Snapshots: Endoplasmic Reticulum Stress in Lipid Metabolism and Cardiovascular Disease

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Abstract
The endoplasmic reticulum (ER) is an organelle present in most eukaryotic cells and plays a pivotal role in lipid metabolism. ER dysfunction, specifically ER stress (ERS), is a pathophysiological response involved in lipid metabolism and cardiovascular lesions. Therefore, suppression of ERS may improve lipid metabolic disorders and reduce cardiovascular risk. Herein, we focus on novel breakthroughs regarding the roles of ERS in lipid metabolism and cardiovascular disease (CVD), as well as the internal mechanisms of ERS and its status as a potential therapeutic target. This review highlights recent advances in ERS, the regulation of which might be helpful for both basic research and clinical drug design for lipid metabolic disorders and CVD.

Introduction
The cardiovascular system consists of the heart and vessels. According to "Heart Disease and Stroke Statistics-2016 Update: A Report from the American Heart Association (AHA)", CVD is the leading cause of mortality in both developed and developing countries (Mozaffarian et al., 2015; Rabani et al., 2017). Annually, approximately 660,000 and 305,000 Americans experience new and recurrent coronary attacks (including angina, myocardial infarction, and acute coronary syndrome), respectively (Jiang et al., 2016; Writing Group et al., 2016).

The ER, first discovered by Porter et al. in 1945 (Porter et al., 1945), forms an interconnected network of flattened membrane-enclosed cisternae (Babour et al., 2010) and plays a significant role in lipid metabolism, calcium homeostasis, protein synthesis, and post-translational modification (Ellgaard et al., 2003; Zhang et al., 2008). There are two types of ER: rough ER (RER) and smooth ER (SER). The SER lacks ribosomes and several major components involved in the process of membrane lipid synthesis, steroid hormone production, and detoxification. ERS, proposed by Gajkowska et al. in 2001, is the disturbance of ER homeostasis in a stressful environment, such as inflammation, oxidative stress, and hyperlipidemia. During ERS, three upstream proteins, IRE1 (inositol requiring 1, the most conserved ER-resident UPR regulator) (Tufanli et al., 2017), activating transcription factor 6 (ATF6), and PERK (RNA-dependent protein kinase-like endoplasmic reticulum kinase) are activated and trigger the unfolded protein response (UPR), usually activated in response to accumulation of unfolded or misfolded proteins in the lumen of the ER (Blond-Elguindi et al., 1993; Xu et al., 2005; Ron et al., 2007; Jiang et al., 2015). Numerous key lipogenic molecules and pathways are located in the ER, and determine the regulatory effects of ER in lipid

metabolism (Fagone et al., 2009; Morgan et al., 2016). The relevant molecules/pathways include S1P (sphingosine-1-phosphate) (Song et al., 2008), SREBP (sterol regulatory element-binding protein) (Kammoun et al., 2009), X box-binding protein 1 (XBP-1) (Todd et al., 2008), the PERK/eukaryotic initiation factor 2 (eIF2α) pathway (Zhang et al., 2011; Xin et al., 2017), and the IRE1α/XBP1 pathway (So et al., 2012). Initially, ERS is protective and helpful for cell repair, whereas chronic or prolonged ERS may initiate caspase signaling, cell apoptosis, and organ injury (Dufey et al., 2015; Tameire et al., 2015; van Vliet et al., 2015; Wang et al., 2017). In this review, we focus on the latter implications of ERS.

Several studies have demonstrated that ERS has a close relationship with lipid metabolism and CVD (Palomer et al., 2014; Qin et al., 2015; Miyagawa et al., 2016; Tampakakis et al., 2016). Disruption of normal ER homeostasis may increase cardiovascular risk (Chaube et al., 2012; Akoumi et al., 2017), induce hepatic lipid accumulation (Li et al., 2014), cause metabolic disorders (Qin et al., 2015), and induce insulin resistance (Diaz et al., 2015). We previously identified roles for ERS in myocardial ischemia (MI) (Yu et al., 2015; Yu et al., 2016), endothelial cells (Wang et al., 2014; Liu et al., 2016), and cell apoptosis (Fan et al., 2016). In this review, we summarize the roles of ERS in lipid metabolism and CVD with respect to recent advances in research. The information compiled here may serve as a comprehensive guide on the mechanisms of ERS in lipid metabolism and provide evidence in support of ERS as a therapeutic target for CVD.

ERS in lipid metabolism
Lipid metabolism involves the synthesis and degradation of lipids in cells (Liu et al., 2015; Das et al., 2017). The ER is the major organelle involved in lipid metabolism, as the ER contains many relevant enzymes for lipid metabolism. ERS is a potential mechanistic link between excess nutrients and lipid accumulation, which is a crucial event in the development of atherosclerosis (Erbay et al., 2009), MI (Perman et al., 2011), and heart failure (Wu et al., 2016).

