Review Article

Role of Endoplasmic Reticulum Stress in Atherosclerosis and Diabetic Macrovascular Complications

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1. Introduction

The endoplasmic reticulum (ER) is a complex cytoplasmic membrane structure presented in eukaryotic cells. ER is involved in protein folding, lipid synthesis, and regulation of the intracellular calcium balance [1]. Secretory and membrane proteins, which are synthesized in ER, undergo proper folding in the ER lumen. ER chaperones such as glucose-regulated protein 78 kDa (GRP78 or BiP) and GRP94, oxidoreductases, and high calcium concentrations are essential for proper protein folding and assembling [2]. GRP78 is a Ca\(^{2+}\)-dependent chaperone that is responsible for the folding of hydrophobic protein regions [3]. Protein disulfide isomerase (PDI) is involved in the formation of disulfide bonds whereas ER thiol oxidase (EROI) initiates disulfide transfer to oxidized proteins [4]. Aging-related changes in ER are associated with stress of this cell organelle [5]. The oxidative protein folding is associated with advanced production of reactive oxygen species (ROS) that may lead to extensive oxidative stress and cell apoptosis [6]. Indeed, the ER is vulnerable to various stressors capable of disturbing the redox homeostasis in the ER lumen.

2. Unfolded Protein Response

Incompletely folded or misfolded proteins are subjected to ER-associated degradation (ERAD) that occurs in cytoplasm. ER-mediated protein synthesis and folding are strictly regulated. Impairments in ER folding capacity may result in the
accumulation of unfolded proteins and induce ER stress. In the ER, three proteins are able to sense increase in misfolded polypeptides and initiate the unfolded protein response (UPR). The UPR sensors include activating transcription factor-6 (ATF6), inositol requiring protein-1 (IRE1), and protein kinase RNA-like ER kinase (PERK). All three proteins have domains exposed to the ER lumen and are capable of binding GRP78 [7]. In normal conditions, GRP78 is bound to the molecules of UPR sensors. In ER stress, GRP78 dissociates from the UPR sensors that leads in turn to induction of UPR (Figure 1).

The UPR applies several mechanisms to minimize ER stress. One of those mechanisms involves the activation of chaperone synthesis in order to improve and intensify the intraluminal protein folding. Another mechanism includes protein translation arrest in order to prevent further protein load into the ER [26]. The ER folding capacity may be also improved indirectly, through stimulating ER biogenesis [6]. In a case of chronic long-term or acute ER stress, when the adaptive UPR is unable to stop the stress, apoptotic pathways are activated in the stressed cell [27]. ER sensor proteins such as IRE1 and PERK are involved in both the adaptive and the proapoptotic UPR pathways.

2.1. IRE1. Among ER stress sensors, IRE1 is the most evolutionarily preserved. In normal conditions, IRE1 and GRP78 interact with each other, and this prevents IRE1 activation [6]. In ER stress, GRP78 becomes released from the complex with IRE1. IRE1 is then activated by self-phosphorylation. The active IRE1 is able to specifically splice mRNA encoding X-box binding protein (XBPI) thereby inducing translation of functionally active XBPI [1]. XBPI induces transcription of chaperones and other UPR-related proteins and enhances the degradation of misfolded proteins [28]. By degrading mRNAs other than XBPI, IRE1 contributes to reducing protein load to the ER [29].

However, in long-lasting ER stress, IRE1 can be involved in activation of proinflammatory pathways and apoptosis. IRE1 forms a complex with the adaptor protein tumor necrosis factor (TNF) receptor-associated factor (TRAF)2 [30] that in turn recruits mitogen-activated protein kinase, apoptosis signal-regulating kinase (ASK) [31], and caspase 12 [32]. The complex activates IκB kinase followed by IκB kinase-mediated suppression of the inhibitor of κB protein and induction of the nuclear factor (NF)-κB. Since NF-κB controls expression of many proinflammatory genes, IRE1 is therefore suggested to provide a link between the ER stress and inflammation [33].

2.2. PERK. This ER stress sensor molecule belongs to the family of serine threonine kinase and has a high degree of homology with IRE1. Both IRE1 and PERK share similar mechanisms of activation involving dissociation of GRP78 from the luminal binding domain and self-phosphorylation upon stress conditions. After activation, PERK downregulates eukaryotic translation initiation factor 2α (eIF2α) that is needed for cap recognition and therefore is essential for further induction of cap-dependent transcription. eIF2α inactivation results in marked decrease of protein load to the ER [6]. Interestingly, phosphorylated eIF2α is responsible for translation of several mRNAs including mRNA for transcriptional factor ATF4. This factor is responsible for the induction of the negative feedback regulatory loop since it activates expression of GADD34, a regulatory subunit of the phosphatase that dephosphorylates eIF2α and restores cap-dependent translation [34]. Indeed, ATF4 regulates protein translation during ER stress.

ATF4 stimulates expression of C/EBPx-homologous protein (CHOP, or GADD153). CHOP expression can be also induced by ATF6 and XBPI, but the PERK-eIF2α-dependent pathway is prevalent [35]. CHOP is a transcription factor that induces apoptosis through several mechanisms including upregulation of ERO1α, which then mediates Ca²⁺-dependent apoptotic pathway, and downregulation of anti-apoptotic factors Bcl-2 and Bnip3 [36, 37].

ERO1α is involved in reoxidation of PDI yielding production of hydrogen peroxide, a byproduct of disulfide bond formation [38]. Therefore, ER stress-induced upregulation of ERO1α may lead to ROS overproduction and advanced oxidative stress that in turn contribute to cell apoptosis [5]. ERO1α activation stimulates inositol-1,4,5-trisphosphate receptor-1 (IP3R1), a ER-associated Ca²⁺ channel [39] that triggers depletion of the intraluminal calcium reservoir. Increase in cytoplasmic Ca²⁺ promotes stimulation of calcium/calmodulin-dependent protein kinase II, which plays a key role in induction of several proapoptotic pathways including activation of the death receptor FAS and mitochondrial release of apoptogens [40]. CHOP is directly involved in induction of expression and translocation to the ER membrane of the proapoptotic protein Bim [41].

2.3. ATF6. Upon initiation of ER stress, ATF6 is cleaved by two (site 1 and site 2) proteases associated with the Golgi complex. After cleavage, the cytosolic N-domain of ATF6 translocates into the nucleus where it triggers expression of the death receptor FAS and mitochondrial release of apoptogens [40]. CHOP is directly involved in induction of expression and translocation to the ER membrane of the proapoptotic protein Bim [41].

3. Role of ER Stress in Atherosclerosis

Prolonged ER stress observed in atherosclerotic lesions is an important contributor to proatherogenic progression [45]. ER stress was found in all major cell type in atherosclerosis including macrophages, vascular smooth muscle cells (VSMCs), and endothelial cells (ECs).

