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.4

SMCs within the vascular wall exist in predominantly 2 phenotypes: a contractile phenotype and a synthetic phenotype. Transformation from the quiescent contractile phenotype to the active synthetic phenotype can be stimulated by the products of coagulation, including platelet-derived growth factors.3 Indeed, it has been shown that aortic allografts undergoing atherosclerotic changes first experience endothelial disruption, then SMC activation and proliferation accompanied by increased synthesis of extracellular matrix proteins.6 The synthetic SMC phenotype is actively involved in de novo protein synthesis and as such may experience endoplasmic reticulum (ER) stress if the protein folding machinery of the ER is overwhelmed by the demand of newly synthesized proteins.7 As a consequence of this ER stress, the cell activates an evolutionarily conserved pathway called the unfolded protein response (UPR). Depending on the duration or severity of ER stress, activation of the UPR can increase cell survival or lead to apoptotic cell death.

The primary function of the ER involves the synthesis and processing of correctly folded secretory or plasma membrane proteins.8 De novo protein synthesis in the ER is under a quality control mechanism that prevents the secretion or incorporation in the membrane of misfolded proteins.9 The correct tertiary structure of a protein is key to its function; thus, aberrantly folded proteins within the ER must be efficiently identified, retained, and disposed of to prevent pathological consequences. The recent emergence of misfolded proteins capable of propagating protein misfolding, namely the autocatalytic theory of prion propagation, represents a pathological condition transmitted by protein misfolding10 and underscores the importance of a functional quality control system in the cell.

Protein folding in the ER is aided by molecular chaperones including GRP78 and CHOP, and thin-walled or ruptured plaques (ruptured). The association between ER stress markers, including GRP78 and CHOP, and thin-walled or ruptured plaques (ruptured) is available at http://circ.ahajournals.org.
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 atherectomy 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. 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 DCF 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. 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 Ca\(^{2+}\) depletion, leading to ER stress. Although it has been demonstrated that 7-KC specifically induces Ca\(^{2+}\) oscillation in SMCs, siRNA knockdown of Nox-4 had no effect on these Ca\(^{2+}\) perturbations. 7-KC–induced ROS production was shown to be detectable with DCFa, whereas polyethylene glycol/superoxide dismutase reduced this effect, 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 Myoishii 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 chaperones 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). 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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**Disclosures**

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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