The liver is a highly active metabolic organ in which ER homeostasis is necessary for lipid metabolism (Ozcan et al., 2004; Canbay et al., 2007; Colgan et al., 2011). Hepatic metabolic disorders contribute to lipid accumulation, hyperlipidemia, and insulin resistance, all of which can increase the risk of CVD

(Cohn et al., 2008). Microcystin-LR, a hepatotoxin commonly used to induce metabolic abnormalities in liver lipids, can increase the levels of serum triglyceride (TG) and low density lipoprotein-cholesterol (LDL-C) by inducing ERS, which is correlated with elevated ATF6 and PERK (Qin et al., 2015). Elevated glucose levels induce lipid accumulation through activation of ERS, whereas AMPK activation prevents high glucose-induced lipid accumulation by inhibiting the mTORC1/ERS pathway (Li et al., 2014; Jiang et al., 2017). Resveratrol could protect against high-fat diet (HFD)-induced hepatic steatosis by inhibiting ERS, which occurs in conjunction with improved dyslipidemia and reduced lipid accumulation (Pan et al., 2015). This suggests that inhibition of ERS is a potential target for alleviating hepatic lipid accumulation. In *Pichia pastoris*, lipid saturation induces the UPR. In return, the activated ERS/UPR pathway also disturbs lipid homeostasis (Zhang et al., 2016). Smoke-exposure causes ERS and lipid accumulation in the retinal pigment epithelium, which is associated with enhanced UPR, elevated glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP) (Kunchithapautham et al., 2014). Scavenger receptor class B, type I (SR-BI) is the main receptor of high-density lipoprotein (HDL) and an emerging atheroprotective candidate. ERS may induce UPR and downregulate the expression of SR-BI in hepatocytes, which might contribute to the unfavorable effects of metabolic disorders on systemic cholesterol homeostasis, a high risk of CVD (Eberhart et al., 2016). In a subclinical hypothyroidism C57BL/6 mice model, elevated thyroid stimulating hormone (TSH) leads to ERS and increases hepatic total cholesterol (TC) and TG contents, thereby exacerbating dyslipidemia and hepatic lipid accumulation. When treated with 4-phenyl butyric acid (4-PBA), an ERS inhibitor, the expression of Bip, p-IRE1α and XBP-1 in 4-PBA-treated mice is significantly downregulated and correlates with improved lipid metabolism (Zhou et al., 2016). In addition, Lebeau and colleagues reported that ERS can activate SREBP-2, an ER-localized transcription factor that directly upregulates sterol-regulatory genes, PCSK9. PCSK9 can contribute to atherosclerosis by targeting LDL receptor degradation. However, tunicamycin blocks PCSK9 secretion and reduces the LDL cholesterol content in plasma (Lebeau et al., 2017). Altogether, these findings suggest that ERS may interfere with lipid metabolism and that blockade of ERS may ameliorate disturbances in lipid metabolism (Figure 1).
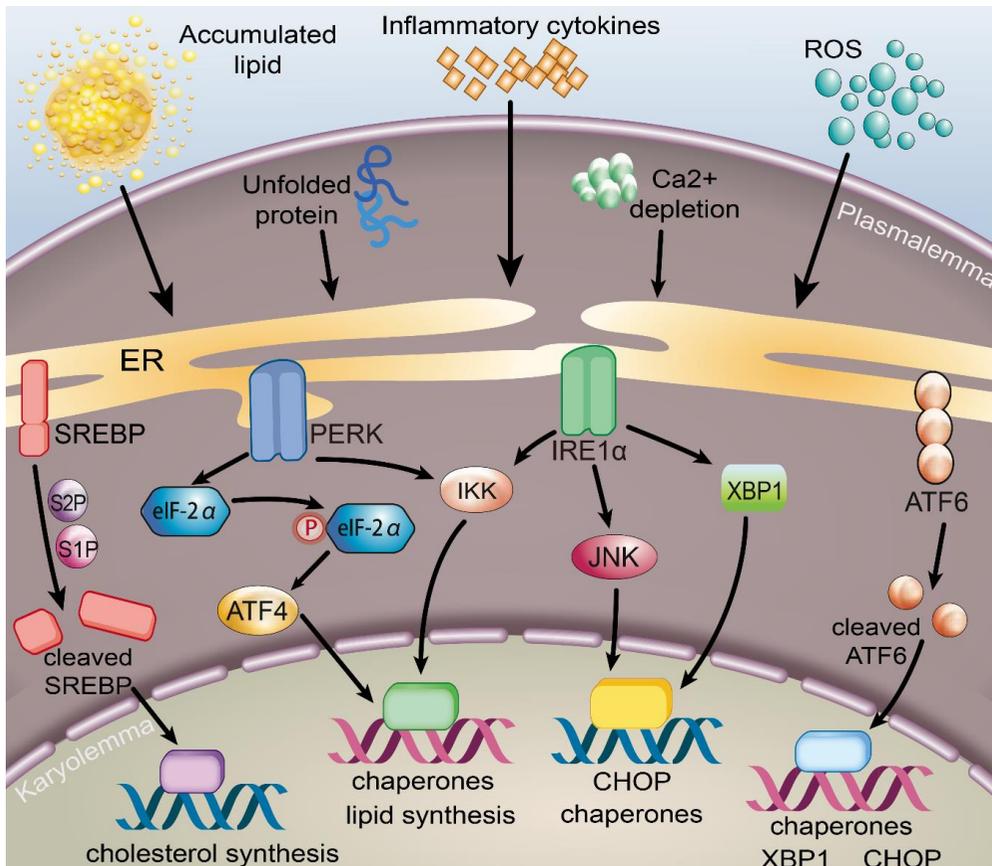
Figure 1. The ERS network. ERS usually acts as a negative regulation factor of lipid metabolism. Initially, extra- and endocellular harmful stimuli contribute to the activation of ERS-related receptors, such as SREBP, PERK, IRE1, and ATF6, all of which induce relevant downstream signalings. Thereafter, activated downstream signalings are transferred to the nucleus and promote the transcription of a series of genes, such as genes of cholesterol synthesis, lipid synthesis, chaperones, CHOP, XBP1, CHOP etc, which may reasult in lipid metabolism dysfunction.