3.1. ER Stress in Macrophages. In normal macrophages, low density lipoprotein (LDL) cholesterol particles are loaded from late endosomes to the ER. In the ER, cholesterol is esterified and accumulates to form inert lipid droplets [46]. In atherosclerotic macrophages, ER-mediated cholesterol reesterification is markedly reduced or failed resulting in heavy intracellular deposits of nonesterified cholesterol in
Figure 1: The adaptive and proapoptotic UPR pathways. (a) Adaptive UPR mechanism. In nonstressed conditions, the ER chaperone GRP78 binds to all three ER stress sensors such as PERK, IRE1, and ATF6. In ER stress, GRP78 dissociates from the ER sensors, and this leads to their activation. eIF2\(\alpha\) is phosphorylated by PERK and dephosphorylated by GADD34. Phosphorylated eIF2\(\alpha\) blocks global protein translation but remains selective translation of several proteins including transcriptional factor ATF4. ATF4 then initiates expression of UPR-related genes. Upon activation, ATF6 translocates from the ER to the Golgi complex where it is cleaved by proteases S1P and S2P. Cleaved ATF6 acts as a transcriptional factor activating expression of several UPR- and non-UPR genes including XBP1. Activated IRE1 specifically splices XBP1 mRNA. Spliced XBP1 shows transcription factor activity to induce UPR- and non-UPR genes. Proteasome plays an important role in degradation of unfolded and misfolded proteins. Thus, production of proteasome components is also stimulated to increase utilization of misfolded proteins through the mechanism of ERAD. (b) Proapoptotic UPR mechanism. The apoptotic pathway is induced in chronic and prolonged ER stress. CHOP plays a key role in mediating ER stress-induced apoptosis. CHOP expression is stimulated by ATF4- and ATF6. CHOP represses expression of antiapoptotic proteins Bcl-2 and Bnip3 and activates translocation of proapoptotic protein Bim to the ER membrane. IRE1\(\alpha\) forms a complex with the adaptor protein TRAF2, which consequently activates ASK1 and JNK. Activation of JNK induces apoptosis cell through phosphorylation of several Bcl-2 family members. The IRE1\(\alpha\)/TRAF2 complex also binds to I\(\kappa\)B kinase, and this results in activation of transcription factor NF-\(\kappa\)B. Prolonged ER stress activates caspase 12 that in turn activates caspase-9/3 thereby leading to the mitochondria-independent apoptotic pathway.

Figure 2: Electron microscopy observations revealed that ER in atherosclerotic macrophages undergoes a remarkable change. In foam cells, intraluminal ER oxidoreductases oxidize cholesterol to 7-ketocholesterol (7-KC) and other oxysterols. Oxysterols are highly cytotoxic and may induce cell death through ROS-mediated oxidative damage and other mechanisms.

Prolonged ER stress contributes to apoptosis of lesional macrophages. Apoptosis associated with robust expression of CHOP was shown in human lesions [45] and atherosclerotic plaques of apolipoprotein (apo)E-deficient mice [49]. Inactivating Chop in apoE-deficient mice leads to decreased rates of macrophage apoptosis and plaque necrosis [50, 51]. CHOP contributes to ER stress-induced macrophage death by inducing Fas activation, depletion of ER-associated calcium stores, and release of apoptogens from mitochondria [52].

In early plaques, apoptotic cells are quickly phagocytized by macrophages [53]. This process is driven by anti-inflammatory cytokines such as transforming growth factor-(TGF-\(\beta\)) and interleukin- (IL-) 10 [54]. In advanced plaques, macrophages cannot efficiently clear dying cells that become necrotic [55]. This results in the formation of the inflammatory necrotic core [56].
In some circumstances, ER stress alone is not strong enough to induce apoptosis in macrophages. Additional stimuli such as the activation of pattern recognition receptors (PRRs) are required to initiate cell death [56]. PRRs include various Toll-like receptors (TLRs) and scavenger receptors. In plaque macrophages, PRRs may be activated by oxidized lipids, and this leads to the induction of apoptosis via CD36-TLR2 pathway and is accompanied with NADPH oxidase-mediated oxidative stress [57]. NADPH oxidase contains a subunit Nox2 whose inhibition minimizes ER stress-induced macrophage death [58]. These findings suggest a central role of this enzyme as a link between the oxidative stress and ER stress in promoting macrophage apoptosis. In addition, upregulation of NADPH oxidase further aggravates apoptotic process through stimulating PERK-CHOP-dependent mechanism.

At low doses, ER stress inducers such as modified (oxidized and acetylated) LDL, 7-KC, and peroxynitrite donor SIN are able to stimulate macrophage PRRs and cause NADPH oxidase-mediated ROS production [56, 59]. In a “two-hit” hypothesis, ER stress in plaque macrophages should be induced by a low-dose ER stressor such as PRR ligands that in turn triggers macrophage apoptosis [57, 59].

Lipoprotein(a) [Lp(a)], an LDL-like lipoprotein, and oxidized phospholipids are established to represent strong risk factors for human atherosclerosis [59]. To initiate apoptosis in ER-stressed macrophages, both atherogenic lipid inducers utilize similar mechanisms involving the activation of CD36-TLR2 signaling and oxidative stress [57]. Lp(a) is a major carrier of oxidized phospholipids in human blood [60]. According to the “two-hit” hypothesis, Lp(a) could therefore mediate apoptosis in human plaque macrophages.

3.2. ER Stress in Endothelial Cells. In EC, various ER stress inducers were shown to initiate UPR. For example, shear stress activates IRE1-dependent UPR [61, 62] whereas oxidized phospholipids and homocysteine induce both IRE1- and CHOP-mediated pathways [63–65]. In dynamic models of shear stress, a variety of UPR-related molecules including ATF6, GRP78, IRE1, and XBPI were upregulated in ECs.
[61, 62, 66, 67]. XBP1 is always overexpressed in advanced plaques, a finding that may reflect a proatherogenic role of long-term XBP1 upregulation whereas limited stimulation of this ER stress effector may be protective against ER stress [61].

ER stress induced by modified (oxidized and glycosylated) LDL results in the development of oxidative stress and oxidation-mediated inhibition of sarcoplasmic/endoplasmic reticulum Ca2+-dependent ATPase (SERCA), a calcium pump resided in the ER [8]. AMP kinase (AMPK) α2 suppresses SERCA oxidation, and inhibition of this kinase in LDL receptor- (Ldlr-) deficient mice leads to advanced ER stress and atherogenesis [9]. Thus, alterations in calcium homeostasis caused by oxidative stress play a crucial role in ER stress-mediated endothelial dysfunction in atherosclerotic vessels. ER stress-induced apoptosis diminishes the barrier function of the vascular endothelium and induces procoagulant phenotypic changes in ECs that may be directly responsible for increased risk of thrombosis and other late atherosclerotic complications [68].

3.3. ER Stress in VSMCs. A stable plaque phenotype may be critically disturbed by apoptosis in VSMCs that alters the formation of the protective fibrous cap [69]. In VSMCs, CHOP-mediated apoptotic mechanism may be induced by numerous ER stressors such as 7-KC, homocysteine, glucosamine, free cholesterol, and others [70–74]. CHOP-dependent apoptosis is accompanied with enhanced formation and release of ROS, and N-acetylcysteine, an anti-oxidant, may therefore protect cultured VSMCs against apoptotic death [73].

