibrous cap and the risk of plaque rupture in ACS.

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None.

References

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Increased Endoplasmic Reticulum Stress in Atherosclerotic Plaques Associated With Acute Coronary Syndrome: A Balancing Act Between Plaque Stability and Rupture

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