ERS in cardiovascular disease

Unlike other cells, cardiomyocytes in the adult preferentially use fatty acids to meet their high energy requirements, which suggests that lipid metabolism is critical for heart tissue (Groenendyk et al., 2010; Han et al., 2016). Animal experiments have revealed that ERS activation is the link from lipid metabolic disorders to the high prevalence of CVD. Despite recent advances, CVD remains the leading cause of mortality in the world and places an immense financial burden on patients. Therefore, a novel molecular target or therapy is greatly needed for the treatment of CVD. Recently, studies have revealed that targeting ERS is a novel avenue to improve lipid metabolism and combat CVD (Son et al., 2010; Haberzettl et al., 2013), and may provide a novel therapeutic target.

Atherosclerosis

Atherosclerosis, also referred to as arteriosclerotic vascular disease, is thought to be a chronic inflammatory arterial disease in the heart, brain, kidney, and mesentery (Lusis, 2000; Libby, 2002; Jiang et al., 2016). The pathogenesis of atherosclerosis initially occurs in the endangium and is subsequently accompanied by lipid accumulation, bleeding, thrombosis, and vessel stenosis. ERS is involved in vascular lipid metabolism, which accelerates the pathogenesis of atherosclerosis.

Fundamental mechanisms

Pathological examination revealed that atherosclerosis is always accompanied by cholesterol and triglyceride accumulation, and endothelial dysfunction (Libby et al., 2002; Rocha et al., 2009). Exogenous lipid administration or other

enzymes may activate ERS and induce endothelial dysfunction. Vascular endothelial injury induced by oxidized low-density lipoprotein (ox-LDL) has been implicated in the early stage of atherosclerosis. Zhou and colleagues reported that ox-LDL rapidly induces the LOX-1/ERS pathway and initiates the rapid dephosphorylation of eNOS at Ser1179, followed by a subsequent decrease in eNOS activity. Alternatively, 4-PBA elevates eNOS levels and alleviates endothelial injury. They also discovered that the effect of ox-LDL/LOX-1/ERS signaling pathway is time-dependent, as the levels of LOX-1 and ERS began to change at 4 h and 12 h after ox-LDL treatment, respectively (Zhou et al., 2013). Treatment of endothelial cells with ox-LDL results in endothelial dysfunction and ERS activation in endothelial cells, which is characterized by elevated ERS sensors IRE1, PERK, and ATF6 (Hong et al., 2014). Saturated fatty acids (SFA) activate toll-like receptor 4 (TLR4), induce ERS, and contribute to endothelial dysfunction and the impairment of insulin-related vasodilatation. Meanwhile, TLR4 knockout mice display improved endothelial function and reduced ERS, indicating that the TLR4/ERS pathway causes damage to the endothelium (Kim et al., 2015). ATP-binding cassette transporter G1 (ABCG1) deficiency alleviates ERS, reduces cholesterol efflux to high-density lipoprotein (HDL) and endothelial apoptosis, whereas downregulation of endothelial ABCG1 inhibits ERS and endothelial apoptosis, thereby reducing atherosclerotic risk (Xue et al., 2013). Activation of hepatic extracellular signal-regulated kinase (ERK) induces endothelial injury via ERS. ERS first increases insulin resistance, and then elevates cholesterol/triglyceride levels, thereby contributing to endothelial dysfunction (Kujiraoka et al., 2013). Moreover, Hong et al used human umbilical vein endothelial cells and discovered that Ox-LDL induces endothelial cell apoptosis via the LOX-1-dependent ERS pathway, and further causes endothelial dysfunction, an initial alteration of atherosclerosis (Hong et al., 2014). Other studies also identified the effects of ERS in endothelial dysfunction (Chaube et al., 2012; Zhou et al., 2013). Together, these findings reveal that ERS causes damage to the endothelium and increases atherosclerotic risk.