Elevated plasma levels of homocysteine are considered to increase atherosclerotic risk in humans and animal models [71, 75]. Hyperhomocysteinemia is believed to induce ER stress through alterations of calcium balance [76] and upregulation of sterol response element binding protein-2 (SREBP-2) that increases lipid deposits in VSMCs [77, 78]. Glucosamine that accumulates in vascular cells in diabetes may have a primary responsibility for ER stress induction in VSMCs of diabetic patients associated with GRP78 upregulation [74]. However, the mechanisms of ER stress-mediated apoptosis in VSMCs are significantly less studied compared to those of macrophages and ECs.

4. ER Stress and Obesity

The human body is able to accumulate extra fat in the adipose tissue to survive in starvation. Normally, fat is deposited in adipocytes. However, regular intake of fat-rich diets and alterations in lipid metabolism may lead to the phenomenon of ectopic fat storage, when fat accumulates not only in adipocytes but also in nonadipocyte cells. In obesity, higher free fatty acids levels may enhance lipid accumulation in macrophages and promote formation of foam cells [79].

In obese people, adipocytes are particularly vulnerable to ER stress and apoptosis due to abnormal fat deposits and upregulated lipid metabolism [80]. Macrophages resided in the adipose tissue phagocytize both the extra fat droplets and apoptotic adipocytes releasing high amounts of ROS by mitochondria. Excessive ROS production drives further progression of cellular stress and increases secretion of adipokines in adipocytes [81]. Adipokines promote preferential differentiation of macrophages towards the proinflammatory M1 phenotype [82].

Adiposity is associated with enhanced M1 macrophage-dependent production of multiple proinflammatory mediators such as IL-1β, IL-6, TNF-α, and CXCL10. M1 macrophages inhibit adipocyte hypertrophy and adipogenesis [83] and support low-grade inflammation in the adipose and nonadipose tissues including vessels [84]. In lesional macrophages, adiposity promotes ER stress by activation of the macrophage fatty acid-binding protein-4, also known as adipocyte fatty acid binding protein aP2 that mediates transfer of saturated fatty acids (SFAs) [19]. Increase in SFA levels leads to the induction of apoptosis in macrophages. ApoE-deficient mice lacking aP2 have reduced atherosclerotic lesions, in which expression of XBP1 and PERK is downregulated and macrophage apoptosis is decreased [85]. Inactivation of aP2 protects macrophages from palmitate-induced ER stress and apoptosis [19]. In aP2-deficient macrophages, expression of transcription factor LXRα is activated, and this factor stimulates transcription of stearoyl-CoA-desaturase 1, an enzyme converting SFAs to monounsaturated fatty acids, which are significantly less potent of inducing ER stress [86]. Indeed, activation of LXRα in aP2-deficient macrophages prevents ER stress while overexpression of aP2 in macrophages and adipocytes, in contrast, supports ER stress induction and atherogenesis.

This protective effect is mediated by increased expression of transcription factor LXRα in aP2-deficient macrophages. This factor activates expression of stearoyl-CoA-desaturase 1, converting SFAs to monounsaturated fatty acids that are significantly less capable of inducing ER stress [86].

5. ER Stress and Diabetes

5.1. Insulin Resistance-Induced ER Stress in Macrophages. In diabetic subjects with atherosclerosis, the proatherogenic role of ER stress and CHOP-mediated macrophage apoptosis is significantly enhanced that results in the development of advanced plaques with the especially large necrotic core [87, 88]. Macrophages were shown to have functional insulin receptors, and insulin resistance (IR) is a potent inducer of chronic ER stress in macrophages [89]. High insulin doses suppress insulin signaling in macrophages [88]. Under diabetic conditions, insulin signal transduction in macrophages is also inhibited by diacylglycerol-dependent activation of protein kinase C [90].

Expression of the scavenger receptor SRA is markedly upregulated in IR macrophages. Indeed, according to the “two-hit” hypothesis, these macrophages should be particularly sensitive to PRR-driven ER stress and apoptosis [91]. Experiments with cultured IR macrophages loaded with lipoprotein-derived free cholesterol do show markedly increased apoptosis that suggest a key role of SRA-induced mechanism of ER stress in mediating death of IR macrophages [92]. In these macrophages, MEK/ERK/cAMP-responsive element-binding protein (CREBP) signaling and
5.2. Glucosamine-Induced ER Stress. Diabetic hyperglycemia significantly increases cardiovascular risk inducing vascular dysfunction through inhibitory effects on proliferation of vascular cells and enhancement of their apoptosis [95–98]. Several pathologic mechanisms link diabetic hyperglycemia and atherosclerosis. Activation of the aldose reductase pathway alters redox homeostasis and promotes oxidative stress-mediated damage of vascular cells [99]. High glucose induces overactivity of protein kinase C that leads to reduced endothelial vasodilation [100] and increased ROS production [101]. Nonenzymatic glycation is markedly increased in diabetic patients, and this results in uncontrolled production of advanced glycation end-products (AGEs) [102] whose accumulation in blood plasma is related to enhanced modification of lipoproteins thereby increasing their atherogenicity [103]. Receptor for AGE (RAGE) is expressed in macrophages, ECs, and VSMCs [104], and AGE-RAGE interaction induces signaling pathways associated with increased ROS production and activation of inflammatory response in vascular cells [105].

In the hexosamine pathway, glutamine : fructose-6-phosphate amidotransferase (GFAT) catalyzes conversion of glucose to glucosamine-6-phosphate (G-6P) [106]. Diabetic hyperglycemia activates the hexosamine pathway that leads to the production of elevated G-6P levels in vascular cells [107, 108]. UDP-N-acetylglucosamine (UDP-GlcNAc), an end-product of the hexosamine pathway, is involved in both O- and N-linked protein glycation. N-glycosylation is an important stage of posttranslational modifications of newly synthesized proteins performed in the ER lumen [109]. Inhibition of N-glycosylation by tunicamycin (UDP-GlcNAc antagonist) activates the UPR [110].

GFAT is a rate-limiting enzyme in the hexosamine pathway. Overactivity of this enzyme in diabetic conditions promotes ER stress via stimulation of expression of UPR-related genes and contributes to downstream events including lipid accumulation and activation of proinflammatory and apoptotic pathways [111]. In contrast, GFAT inhibition attenuates ER stress [74]. Cultured human aortic VSMCs and macrophages treated with glucosamine develop apoptosis [74, 112, 113]. Therefore, suppression of GFAT could have a therapeutic potential in prevention of glucosamine-induced ER stress and apoptosis.

Glycogen synthase kinase (GSK)-3 whose expression is activated in glucosamine-induced ER stress may represent another potential target for antiatherogenic therapy [114]. GSK-3β activation in the aorta apoE-deficient hyperglycemic hyperhomocysteinemic mice fed on high-fat diet correlates with advanced atherosclerosis [115]. GSK-3α and β are two enzyme isoforms implicated in a variety of signaling pathways [116]. Upon the UPR induction, the inactive enzyme phosphorylated at Ser(21/9) is rapidly degraded in lysosomes that yields increase in GSK-3 activity [117]. Inhibition of GSK-3 displays both atheroprotective and anti-ER stress effects in cell cultures [118, 119] and hyperglycemic murine models [120].

