Research Highlight
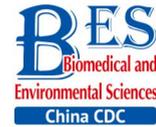
The Role of Endoplasmic Reticulum Stress-related Apoptosis in Vascular Endothelium Pathogenesis[*]

TAO Yong Kang[1], SHI Jing[2], YU Pu Lin[2,#], and ZHANG Guo Qiang[1,#]

Vascular endothelium refers to a single layer of endothelial cells that line the inner surface of blood vessels, serving as barriers and transducers between the circulating blood in the lumen and the rest of the vessel wall. Endothelial cells play essential roles in many aspects of vascular biology, such as barrier functions, thrombosis/fibrinolysis, inflammation, angiogenesis, vasoconstriction and vasodilation, and repair of damaged organs. The endothelium also produces active factors, including nitric oxide (NO), bradykinin and prostacyclin, endothelin, reactive oxygen species (ROS), prostaglandin H2, thromboxane A2, and angiotensin II[1]. Endothelial dysfunction is a strong independent predictor of both cardiovascular events and mortality in various populations. Dysfunctional endothelium is also related to numerous pathological conditions, including aging, obesity, hypertension, and type 2 diabetes[2]. Since the roles of the endothelium are critical, any noxious stimulating factor could impair its normal function, of which the exact mechanisms have not yet been completely revealed. However, several studies suggest that a disproportionate activation of the endoplasmic reticulum (ER) stress response may play a central role by inducing endothelial apoptosis.

Molecular Mechanisms of the ER Stress Response and Apoptosis

Pathways of ER Stress   The ER is an essential subcellular network of flattened, membrane-enclosed sacs or tube-like compartments. The ER is responsible for lipid biosynthesis, calcium sequestration, and the synthesis and folding of almost one-third of proteins. Different physiological and pathological perturbations, such as oxidative stress, ischemia, and disturbance of calcium homeostasis could interfere with protein folding processes in the ER lumen, lead to accumulation of unfolded or misfolded proteins, and result in a cellular condition known as ER stress. The ER responds to ER stress by further activating intracellular signal transduction pathways, called the unfolded protein response (UPR). The UPR contributes to restoring ER homeostasis by reducing protein synthesis, increasing ER chaperone levels, and clearing misfolded or unfolded proteins[3]. Nevertheless, despite its protective role, when strenuous perturbations are prolonged or excessive for the compensation mechanism, the UPR then triggers intracellular signal cascades that lead to the activation of inflammatory pathways and eventually cell apoptosis[4]. The ER stress also stimulates transmembrane stress sensors: protein kinase-like ER kinase (PERK), inositol-requiring kinase 1 α (IRE1α), and activating transcription factor 6 (ATF6), which bind to ER chaperone glucose-regulated protein 78 kD protein/immunoglobulin binding protein (GRP78/BiP) under unstressed conditions[4].

PERK is an ER transmembrane protein kinase that oligomerizes to activate its kinase domain during ER stress. PERK inactivates the eukaryotic translation initiation factor 2 α-subunit (eIF2α) by serine phosphorylation. This inactivation globally shuts off the translation of 90% of cellular mRNAs, thus attenuating protein load to the ER[5]. Paradoxical to its suppression of global protein translation, the phosphorylation of eIF2α leads to translation of several mRNAs containing open reading frames, such as activating transcription factor-4 (ATF4). ATF4 binds to promoter/enhancer regions and transcriptionally augments the expression of a subset of UPR genes including C/EBP homologous protein (CHOP), vascular endothelial growth factor (VEGF) A, TRB3, E-selectin, and genes important in amino acid metabolism[6].