Additionally, ERS also regulates macrophages and leukocytes, which can exacerbate atherosclerosis. Macrophages are particularly vulnerable to lipid-induced toxicity; differentiation and recruitment of macrophages to plaques accelerate the development of atherosclerosis (Hotamisligil et al.,

2008; Wu et al., 2015; Ren et al., 2017). Erbay and colleagues reported that mitigation of ERS with a chemical lipid chaperone (aP2) results in significant protection against dying lipotoxic macrophages and prevents atherosclerosis, while these effects are reversed in the aP2 knockout mouse. These findings suggest that lipid chaperones regulate ER homeostasis to prevent atherosclerosis, both genetically and chemically (Erbay et al., 2009). Suppression of ERS promotes the differentiation of M2 macrophages toward a M1 phenotype and subsequently suppresses the formation of foam cells by increasing HDL- and apoA-1-induced cholesterol efflux (Oh et al., 2012). Tampakakis and coworkers discovered that intravenous intralipid infusion induces early ERS activation in leukocytes from 21 healthy subjects, which was evidenced by activation of ATF6 and phosphoinositol requiring kinase 1 (pIRE1). Inflammation contributes to endothelial injury and dysfunction, but the authors did not detect inflammatory molecules associated with leukocytes. In our early studies, we found that ERS has a close relationship with the adhesion of monocytes to HUVECs and subsequently elevated inflammatory levels. However, whether monocyte-related inflammation is ERS-dependent remains unknown (Wang et al., 2014; Liu et al., 2016). Thus far, preliminary research suggests that leukocyte-mediated inflammation is not an ERS-dependent effect (Erbay et al., 2009; Dai et al., 2014; Mozzini et al., 2014; Liu et al., 2016). Further studies are required to confirm whether ERS induces endothelial dysfunction via an ERS-dependent pathway or not (Tampakakis et al., 2016). Altogether, these findings suggest that ERS regulates endothelial cells, macrophages, and leukocytes to induce lipid metabolic dysfunction and atherosclerosis.

Pro-atherosclerotic effects
Numerous studies have demonstrated the pro-atherosclerotic effects of ERS by increasing TG or TC levels. Hydrogen sulfide is a novel gastrotransmitter that plays an important anti-atherosclerotic role. Compared with control mice, ApoE knockout mice fed an HFD diet exhibit increased plasma levels of TC, TG and LDL, increased aortic plaque size and expression of GRP78 and caspase-12. Compared with ApoE knockout mice, hydrogen sulfide donor-treated ApoE knockout mice display a decreased ERS response, lower plasma LDL levels and less plaque necrosis. These findings reveal that hydrogen sulfide plays a suppressive role in aortic ERS and reduces atherosclerotic lesions in ApoE knockout

mice (Chen et al., 2011). Hyperglycemia, hyperhomocysteinemia, and HFD significantly enhance ERS/glycogen synthase kinase (GSK) 3β activity and exacerbate atherosclerotic lesions compared with controls, whereas valproate (a medication primarily used to treat epilepsy and bipolar disorder) supplementation reduces lipid accumulation by blocking the ERS/GSK3β pathway (McAlpine et al., 2012). Quercetin, found in natural botanical plants, is under basic and early-stage clinical research for a variety of disease conditions (Miles et al., 2014). It can protect RAW264.7 macrophages from glucosamine-induced lipid accumulation through the suppression of ERS, which is evidenced by reduced CHOP and ATF6 levels (Cai et al., 2015).

ERS may decrease the stability of atherosclerotic plaques and increase the size and number of plaques (Dickhout et al., 2007). In a retrospective study, plaque stability was negatively correlated with ERS in carotid atherosclerosis patients (Saksi et al., 2014). Fibroblast growth factor 21 (FGF-21) treatment remarkably reduces the number and size of aortic plaques via the suppression of ERS, which attenuates aortic endothelium apoptosis and atherosclerosis in ApoE knockout mice (Wu et al.,