6. Therapeutic Targeting of UPR Components and Its Clinical Potential

Targeting of proteins in ER stress and ER stress-induced apoptosis may be of high therapeutic value for treatment of human diseases in which ER stress plays a substantial role (Table 1). Promoters of GRP78 and GRP94 genes share significant sequence homology that explains the high concordance in coordinated expression of both ER chaperones [121]. Activation of ER chaperones plays an important role in adaptive UPR since it improves protein folding and prevents ER stress-induced apoptosis. Overexpression or stimulation of GRP78/94 had beneficial effect on ER-stressed cardiomyocytes [122–124] and showed cardioprotective properties in experiments in vivo [11–13].

Chemical chaperones such as phenylbutyrate and tau-roursodeoxycholic acid (TUDCA) are able to stabilize proteins in their native conformation thereby mimicking properties of native ER chaperones [125]. Murine macrophages and adipocytes treated with chemical chaperones showed resistance to ER stress [19]. At present, phenylbutyric acid (PBA) in its sodium salt form is approved for therapy of urea cycle disorders [126] and is in process of clinical testing for treatment of some genetic disorders related to protein misfolding [127, 128]. PBA was shown to reduce ER stress and normalize glucose levels in diabetic mice [129]. Taking into account clinical approval of PBA for therapy of several diseases, it would be interesting to check whether PBA is efficient for treatment of cardiovascular pathology.

TUDCA was shown to display antiapoptotic and anti-ER stress properties for many types of cells and many diseases including atherosclerosis. TUDCA was able to block ER stress and slow lesion progression in Ldlr-deficient mice [9] and efficiently prevent apoptosis and ER stress induced by oxidized LDL in murine macrophages transgenic for human APOE4, a genetic risk variant for Alzheimer disease and atherosclerosis [130]. The antiapoptotic function of TUDCA
| Drug | Mechanism | Potential indication | Reference |
|------|-----------|----------------------|-----------|
| 5-Aminoimidazole-4-carboxyamide-1-β-D-ribofuranoside (AICAR) | Reduction of ER stress by AMPK activation | Ischemic heart disease, heart failure, cardiac hypertrophy, atherosclerosis | [8–10] |
| BiP inducer X | Induction of GRP78 | Heart failure, stroke | [11, 12] |
| Curcumin | Induction of GRP94 | Heart failure, atherosclerosis, thrombosis, diabetes, diabetic cardiomyopathy, inflammation, dyslipidemia | [13] |
| CS-866 | Reduction of ER stress by pressure-overload | Heart failure, cardiac hypertrophy | [14] |
| EN460 | ERO1α inhibitor | Prevention/reduction of ER stress-induced oxidative stress | [15, 16] |
| Benzodiazepinones | ASK1 inhibitor | Atherosclerosis, cerebrovascular ischemia | [16] |
| QM295 | ERO1α inhibitor | Prevention/reduction of ER stress-induced oxidative stress | [15] |
| Isoproterenol | Proteasome activation and assembly | Heart failure, atherosclerosis | [17] |
| Pioglitazone | Reduction of ER stress | Heart failure, atherosclerosis | [18] |
| Phenylbutyrate | Chemical chaperone | Heart failure, atherosclerosis, pulmonary hypertension | [19–21] |
| Pravastatin | Reduction of ER stress by pressure-overload | Heart failure, cardiac hypertrophy | [22] |
| Salubrinal | Prevention of elf2α dephosphorylation | Heart failure, cardiac hypertrophy | [23] |
| SB203580 | CHOP phosphorylation | Heart failure, cardiac hypertrophy, atherosclerosis | [24] |
| SP600125 | Prevention of CHOP induction by stretch | Heart failure, cardiac hypertrophy, atherosclerosis | [24] |
| Sunitinib | IRE1 activation | Heart failure, atherosclerosis | [25] |
| Tauroursodeoxycholic acid (TUDCA) | Chemical chaperone | Heart failure, atherosclerosis | [19] |

can be released through restoring calcium homeostasis and SERCA activity [131] and downregulation of proapoptotic protein Bad [132].

Salubrinal specifically inhibits elf2α phosphatases [23] and therefore supports blocking protein synthesis mediated by phosphorylated elf2α [133]. Salubrinal is able to stop ER stress-induced apoptosis by inhibiting synthesis of members of proapoptotic signaling such as CHOP and caspase-12 in cardiac myocytes [134] and upregulating GRP78 in neurons [23]. However, in pancreatic β-cells, salubrinal induced activation of ATF4-CHOP mechanism that resulted in severe ER stress and apoptosis [133]. Thus, various cell types differently respond to salubrinal, and this limits its utility as a broad spectrum antiapoptotic drug [135].

CHOP is crucial in inducing ER stress-mediated apoptosis and hence development of CHOP inhibitors would be beneficial in prevention of atherosclerosis and treatment of heart failure and cardiac hypertrophy [50]. To date, no pharmacological agents specific for CHOP are available but there are drugs able to target molecular components of CHOP-mediated signaling. For example, SB203580, an inhibitor of p38 mitogen-activated protein kinase disrupts CHOP phosphorylation [136]. Mitogen-activated protein kinase blockers indirectly inhibit CHOP-dependent signaling in ER stress-induced apoptosis. JNK inhibitor SP600125 showed ability to suppress mechanical stretch-induced activation of CHOP [24].

Inhibition of ERO1α results in disruption of ER stress induced by oxidative stress and CHOP. Furthermore, several selective ERO1α inhibitors were developed. The inhibitor EN460 inactivates ERO1α by blocking its reoxidation [15]. Inhibitors EN460 and QM295 are able to launch the adaptive UPR signaling that prevents ER stress [15, 16]. Advanced ROS production induced by ERO1α overactivation may be
efficiently suppressed by the antioxidant N-acetylcysteine [137] and by the treatment with curcumin and masoprocil that protect PDI from oxidative inactivation [138].

Restoring proteasome function, which is inhibited in ER stress [139], by protein kinase A activators such as iso-proterenol or forskolin helps to attenuate ER stress-induced apoptosis [17]. TNF-α is significantly upregulated in ER stress, and inhibition of this cytokine by pravastatin [22] or TNF-α-specific antibody [24] results in significant protection of cardiomyocytes and other cells against apoptotic death. Hyperactivity of ASK1, a downstream target of IRE1-mediated signaling, contributes to the development of cardiac hypertrophy and heart failure, and inhibition of ASK1 by benzodiazebinones may be helpful for therapy of these cardiopathies [140, 141].

AMPK regulates switching from anabolic pathways (fatty acid synthesis, protein synthesis, etc.) to catabolism (fatty acid oxidation, glucose transport, etc.) thereby elevating energy levels in the cell [142]. The RNase activity of IRE1 is probably required to activate AMPK that leads to the induction of the proper UPR and increases cell survival [143]. AMPK activation has the cardioprotective effect through reducing cardiac ER stress [10, 144]. Inactivation of AMPK is associated with severe ER stress and atherosclerosis that can be reduced by ER stress suppressors such as tempol or TUDCA [8, 9]. In contrast, multiple AMPK agonists such as 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), atorvastatin, A-769662, and PTI reduce cardiovascular disease by blocking ER stress [10, 145]. Currently, AMPK activators are implicated in the treatment of obesity and metabolic syndrome. However, these drugs may be very helpful in antiatherogenic and cardioprotective therapy [146].