IRE1α is a transmembrane protein, which contains both a Ser/Thr kinase domain and an endoribonuclease domain. IRE1α oligomerizes and autophosphorylates when dissociated from GRP78/BiP during ER stress. Furthermore, misfolded or unfolded proteins may directly stimulate IRE1α by the domain in the ER chamber. The activated endoribonuclease domain of IRE1α splices a 26-base intron from X-box binding protein 1 (XBP1) mRNA, rendering it competent for translation to produce 41-kDa XBP1 protein, a bZIP-family transcription factor. Spliced XBP1 can promote the expression of UPR genes implicated in ER-associated protein degradation. Moreover, IRE1α can interact with tumor necrosis factor (TNF)-receptor-associated factor 2 (TRAF-2) as IRE1/TRAF-2 complex. The complex IRE1/TRAF-2 recruits the inhibitor of kB (IkB) kinase (IKK) and consequently activates nuclear factor kB (NF-kB), which is a transcriptional factor that promotes the expression of inflammation genes. Furthermore, TRAF-2 both promotes the activation of apoptosis-signaling-kinase 1 (ASK1) and enhances the activity of the stress kinase Jun-N-terminal kinase (JNK), which is known to induce insulin resistance, inflammatory pathways, and apoptosis. ASK1 also promotes the activity of p38 mitogen-activated protein kinase (MAPK) that in turn activates CHOP[7].

The third ER transmembrane sensor, ATF6, acts as a potent transcription factor and performs a different mechanism of protein activation when compared with both PERK and IRE1α. Upon its dissociation from GRP78/BiP, ATF6 is transported to the Golgi apparatus and is cleaved by proteases. Then, the cleaved N-terminal cytosolic domain of ATF6 translocates to the nucleus and upregulates genes involved in protein folding and degradation, including CHOP, NF-kB, GRP78/BiP, and XBP1[2].

ER Stress-related Apoptosis

ER stress can induce widespread pathologic apoptosis under prolonged noxious stimulation. Non-selective ER stress-induced apoptosis is considered a crucial pathogenic factor in several diseases, including neurodegenerative diseases, diabetes, atherosclerosis, and renal disease[8]. Activation of CHOP and IRE1α could induce apoptosis in cells under conditions of stress overload[9]. As discussed above, CHOP could be activated in three branches of the ER stress signaling pathway, of which PERK-eIF2α-ATF4 pathway is the

best known as well as the major branch. As mentioned before, IRE1α can interact with TRAF-2 to become IRE-1/TRAF-2 complex, which could activate both ASK1 and JNK, hence causing apoptosis. Moreover, p38MAPK is also able to up-regulate CHOP in the IRE1α pathway. Prolonged ER stress in vascular endothelial cells of the brain induces a significant flow of Ca^{2+} from the ER to the mitochondria, leading to mitochondrial Ca^{2+} overload, and then activation of mitochondria-dependent apoptosis. This last event is rescued by blocking the mitochondrial Bax channel. Failure of Ca^{2+} homeostasis can increase production of ROS, the resulting elevated levels of ROS can deregulate Ca^{2+} homeostasis, thereby leading to apoptosis[10-11]. Caspase-12 is included in ER-stress-mediated apoptosis of rodent species. This mechanism involves the interaction of pro-caspase-12 with the IRE1-TRAF-2 complex, CHOP-ERO1α-IP3R-calcium-calpain pathway might also contribute to caspase-12 activation. In humans, caspase-4 belongs to the caspase-1 subfamily, is an analog of rodent caspase-12, and performs the functions usually ascribed to rodent caspase-12 in the context of human ER stress[12].

ER Stress-related Apoptosis as an Underlying Molecular Mechanism in Endothelial Cell Pathogenesis

A series of experiments explored how ER stress affected endothelial cells by different mechanisms. In the sections below, recent studies about ER stress-related apoptosis in endothelial pathogenesis are reviewed.

Hyperlipidemia

Dickkopf1 (DKK1) expression is upregulated in response to ox-LDL treatment in a time- and concentration-dependent manner in human umbilical vein endothelial cells (HUVECs). The interference of DKK1 reverses ox-LDL-induced apoptosis in HUVECs. The mechanism underlying this effect is DKK1's activation of the JNK signal transduction pathway and inhibition of canonical Wnt signaling, followed by activation of IRE1α and eif2α/CHOP pathways. DKK1 promotes plaque formation and vulnerability partly by inducing apoptosis in endothelial cells. Endothelial apoptosis is partly induced through the induction of the JNK-endoplasmic reticulum stress pathway and the inhibition of canonical Wnt signaling[13].