2015). Cold stress exposure contributes to the instability of atherosclerotic plaques and increased numbers of apoptotic cells in atherosclerotic plaques by activating ERS, which is correlated with elevated levels of CHOP and GRP78. Meanwhile, cold stress promotes angiogenesis and infiltration of monocytes into atherosclerotic plaques, further exacerbating the instability of atherosclerotic plaques via ERS (Dai et al., 2014). Hydrogen or simvastatin significantly enhances plaque stability by inducing ERS, which is evidenced by increased collagen and smooth muscle cells (SMC). Meanwhile, 4-PBA also achieves the same effects, suggesting that the inhibition of ERS enhances the stability of atherosclerotic plaques (Song et al., 2015). Tufanli and colleagues reported that IRE1 inhibitors uncouple lipid-induced ERS from inflammasome activation in both mouse and human macrophages, which leads to a significant decrease in hyperlipidemia-induced IL-1β and IL-18 production, lowers T-helper type-1 immune responses, and reduces atherosclerotic plaque size without altering the plasma lipid profiles in apolipoprotein E-deficient mice. These results reveal that pharmacologic modulation of IRE1 alleviates ERS, lipid metabolic disorders, and atherosclerosis (Tufanli et al., 2017). Clec4e is
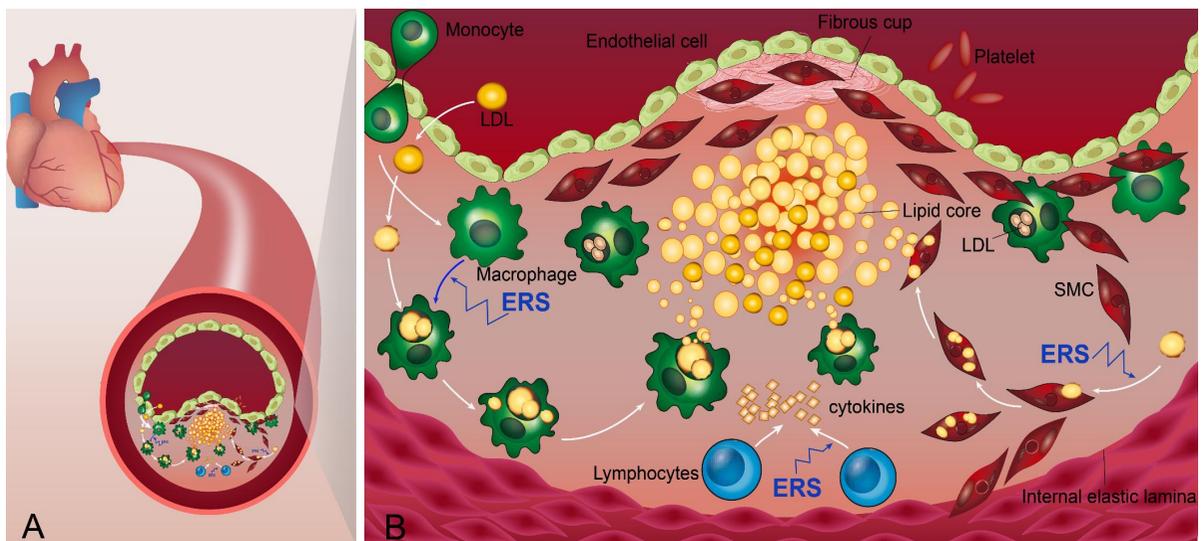

Figure 2. Roles of ERS in the pathogenesis of atherosclerosis. Atheroclerosis is a type of chronic cardiovascular diseases characterized by lipid accumulation and metabolic dysfunction. ERS plays a core part in lipid metabolism dysfunction and pathogenesis of atheroclerosis. A. This figure demonstrates the composition of damaged coronary artery. B. ERS almost participates in all processes of atherogenesis. ERS contributes to endothelial injury, promotes adhesion of monocytes to the endothelium, SMC migration, as well as the macrophages differentiation towards a M2 phenotype. These alterations above altimately result in the formation of foam cells and fibrous core, thereby exacerbating the progression of atherosclerosis.

expressed within human and mouse atherosclerotic lesions, and is activated by necrotic lesion extracts. Clec4e induces UPR in macrophages, promotes cholesterol accumulation and plaque instability whereas CHOP and IRE1a deficiencies significantly limit Clec4e-dependent effects (Clement et al., 2016). Moreover, it was demonstrated that ERS accelerates the development of atherosclerosis (Wu et al., 2015; Xiao et al., 2015). These findings further suggest that the pro-atherosclerosis effects of ERS are mediated by increasing the size and number of plaques (Figure 2).