7. Conclusion: Limitations and Challenges in Anti-ER Stress Therapy

The UPR can be targeted by two ways including the activation of components of the adaptive mechanism of UPR and inhibition of those involved in the proapoptotic pathways of UPR. However, several questions should be answered to increase our understanding of mechanisms by which UPR targeting may help in therapy of cardiovascular disease. For example, one ER stressor (ATF6) has a cardioprotective role [147, 148] while two others (IRE1α and PERK) are involved in both the adaptive and proapoptotic UPR pathways. To date, the mechanisms controlling the switch from cell survival to death are not fully understood. Indeed, we do not know precisely when to activate or inhibit ER stress sensor proteins for treatment.

A variety of chemical inhibitors of protein kinases including receptor tyrosine kinase inhibitors are available. Some of those including sunitinib can directly activate IRE1 that results in XBP1 splicing and decreased ER stress [25]. Sunitinib malate is approved for use in treating renal cell carcinoma and gastrointestinal stromal tumor. However, in patients with a previous history of hypertension and coronary heart disease, sunitinib increases risk for cardiovascular events [149]. Thus, kinase inhibitors especially those that have a broad target spectrum should be carefully evaluated to prevent acute side effects.

In preclinical studies, chemical chaperones showed promising results in the improvement of ER folding capacity [125]. However, there are some limitations that seriously affect the therapeutic efficiency of these agents. Typically, high doses of these small molecule drugs are required to reach the desired effect. In addition, the UPR components are broadly expressed and their inhibition/activation in one tissue or organ may negatively influence the function of another tissue or organ. Targeting cell-specific ER components such as cAMP-responsive element-binding transcription factor H (CREBH) may be a promising strategy. The implementation of nanotherapeutic targeting approaches would be helpful for resolving these problems and providing new advances in efficient prevention of ER stress and treatment of ER stress-related diseases.

Using nanoparticles loaded with a therapeutic agent and coated with a monoclonal antibody against a tissue-specific antigen is a promising strategy for targeted delivery of a drug at high local concentrations. However, the development of nanotherapeutic tools for targeting cardiovascular ER stress-induced apoptosis is still in its infancy. Delie et al. [150] constructed polymeric nanoparticles capable of recognizing the COOH-terminal ER retention domain of GRP78, which is markedly overexpressed in prostate and ovarian cancer. The nanoparticles were able to deliver a cytotoxic agent, paclitaxel, to GRP-78-positive cancer cells. Niu et al. [151] reported a cardioprotective effect of nanoparticles loaded with cerium oxide (CeO₂), a ROS scavenger, in transgenic mice with cardiac-specific expression of monocyte chemoattractant protein-1 (MCP-1) that causes ischemic cardiomyopathy associated with the activation of ER stress. In heart failure, MCP-1 is involved in cardiomyocyte death through ROS-induced ER stress and apoptosis mediated by MCP-1-induced protein (MCPIP), a proapoptotic transcription factor [152]. CeO₂ nanoparticles injected intravenously inhibited progressive left ventricular dysfunction and dilatation in MCP mice by reducing oxidative stress and ER stress associated with suppression of expression of key ER-stress-related proteins [151].

The development of therapeutic nanoparticles capable of prolonged circulation in the bloodstream may provide an effective alternative method for treating ER stress in atherosclerosis and other cardiovascular diseases. For example, liposomal encapsulation of a drug and further liposomal pegylation significantly increase drug stability and residence time in blood as well as decreasing its cardiotoxicity [153]. In a rat ischemia/reperfusion model of cardiac injury, Takahama et al. [154] showed significantly advanced cardioprotective properties for prolonged adenosine encapsulated in pegylated liposomes compared to free adenosine. Knowledge regarding the mechanisms of the UPR and ER-stress-related diseases has rapidly accumulated in recent years, but many questions remain unanswered. Investigations of the mechanisms and pharmacological actions of ER stress are important in providing new mechanistic insights and developing novel targets for ER stress-related diseases. We believe that a more
deep understanding of ER stress will open promising avenues for the development of clinically useful drugs.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| AGE          | Advanced glycation end-product |
| AICAR        | 5-Aminoisoxazole-4-carboxamide-1-β-D-ribofuranoside |
| AMPK         | AMP kinase |
| Apo          | Apolipoprotein |
| ASK1         | Apoptosis signal-regulating kinase-1 |
| ATF          | Activating transcription factor |
| Bcl-2        | B cell lymphoma-2 |
| Bin          | BH3-only protein |
| CHOP         | C/EBPα-homologous protein |
| CREBH        | cAMP-responsive element-binding transcription factor H |
| CREBP        | cAMP-responsive element-binding protein |
| eIF2α        | Eukaryotic translation initiation factor 2α |
| EC           | Endothelial cell |
| ER           | Endoplasmic reticulum |
| ERAD         | ER-associated degradation |
| ERO1         | ER oxidoase 1 |
| GFAT         | Glutamine:fructose-6-phosphate amidotransferase |
| GLP-1        | Glucagon-like peptide 1 |
| G6P          | Glucosamine-6-phosphate |
| GRP          | Glucose-regulated protein |
| GSK-3        | Glycogen synthase kinase-3 |
| IL           | Interleukin |
| IP3R1        | Inositol-1,4,5-trisphosphate receptor-1 |
| IRE1         | Inositol requiring protein-1 |
| 7-KC         | Kettocholesterol |
| LDL          | Low density lipoprotein |
| Ldlr         | LDL receptor |
| Lp(a)        | Lipoprotein(a) |
| MCP-1        | Monocyte chemotactic protein-1 |
| MCPIP         | MCP-1-induced protein |
| PARM-1       | Prostate androgen-regulated mucin-like protein 1 |
| PBA          | Phenylbutyrate |
| PERK         | Protein kinase RNA-like ER kinase |
| PDI          | Protein disulfide isomerase |
| PRR          | Pattern recognition receptor |
| RAGE         | AGE receptor |
| ROS          | Reactive oxygen species |
| SERCA        | Sarcoplasmic/endoplasmic reticulum Ca2+-dependent ATPase |
| SFA          | Saturated fatty acid |
| SRA          | Scavenger receptor |
| SREBP-2      | Sterol response element binding protein-2 |
| TLR          | Toll-like receptor |
| TNF          | Tumor necrosis factor |
| TRAF2        | TNF receptor-associated factor 2 |
| TUDCA        | Tauroursodeoxycholic acid |
| UDP-GlcNAc   | UDP-N-acetylglucosamine |
| UPR          | Unfolded protein response |
| VSMC         | Vascular smooth muscle cell |
| XBPI         | X-box binding protein 1. |

**Conflict of Interests**

The authors report no conflict of interests.

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**References**

[1] T. Minamino and M. Kitakaze, “ER stress in cardiovascular disease,” *Journal of Molecular and Cellular Cardiology*, vol. 48, no. 6, pp. 1105–1110, 2010.