Hyperlipidemic Apoe knockout mice with endothelial-specific gain of transient receptor potential canonical 3 channel function (TgESTRPC3/ApoeKO) have increased burden of advanced aortic atherosclerosis with 16 weeks' high-fat diet compared with nontransgenic ApoeKO littermate controls (non-Tg/ApoeKO), the livers of TgESTRPC3/ApoeKO mice show steatosis, fibrosis, and altered hepatic enzymes compared with non-Tg/ApoeKO animals at an early stage. *In vitro*, downregulation of TRPC3 in liver sinusoid endothelial cells reduces their susceptibility to ER stress-induced apoptosis, suggesting that a proapoptotic effect of TRPC3 might add to other fibrogenic factors[14].

SS-31, a tetrapeptide targeting cardiolipin and protecting mitochondrial cristae, could overcome lipotoxicity in all kidney cells including endothelial cells, restores renal AMP kinase activity, and prevents intracellular lipid accumulation, endoplasmic reticulum stress, and apoptosis[15].

A previous study also provided evidence that ox-LDL induces apoptosis in vascular endothelial cells mediated largely via the PERK/eIF2α/CHOP ER-stress pathway[16].

Hyperglycemia

Maamoun et al.[17] reported that hemeoxygenase-1 (HO-1) induction prevents the high glucose-mediated increase of mRNA and protein expression of key ER stress markers. Cells incubated with high glucose exhibited high levels of oxidative stress, activation of major inflammatory and apoptotic responses (NF-κB and JNK) and increased rate of apoptosis; however, cells pre-treated with cobalt (III) protoporphyrin IX chloride (CoPP, HO-1 inducer) or 4-phenyl butyric acid (PBA, ER stress inhibitor) were adequately protected. In addition, high glucose enhances caspase-3 and caspase-7 cleavage and activity, and augments cleaved poly ADP ribose polymerase (PARP) expression, whereas HO-1 induction prevents these effects. Finally, HO-1 induction and ER stress inhibition prevents high glucose-induced reduction in NO release and impairs angiogenic capacity of endothelial cells, and enhances vascular endothelial growth factor (VEGF)-A expression.

Fiorentino et al.[18] recently demonstrated that elevated glucosamine, which could be produced via activation of the hexosamine biosynthetic pathway (HBP) secondary to hyperglycemia, induces endothelial cell apoptosis and inflammation that is, at least partly, mediated by ER stress.

Homocysteinemia

Zhang et al.[19] reported that homocysteine induces apoptosis in HUVECs by activation of the UPR and is signaled through IRE1α in 2001.

Homocysteine treatment decreases endothelial cell viability and increased apoptosis as verified by expression of GRP78/Bip, CHOP, and XBP1 splicing, which is inhibited by l-serine. The effects of l-serine on homocysteine-induced ER stress are not attributed to intracellular homocysteine metabolism, but instead, to decreased homocysteine uptake. Glycine exerts the same effects on homocysteine-induced ER stress, apoptosis, and cell viability; however, the mechanism remains unclear[20].

Kil et al.[21] revealed that homocysteine increases annexin V-positive cells, DHE oxidation, GRP78 and CHOP expression, and XBP1 mRNA splicing, indicating that homocysteine induces apoptosis, oxidative stress, and ER stress, which could be inhibited by pretreatment with piceatannol via activating nuclear factor-E2-related factor 2 (Nrf2) followed by up-regulation of heme oxygenase-1 expression. These results suggest that piceatannol may protect endothelial cells against homocysteine-induced apoptosis, oxidative stress, and ER stress via Nrf2-dependent heme oxygenase-1 expression.

NO and ROS

ER stress is likely an essential initiator of endothelial dysfunction through the induction of oxidative stress and a reduction in NO synthesis. 3',4'-dihydroxyflavonol, which is a synthetic flavonol with antioxidant activity, directly protects against ER stress-induced injury. 3',4'-dihydroxyflavonol also inhibited tunicamycin-induced ER stress and apoptosis in cultured human endothelial cells[22].