Cardiac lipotoxicity
Cardiac lipotoxicity is the result of an imbalance between lipid uptake and utilization, usually manifested as accumulated lipid in the myocardium. Intracellular accumulation of myocardial lipids occurs after MI, which decreases heart function (Schaffer, 2003; Perman et al., 2011). Pig MI promotes the accumulation of cholesteryl esters in the infarct and the peri-infarct myocardium, which is concomitant with enhanced ERS and elevated LDL receptor (LDLR) levels (Drevinge et al., 2013). Expression of the VLDL receptor (VLDLR) promotes total TG accumulation and increases mortality in mice by increasing ERS in hypoxic cardiomyocytes and ischemic mouse hearts. ERS and lipid accumulation are diminished in VLDLR knockout mice, followed by improved cardiac lipotoxicity, pathological remodeling, and cardiac function, which suggests that normalizing lipid levels by inhibiting ERS after MI might improve myocardial function and serve as a target for the treatment of cardiac lipotoxicity (Perman et al., 2011). Endogenous H_2S levels in serum of DCM patients and DCM rats are significant low. Deficiency of endogenous H_2S might contribute to cardiac lipotoxicity of diabetic cardiomyopathy (DCM) SD rats. However, pretreatment of AC16 cardiomyocytes with NaHS (a donor of H_2S) inhibits ERS and suppresses the palmitate-induced myocardial lipotoxicity injury (Guo et al., 2017). In addition, palmitate can induce cardiomyocyte lipotoxicity via the ERS-mediated apoptosis pathway, which is characterized by increased expression of GRP78, eIF2α, and CHOP, whereas this effect was reversed by a specific ERS inhibitor (4phenyl butyric acid) (Zou et al., 2017).

Other cardiovascular injuries
Among obese patients (Haslam et al., 2005), excessive fatty acid uptake by cardiomyocytes induces cardiac hypertrophy, a mechanism contributing to hypertrophic cardiomyopathy. ERS

and collagen deposition are significantly increased in HFD mouse hearts, accompanied by hypertrophic and fibrotic responses and myocardial dysfunction. Disruption of calpain prevents lipotoxicity-induced apoptosis by restraining ERS in cardiomyocytes and cardiac hypertrophy in mice fed an HFD. Additionally, pharmacological inhibition of ERS achieves the same effects, suggesting that restraining ERS may inhibit cardiac hypertrophy and hypertrophic cardiomyopathy (Li et al., 2016; Li et al., 2016). Akoumi' group treated H9C2 cardiomyocytes with 300 μM for 8 h and discovered that palmitate results in significantly accumulated intracellular diacylglycerol, mostly in the ER, which is correlated with elevated levels of ERS. Furthermore, acute administration of MG132 significantly attenuats palmitate-mediated ERS, lipid accumulation, and cell death, thereby ameliorating injury to cardiomyoxytes (Akoumi et al., 2017).

Experimental studies have demonstrated that anti-HIV drugs may promote ERS (Zha et al., 2013) and contribute to elevated cardiovascular risk (Gresele et al., 2012; Lake et al., 2013). Efavirenz and nelfinavir are classic first-generation drugs for HIV (Walmsley et al., 2002; Jamaluddin et al., 2010). Pharmacologically relevant concentrations of efavirenz induce ERS and result in reduced proliferation and viability of human umbilical vein endothelial cells (HUVEC). Surprisingly, the combination of these two drugs results in higher ERS levels in endothelial cells even at lower concentrations, further increasing endothelial dysfunction and relevant cardiovascular risk (Weiss et al., 2016).

Our group has confirmed that ERS exacerbates the symptoms of MI. Compared with the MI/reperfusion (MIR) group, melatonin effectively alleviates myocardial ERS, ischemic injury, and improves cardiac function. Moreover, we discovered that melatonin pretreatment attenuates ERS via the PERK/eIF2α/ATF4 pathway during MIR injury (Yu et al., 2016). Type 2 diabetic rats exhibit a significantly elevated ERS response. When subjected to MIR surgery, ERS and myocardial injury are enhanced (Yu et al., 2015). Melatonin markedly reduces MIR injury by downregulating ERS, decreasing myocardial apoptosis, and improving cardiac functional recovery. This suggests that melatonin ameliorates reperfusion-induced myocardial injury via inactivation of ERS (Yu et al., 2015) (Figure 1, Table 1).

Table 1. Roles of ERS in cardiovascular disease.