[2] I. Kim, W. Xu, and J. C. Reed, “Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities,” *Nature Reviews Drug Discovery*, vol. 7, no. 12, pp. 1013–1030, 2008.

[3] M.-J. Gething, “Role and regulation of the ER chaperone BiP,” *Seminars in Cell and Developmental Biology*, vol. 10, no. 5, pp. 465–472, 1999.

[4] T. Simmen, E. M. Lynes, K. Gesson, and G. Thomas, “Oxidative protein folding in the endoplasmic reticulum: tight links to the mitochondria-associated membrane (MAM),” *Biochimica et Biophysica Acta: Biomembranes*, vol. 1798, no. 8, pp. 1465–1473, 2010.

[5] N. Najdoo, “ER and aging—protein folding and the ER stress response,” *Ageing Research Reviews*, vol. 8, no. 3, pp. 150–159, 2009.

[6] D. Ron and P. Walter, “Signal integration in the endoplasmic reticulum unfolded protein response,” *Nature Reviews Molecular Cell Biology*, vol. 8, no. 7, pp. 519–529, 2007.

[7] A. Bertolotti, Y. Zhang, L. M. Hendershot, H. P. Harding, and D. Ron, “Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response,” *Nature Cell Biology*, vol. 2, no. 6, pp. 326–332, 2000.

[8] Y. Dong, M. Zhang, S. Wang et al., “Activation of AMP-activated protein kinase inhibits oxidized LDL-triggered endoplasmic reticulum stress in vivo,” *Diabetes*, vol. 59, no. 6, pp. 1386–1396, 2010.

[9] Y. Dong, M. Zhang, B. Liang et al., “Reduction of AMP-activated protein kinase α2 increases endoplasmic reticulum stress and atherosclerosis in vivo,” *Circulation*, vol. 121, no. 6, pp. 792–803, 2010.

[10] K. Terai, Y. Hiramoto, M. Masaki et al., “AMP-activated protein kinase protects cardiomyocytes against hypoxic injury through attenuation of endoplasmic reticulum stress,” *Molecular and Cellular Biology*, vol. 25, no. 21, pp. 9554–9575, 2005.

[11] T. Kudo, S. Kanemoto, H. Haru et al., “A molecular chaperone inducer protects neurons from ER stress,” *Cell Death and Differentiation*, vol. 15, no. 2, pp. 364–375, 2008.

[12] Y. Oida, J. Hamanaka, K. Hyakko et al., “Post-treatment of a BiP inducer prevents cell death after middle cerebral artery occlusion in mice,” *Neuroscience Letters*, vol. 484, no. 1, pp. 43–46, 2010.
[44] H. Su and X. Wang, “The ubiquitin-proteasome system in cardiac proteinopathy: a quality control perspective,” Cardiovascular Research, vol. 85, no. 2, pp. 253–262, 2010.

[45] M. Myoshi, H. Hao, T. Minamino et al., “Increased endoplasmic reticulum stress in atherosclerotic plaques associated with acute coronary syndrome,” Circulation, vol. 116, no. 11, pp. 1226–1233, 2007.

[46] M. S. Brown and J. L. Goldstein, “Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis,” Annual Review of Biochemistry, vol. 52, pp. 223–261, 1983.

[47] F. R. Maxfield and I. Tabas, “Role of cholesterol and lipid organization in disease,” Nature, vol. 438, no. 7068, pp. 612–621, 2005.

[48] B. Feng, P. M. Yaol, Yi. Li et al., “The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages,” Nature Cell Biology, vol. 5, no. 9, pp. 781–792, 2003.

[49] J. Zhou, Š. Lhoták, B. A. Hilditch, and R. C. Austin, “Activation of the unfolded protein response occurs at all stages of atherosclerotic lesion development in apolipoprotein E-deficient mice,” Circulation, vol. III, no. 14, pp. 1814–1821, 2005.

[50] E. Thorp, G. Li, T. A. Seimon, G. Kuriakose, D. Ron, and I. Tabas, “Reduced apoptosis and plaque necrosis in advanced atherosclerotic lesions of ApoE−/− and Ldlr−/− mice lacking CHOP,” Cell Metabolism, vol. 9, no. 5, pp. 474–481, 2009.

[51] H. Tsukano, T. Gotoh, M. Endo et al., “The endoplasmic reticulum stress-C/EBP homologous protein pathway-mediated apoptosis in macrophages contributes to the instability of atherosclerotic plaques,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 30, no. 10, pp. 1925–1932, 2010.

[52] L. Ozcan and I. Tabas, “Pivotal role of calcium/calmodulin-dependent protein kinase II in ER stress-induced apoptosis,” Cell Cycle, vol. 9, no. 2, pp. 223–224, 2010.

[53] D. M. Schrijvers, G. R. Y. De Meyer, A. G. Herman, and W. Martinet, “Phagocytosis in atherosclerosis: molecular mechanisms and implications for plaque progression and stability,” Cardiovascular Research, vol. 73, no. 3, pp. 470–480, 2007.

[54] P. M. Henson, D. L. Bratton, and V. A. Fadok, “Apoptotic cell removal,” Current Biology, vol. II, no. 19, pp. R795–R805, 2001.

[55] I. Tabas, “Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 25, no. II, pp. 2255–2264, 2005.

[56] T. Devries-Seimon, Y. Li, P. M. Yoa et al., “Cholesterol-induced macrophage apoptosis requires ER stress pathways and engagement of the type A scavenger receptor,” The Journal of Cell Biology, vol. 171, no. 1, pp. 61–73, 2005.

[57] T. A. Seimon, M. J. Nadolski, X. Liao et al., “Atherogenic lipids and lipoproteins trigger CD36-TLR2-dependent apoptosis in macrophages undergoing endoplasmic reticulum stress,” Cell Metabolism, vol. 12, no. 5, pp. 467–482, 2010.

[58] G. Li, C. Scull, L. Ozcan, and I. Tabas, “NADPH oxidase links endoplasmic reticulum stress, oxidative stress, and PKR activation to induce apoptosis,” Journal of Cell Biology, vol. 191, no. 6, pp. 1113–1125, 2010.

[59] T. A. Seimon, A. Obstfeld, K. J. Moore, D. T. Golenbock, and I. Tabas, “Combinatorial pattern recognition receptor signaling alters the balance of life and death in macrophages,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 52, pp. 19794–19799, 2006.

[60] S. Erkou, S. Kaptoge, P. L. Perry et al., “Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality,” Journal of the American Medical Association, vol. 302, no. 4, pp. 412–423, 2009.

[61] L. Zeng, A. Zampetaki, A. Margariti et al., “Sustained activation of XBPI splicing leads to endothelial apoptosis and atherosclerosis development in response to disturbed flow,” Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 20, pp. 8326–8331, 2009.

[62] M. Civelek, E. Manduchi, R. J. Riley, C. J. Stoeckert Jr., and P. F. Davies, “Chronic endoplasmic reticulum stress activates unfolded protein response in arterial endothelium in regions of susceptibility to atherosclerosis,” Circulation Research, vol. 105, no. 5, pp. 453–461, 2009.