Models	Types	Molecular/cellular evidence	Symptomatic evidence	Reference
HUVECs	Atherosclerosis	Elevated activity of IRE1, PERK, and ATF6	Ox-LDL induces ERS and endothelial dysfunction	Hong et al., 2014
Bovine aortic endothelial cells	Atherosclerosis	Saturated fatty acids activate the TLR4/ERS pathway	Increased damage to the endothelium	Kim et al., 2015
aP2 knockout mice	Atherosclerosis	Mitigation of ERS protects against lipotoxic macrophages death	Ameliorated progression of atherosclerosis	Erbay et al., 2009
Peritoneal macrophages of CD36 knockout mice	Atherosclerosis	Suppression of ERS promotes the M2-M1 differentiation and suppresses the formation of foam cell	Ameliorated progression of atherosclerosis	Oh et al., 2012
C57BL/6J mice	Atherosclerosis	FGF-21 treatment remarkably reduces the number and size of aortic plaques by suppressing ERS	Attenuated apoptosis of aortic endothelium and atherosclerosis in ApoE knockout mice	Wu et al., 2015
C57BL/6 mice	Cardiac lipotoxicity	VLDLR promotes total TG accumulation and increases mortality in mice by increasing ERS	Improved cardiac lipotoxicity, pathological remodeling, and cardiac function	Perman et al., 2011
C57BL/6 mice	Cardiac hypertrophy	ERS and collagen deposition are significantly increased in HFD-fed mouse hearts, accompanied by hypertrophic and fibrosis response	Pharmacological inhibition of ERS prevents lipotoxicity-induced apoptosis and cardiac hypertrophy	Li et al., 2016
HUVECs	Anti-HIV drugs-related cardiac injury	The combination of efavirenz and nelfinavir increases the ERS levels in HUVECs	Increased endothelial dysfunction	Weiss et al., 2016
C57Bl/6 mice	MI	Melatonin effectively alleviates myocardial ERS via PERK/eIF2α/ATF4 pathway	Improved ischemic injury, and cardiac function	Yu et al., 2016
SD rats	MI	Melatonin suppresses myocardial apoptosis by downregulating ERS	Improved MIR injury and cardiac function	Yu et al., 2015

Further perspectives and potential directions

Initially, we hypothesized that ERS acts a negative regulatory factor in lipid metabolism and cardiovascular system. Particularly, ERS is activated after exposure to harmful stimuli, which induces lipid metabolic disorders and CVD, the leading cause of death among chronic kidney disease (CKD) patients (Heine et al., 2012; Gansevoort et al., 2013). CKD is characterized by elevated serum creatinine, reduced glomerular filtration rate (GFR), hypertension, and cachexia. CKD is also a dependent risk factor for CVD, such as atherosclerosis (Stubbs et al., 2016), myocardial infarction (Tonelli et al., 2013), and heart failure (Manzano-Fernandez et al., 2013) N-acetylcysteine prevents ERS and attenuates the deleterious effects of CKD on lipid accumulation in macrophages, which prevents atherogenesis and improves CKD symptoms (Machado et al., 2014). Treatment with simvastatin plus ezetimibe strongly reduces the levels of serum oxysterols and attenuates CKD-dependent atherosclerosis, vascular calcification, and cardiac dysfunction in ApoE knockout mice via suppression of ERS, suggesting that inhibiting ERS is a potential target for CKD-related CVD (Miyazaki-Anzai et al., 2014). Moreover, flaxseed oil rich in omega-3, may protect aorta against inflammation and ERS partially mediated by GPR120 receptor in obese, diabetic and dyslipidemic mice, suggesting that healthy unsaturated fat also has the ability of cardioprotection via inhibiting ERS and maintaining lipid homeostasis (Moura-Assis et al., 2017).

Cardiologists and other medical workers are committed to cardioprotection by targeting ERS and lipid metabolism. Miyazaki-Anzai et al. enrolled 10 patients with stages 3/4 CKD and 10 healthy subjects. They discovered that CKD increases the levels of serum 7-ketocholesterol and enhances the ERS response in patients (Miyazaki-Anzai et al., 2014). Saksi's group carried out a retrospective study involving 7491 patients with low expression of fatty acid-binding protein 4 (FABP4), 3432 patients with myocardial infarction, and 92 patients with carotid atherosclerosis. They discovered that low expression of FABP4 is associated with reduced serum total-C levels, stable carotid stenosis, reduced atheroma and the prevalence of atherosclerosis. Moreover, high FABP4 expression in carotid plaques is associated with enhanced ERS, lipid accumulation, and increased cardiovascular events (carotid atherosclerosis and stroke). This study suggests that low expression of FABP4 may contribute to reduced cardiovascular risk, probably by inhibiting ERS and reducing lipid accumulation (Saksi et al., 2014; Lv et al., 2017). Mozzini et al. enrolled 29 stable coronary artery disease (CAD) patients and 28 healthy subjects and discovered that ERS, LDL and oxLDL levels were significantly higher in the CAD group than the controls ($p<0.01$). Increasing amounts of lipids induce a dose-dependent increase in CHOP levels and enhancement of the ERS response ($p<0.01$) (Mozzini et al., 2014). Vitamin D-deficient subjects usually have a pro-atherogenic macrophage phenotype compared with vitamin D-sufficient subjects in patients with T2DM. Specifically, Vitamin D-sufficient patients have lower monocyte ERS and a predominance of M1 macrophage membrane receptors, whereas activation of ERS increases adhesion molecule and induces an M2-predominant phenotype in vitamin D-deficient macrophages. Conversely, deletion of the vitamin D receptor in macrophages from diabetic patients activates ERS, accelerates adhesion, and increases atherosclerotic risk, while the absence of the ERS protein CHOP ameliorates these issues. Thus, vitamin D is a natural ERS reliever that induces an anti-atherogenic M1 macrophage phenotype (Riek et al., 2012).