[63] P. S. Gargalovic, N. M. Gharavi, M. J. Clark et al., “The unfolded protein response is an important regulator of inflammatory genes in endothelial cells,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 26, no. II, pp. 2490–2496, 2006.

[64] S. Gora, S. Maouche, R. Atout et al., “Phospholipidylated LDL induces an inflammatory response in endothelial cells through endoplasmic reticulum stress signaling,” FASEB Journal, vol. 24, no. 9, pp. 3284–3297, 2010.

[65] P. A. Outtinen, S. K. Sood, S. I. Pfeifer et al., “Homocysteine-induced endoplasmic reticulum stress and growth arrest leads to specific changes in gene expression in human vascular endothelial cells,” Blood, vol. 94, no. 3, pp. 959–967, 1999.

[66] R. E. Feaver, N. E. Hastings, A. Pryor, and B. R. Blackman, “GRP78 upregulation by atheroprone shear stress via p38-, a2b1-dependent mechanism in endothelial cells,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 28, no. 8, pp. 1534–1541, 2008.

[67] S. Chien, “Effects of disturbed flow on endothelial cells,” Annals of Biomedical Engineering, vol. 36, no. 4, pp. 554–562, 2008.

[68] T. Bombeli, B. R. Schwartz, and J. M. Harlan, “Endothelial cells undergoing apoptosis become proadhesive for nonactivated platelets,” Blood, vol. 93, no. II, pp. 3831–3838, 1999.

[69] M. C. Clarke, N. Figg, J. J. Maguire et al., “Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis,” Nature Medicine, vol. 12, no. 9, pp. 1075–1080, 2006.

[70] W. P. Cheng, H. F. Hung, B. W. Wang, and K. G. Shyu, “The molecular regulation of GADD153 in apoptosis of cultured vascular smooth muscle cells by cyclic mechanical stretch,” Cardiovascular Research, vol. 77, no. 3, pp. 551–559, 2008.

[71] J. Zhou, G. H. Werstuck, Š. Lhoták et al., “Association of multiple cellular stress pathways with accelerated atherosclerosis in hyperhomocysteinemic apolipoprotein E-deficient mice,” Circulation, vol. 110, no. 2, pp. 207–213, 2004.

[72] X. Kedi, Y. Ming, W. Yongping, Y. Yi, and Z. Xiaoxiang, “Free cholesterol overloading induced smooth muscle cells death and activated both ER- and mitochondrial-dependent death pathway,” Atherosclerosis, vol. 207, no. 1, pp. 123–130, 2009.

[73] E. Pedrazzi, C. Guichard, V. Ollivier et al., “NAD(P)H oxidase Nox-4 mediates 7-ketocholesterol-induced endoplasmic reticulum stress and apoptosis in human aortic smooth muscle cells,” Molecular and Cellular Biology, vol. 24, no. 24, pp. 10703–10717, 2004.

[74] G. H. Werstuck, M. I. Khan, G. Femia et al., “Glucosamine-induced endoplasmic reticulum dysfunction is associated with accelerated atherosclerosis in a hyperglycemic mouse model,” Diabetes, vol. 55, no. I, pp. 93–101, 2006.
B. C. Bergman, D. M. Hunerdosse, A. Kerege, M. C. Playdon, and C. Zhang, "Emerging role of adipokines as mediators in atherosclerosis," BioFactors, vol. 35, no. 2, pp. 120–129, 2009.

J. G. Dickhout, S. K. Sood, and R. C. Austin, "Role of endoplasmic reticulum calcium disequilibria in the mechanism of homocysteine-induced ER stress," Antioxidants and Redox Signaling, vol. 9, no. 11, pp. 1863–1873, 2007.

S. M. Colgan, D. Tang, G. H. Werstuck, and R. C. Austin, "Role of ERO1-α-mediated stimulation of inositol 1,4,5-trisphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis," International Journal of Biochemistry and Cell Biology, vol. 39, no. 10, pp. 1843–1851, 2007.

G. H. Werstuck, S. R. Lentz, S. Dayal et al., "Homocysteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglyceride biosynthetic pathways," Journal of Clinical Investigation, vol. 107, no. 10, pp. 1263–1273, 2001.

M. Cnop, F. Fournelle, and L. A. Vellosolo, "Endoplasmic reticulum stress, obesity and diabetes," Trends in Molecular Medicine, vol. 18, no. 1, pp. 59–68, 2012.

M. Keuper, M. Blüher, M. R. Schön et al., "An inflammatory micro-environment promotes human adipocyte apoptosis," Molecular and Cellular Endocrinology, vol. 339, no. 1-2, pp. 105–113, 2011.

X. Cheng, E. J. Folco, K. Shimizu, and P. Libby, "Adiponectin induces pro-inflammatory programs in human macrophages and CD4+ T cells," Journal of Biological Chemistry, vol. 287, no. 44, pp. 36896–36904, 2012.

J. Oh, A. E. Riek, S. Weng et al., "Endoplasmic reticulum stress controls M2 macrophage differentiation and foam cell formation," The Journal of Biological Chemistry, vol. 287, no. 15, pp. 11629–11641, 2012.

C. N. Lumeng, S. M. DeYoung, J. L. Bodzin, and A. R. Saltiel, "Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity," Diabetes, vol. 56, no. 1, pp. 16–23, 2007.

H. Zhang, J. Cui, and C. Zhang, "Emerging role of adipokines as mediators in atherosclerosis," World Journal of Cardiology, vol. 2, pp. 370–376, 2010.

L. Makowski, J. B. Boord, K. Maeda et al., "Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis," Nature Medicine, vol. 7, no. 6, pp. 699–705, 2001.

K. H. Hellemans, J. Hannaert, B. Denys et al., "Susceptibility of pancreatic beta cells to fatty acids is regulated by LXR/PPARα-dependent stearoyl-coenzyme a desaturase," PLoS ONE, vol. 4, no. 9, Article ID e7266, 2009.

I. Tabas, A. Tall, and D. Accili, "The impact of macrophage insulin resistance on advanced atherosclerotic plaque progression," Circulation Research, vol. 106, no. 1, pp. 58–67, 2010.

C. P. Liang, S. Han, T. Senokuchi, and A. R. Tall, "The macrophage at the crossroads of insulin resistance and atherosclerosis," Circulation Research, vol. 100, no. 11, pp. 1546–1555, 2007.

C.-P. Liang, S. Han, G. Li, I. Tabas, and A. R. Tall, "Impaired MEK signaling and SERCA expression promote ER stress and apoptosis in insulin-resistant macrophages and are reversed by exenatide treatment," Diabetes, vol. 61, no. 10, pp. 2609–2620, 2012.

B. C. Bergman, D. M. Hunerdosse, A. Kerege, M. C. Playdon, and L. Perreault, "Localization and composition of skeletal muscle diacylglycerol predicts insulin resistance in humans," Diabetologia, vol. 55, no. 4, pp. 1140–1150, 2012.

C. Liang, S. Han, H. Okamoto et al., "Increased CD36 protein as a response to defective insulin signaling in macrophages," Journal of Clinical Investigation, vol. 113, no. 5, pp. 764–773, 2004.