Every downstream signaling factor of ERS has its own contribution or synergistic actions with other signaling molecules in lipid metabolism. In CHOP deficiency mice with normal renal function, atherosclerosis is inhibited (Masuda et al., 2013), while CHOP deficiency fails to suppress atherosclerosis in ApoE knockout mice with CKD.

These data indicate that CHOP inhibition alone is not enough to suppress CKD-dependent atherosclerosis. Other deleterious downstream signaling molecules of ERS, such as ATF4 and IRE1α, may also be involved in the development of CKD-dependent atherosclerosis (Miyazaki-Anzai et al., 2014). SREBP-1c is a downstream transcriptional factor of ERS that activates lipogenic genes. Microcystin-LR exposure contributes to lipid accumulation and suppression of SREBP-1c, while Qin et al. observed significantly higher expression of the downstream targets of SREBP-1c, such as ACACA and GSK-3β. These findings reveal that other downstream targets of ERS might activate the expression of genes downstream of SREBP-1c (Qin et al., 2015). VLDLR promotes hypoxia-induced ERS in HL-1 cardiomyocytes and mouse heart tissue. Meanwhile, ERS also induces the expression of VLDLR in HL-1 cells, indicating that there is a positive feedback loop, i.e., the hypoxia-induced increase in VLDLR results in ERS, which in turn promotes the expression of the VLDLR (Perman et al., 2011). Hong and colleagues discovered that ox-LDL induces endothelial dysfunction and apoptosis via the LOX-1/ERS pathway (Hong et al., 2014), while Ishiyama et al. recently demonstrated that palmitic acid causes upregulation of LOX-1 via activation of the ERS response (Ishiyama et al., 2011). These findings suggest that there may be a positive feedback loop between ERS and LOX-1 expression. Thus, potential ERS inhibitors do have a closed relationship with their downstream/upstream targets and their internal interactions.

Notably, Devarajan and colleagues discovered that the normal function of the ER mainly depends on mitochondrial function and calcium overload. They discovered that paraoxonase 2 deficiency ameliorates ERS and lipid accumulation, and aggravates atherosclerosis in macrophages from ApoE knockout mice fed with a western diet, concomitant with reduced mitochondrial dysfunction and calcium overload. Treatment with a mitochondrial calcium uptake inhibitor, RU360, attenuates mitochondrial dysfunction and calcium overload via targeting ERS, as evidenced by decreased CHOP and GRP78 levels. This finding shows that ERS and mitochondrial calcium metabolism are interconnected, which indicates that other organelles and relevant biological processes should be considered when searching for an ERS inhibitor (Devarajan et al., 2012). Additionally, animal studies have revealed that maternal hyperglycemia may trigger ERS in the embryonic

heart (Wang et al., 2015). Elevated ERS response in maternal mice with type 2 diabetes mellitus (T2DM) triggers postnatal congenial heart failure (CHD) by inducing ERS, which is evidenced by elevated CHOP, BiP, and XBP1 levels. This indicates that proper hypoglycemic management of pregnant women could reduce the prevalence of cardiovascular risk for the fetus, partially due to suppressed ERS under normal blood glucose conditions (Shah et al., 2016; Wu et al., 2016).

Concluding remarks

Based on current literature and our reflections, we first provided the general background of ERS, lipid metabolism and CVD. Thereafter, we introduced the actions of ERS in lipid metabolism and CVD. We also discussed the clinical evidence and upstream/downstream signaling of ERS. Eventually, we summarized the potential directions of ERS in lipid metabolism and CVD and made a conclusion. Because clinical research on this topic is limited, we require more research involving clinical trials or other retrospective studies. We think that the diversity and complicated nature of the ERS network are the greatest difficulties for the discovery of drug targets for lipid metabolic disorders and CVD. Unfortunately, the biological mechanisms of ERS are still not completely understood. Future studies may include: 1) elucidating the upstream/downstream molecules and their crosstalk in the ERS network; 2) combination of basic and clinical research to develop a new ERS inhibitor for lipid metabolic disorders and CVD; 3) a systemic assessment of the positive and negative actions of ERS in lipid metabolism and CVD for relevant clinical applications; 4) exploring the application of drugs involving ERS signaling in CKD-related CVD; 5) and elucidating the relationship between metabolic syndromes and lipid disorder-related CVD. Altogether, current studies involving ERS have significantly enriched our knowledge of lipid metabolism and may inform future studies and clinical treatment of CVD.

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