S. Han, C. Liang, T. DeVries-Seimon et al., "Macrophage insulin receptor deficiency increases ER stress-induced apoptosis and necrotic core formation in advanced atherosclerotic lesions," Cell Metabolism, vol. 3, no. 4, pp. 257–266, 2006.

T. Senokuchi, C. P. Liang, T. A. Seimon et al., "Forkhead transcription factors (FoxOs) promote apoptosis of insulin-resistant macrophages during cholesterol-induced endoplasmic reticulum stress," Diabetes, vol. 57, no. 11, pp. 2967–2976, 2008.

A. A. Beg and D. Baltimore, "An essential role for NF-κB in preventing TNF-α-induced cell death," Science, vol. 274, no. 5288, pp. 782–784, 1996.

S. Lehto, T. Rönnemaa, S. M. Haffner, K. Pyörälä, V. Kallio, and M. Laakso, "Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM," Diabetes, vol. 46, no. 8, pp. 1354–1359, 1997.

S. M. Haffner, "The importance of hyperglycemia in the nonfasting state to the development of cardiovascular disease," Endocrine Reviews, vol. 19, no. 5, pp. 583–592, 1998.

L. Quagliaro, L. Piconi, R. Assaloni, L. Martinelli, E. Motz, and A. Ceriello, "Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation," Diabetes, vol. 52, no. 11, pp. 2795–2804, 2003.

D. Popov and M. Simionescu, "Cellular mechanisms and signalling pathways activated by high glucose and AGE-albumin in the aortic endothelium," Archives of Physiology and Biochemistry, vol. 112, no. 4-5, pp. 265–273, 2006.

C. A. Gleissner, J. M. Sanders, J. Nadler, and K. Ley, "Upregulation of aldose reductase during foam cell formation as possible link among diabetes, hyperlipidemia, and atherosclerosis," Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 28, no. 6, pp. 1137–1143, 2008.

B. Tesfamariam, M. L. Brown, and R. A. Cohen, "Elevated glucose impairs endothelium-dependent relaxation by activating protein kinase C," Journal of Clinical Investigation, vol. 87, no. 5, pp. 1643–1648, 1991.

T. Inoguchi, P. Li, F. Umeda et al., "High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells," Diabetes, vol. 49, no. 11, pp. 1939–1945, 2000.

K. J. Wells-Knecht, D. V. Zyzak, J. E. Litchfield, S. R. Thorpe, and J. W. Baynes, "Mechanism of autoxidative glycosylation: identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucose," Biochemistry, vol. 34, no. 11, pp. 3702–3709, 1995.

A. J. Smit, J. W. Hartog, A. A. Voors, and D. J. van Veldhuisen, "Advanced glycation endproducts in chronic heart failure," Annals of the New York Academy of Sciences, vol. 1126, pp. 225–230, 2008.

S. F. Yan, R. Ramosamy, Y. Naka, and A. M. Schmidt, "Glycation, inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond," Circulation Research, vol. 93, no. 12, pp. 1159–1169, 2003.

M. Brownlee, "The pathobiology of diabetic complications: a unifying mechanism," Diabetes, vol. 54, no. 6, pp. 1615–1625, 2005.
C. Liu, X. Li, G. Hu et al., “Salubrinal protects against tunicamycin and hypoxia induced cardiomyocyte apoptosis via the PERK-eIF2α signaling pathway,” Journal of Geriatric Cardiology, vol. 9, no. 3, pp. 258–268, 2012.

X. Wang and D. Ron, “Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP kinase,” Science, vol. 272, no. 5266, pp. 1347–1349, 1996.

R. Guo, H. Ma, F. Gao, L. Zhong, and J. Ren, “Metallothionein alleviates oxidative stress-induced endoplasmic reticulum stress and myocardial dysfunction,” Journal of Molecular and Cellular Cardiology, vol. 47, no. 2, pp. 228–237, 2009.

R. Pal, E. A. Cristan, K. Schnittker, and M. Narayan, “Rescue of ER oxidoreductase function through polyphenolic phytochemical intervention: Implications for subcellular traffic and neurodegenerative disorders,” Biochemical and Biophysical Research Communications, vol. 392, no. 4, pp. 567–571, 2010.

O. Tsukamoto, T. Minamino, K. Okada et al., “Depression of proteasome activities during the progression of cardiac dysfunction in pressure-overloaded heart of mice,” Biochemical and Biophysical Research Communications, vol. 340, no. 4, pp. 1125–1133, 2006.

S. Hikoso, Y. Ikeda, O. Yamaguchi et al., “Progression of heart failure was suppressed by inhibition of apoptosis signal-regulating kinase 1 via transcoronary gene transfer,” Journal of the American College of Cardiology, vol. 50, no. 5, pp. 453–462, 2007.

K. Homma, K. Katagiri, H. Nishitoh, and H. Ichijo, “Targeting ASK1 in ER stress-related neurodegenerative diseases,” Expert Opinion on Therapeutic Targets, vol. 13, no. 6, pp. 653–664, 2009.

W. W. Winder, “Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle,” Journal of Applied Physiology, vol. 91, no. 3, pp. 1017–1028, 2001.

G. P. Meares, K. J. Hughes, A. Naatz et al., “IRE1-dependent activation of AMPK in response to nitric oxide,” Molecular and Celllar Biology, vol. 31, no. 21, pp. 4286–4297, 2011.

H. Sasaki, H. Asanuma, M. Fujita et al., “Metformin prevents progression of heart failure in dogs role of AMP-activated protein kinase,” Circulation, vol. 119, no. 19, pp. 2568–2577, 2009.

F. Jia, C. Wu, Z. Chen, and G. Lu, “Atorvastatin inhibits homocysteine -induced endoplasmic reticulum stress through activation of AMP-activated protein kinase,” Cardiovascular Therapeutics, vol. 30, no. 6, pp. 317–325, 2012.

M. Kim and R. Tian, “Targeting AMPK for cardiac protection: opportunities and challenges,” Journal of Molecular and Cellular Cardiology, vol. 51, no. 4, pp. 548–553, 2011.

J. J. Martindale, R. Fernandez, D. Thuerauf et al., “Endoplasmic reticulum stress gene induction and protection from ischemia/reperfusion injury in the hearts of transgenic mice with a tamoxifen-regulated form of ATF6,” Circulation Research, vol. 98, no. 9, pp. 1186–1193, 2006.

H. Toko, H. Takahashi, Y. Kayama et al., “ATF6 is important under both pathological and physiological states in the heart,” Journal of Molecular and Cellular Cardiology, vol. 49, no. 1, pp. 113–120, 2010.

R. Gupta and M. L. Maitland, “Sunitinib, hypertension, and heart failure: a model for kinase inhibitor-mediated cardiotoxicity,” Current Hypertension Reports, vol. 13, no. 6, pp. 430–435, 2011.

F. Delie, P. Ribaux, P. Petignat, and M. Cohen, “Anti-KDEL-coated nanoparticles: a promising tumor targeting approach for ovarian cancer?” Biochimie, vol. 94, no. 11, pp. 2391–2397, 2012.