Treatment with a lethal concentration of sibutramine facilitates the production of ROS, alters expression of cristae structure the ER stress response genes (heat shock protein 70 and C/EBP homologous protein), and inactivates 26S proteasome-based proteolysis provoking cells apoptosis. The treatment also decreases the cellular level of NO through lowering both the expression and activity of endothelial NO synthase. It might well suggest that ROS production and depletion of NO are crucial events in the apoptotic mechanism and are

likely to be linked to the pathogenesis of vasoconstriction elicited by the drug[23].

Hu et al.[24] demonstrated that AngII upregulates the expression levels of inducible nitric oxide synthase (iNOS), stimulates ROS production, and increases ER stress biomarkers (GRP/Bip, CHOP) and cell apoptosis. Furthermore, hydrogen peroxide (H_2O_2; an exogenous ROS) has similar effects as AngII, whereas the inhibitory effects of AngII are completely suppressed by N-acetyl-L-cysteine (a ROS scavenger). Conversely, sodium hydrosulfide (NaHS; an H_2S donor) reduces ROS production, inhibits CHOP and GRP78 expression, and decreases cell apoptosis. The present study indicated that AngII induces endothelial dysfunction via the activation of ER stress in HUVECs. In addition, the effects of AngII on ER stress could be suppressed by H_2S.

Mechanical Stress

Pan et al.[25] demonstrated that artificial shear stress induces ER stress, interleukin‑1β production, and apoptosis in human aortic endothelial cells in a time‑dependent manner. The inhibition of ER stress and treatment with both an interleukin‑1 receptor antagonist protein and siRNA against interleukin‑1 receptor-associated kinase 2 attenuates shear stress-induced CHOP signaling-mediated cellular apoptosis. Therefore, overproduction of interleukin‑1β exacerbates shear stress‑induced ER stress‑mediated apoptosis via the IRAK2/CHOP signaling pathway in endothelial cells.

Zeng et al.[26] reported that the disturbed flow in *ApoE$^-/^-$* mice exhibit increased expression and splicing of XBP1. Also, over-expression of spliced XBP1 induces endothelial cell apoptosis and accelerates the development of atherosclerotic lesions.

Substantial evidence exists that ER stress is activated and apoptosis is induced in endothelial pathogenesis. Protecting endothelial cells from ER stress-related apoptosis could become a promising therapeutic approach to prevent or improve pathological conditions of the endothelium.

#Correspondence should be addressed to ZHANG Guo Qiang, MD, Tel: 86-10-84205174, E-mail: zhangchong2003@vip.sina.com; YU Pu Lin, PHD, Tel: 86-10-85111151, E-mail: pulin_yu@163.com

REFERENCES

1. Cines DB, Pollak ES, Buck CA, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood, 1998; 91, 3527-61.

2. Battson ML, Lee DM, Gentile CL. Endoplasmic reticulum stress and the development of endothelial dysfunction. Am J Physiol Heart Circ Physiol, 2017; 312, H355-67.

3. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol, 2007; 8, 519-29.

4. Rao RV, Ellerby HM, Bredesen DE. Coupling endoplasmic reticulum stress to the cell death program. Cell Death Differ, 2004; 11, 372-80.

5. Arensdorf AM, Diedrichs D, Rutkowski DT. Regulation of the transcriptome by ER stress: non-canonical mechanisms and physiological consequences. Front Genet, 2013; 4, 256.

6. Cunard R. Endoplasmic Reticulum Stress, a Driver or an Innocent Bystander in Endothelial Dysfunction Associated with Hypertension? Curr Hypertens Rep, 2017; 19, 64.

7. Cimellaro A, Perticone M, Fiorentino TV, et al. Role of endoplasmic reticulum stress in endothelial dysfunction. Nutr Metab Cardiovasc Dis, 2016; 26, 863-71.

8. Kaufman RJ. Orchestrating the unfolded protein response in health and disease. J Clin Invest, 2002; 110, 1389-98.

9. Szegezdi E, Logue SE, Gorman AM, et al. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep, 2006; 7, 880-5.

10. Fonseca AC, Cardoso SM, Pereira CF. Calcium and redox homeostasis in Alzheimer's disease: a focus on the endoplasmic reticulum. Therapeutic Targets for Neurological Diseases, 2014; 1, e428.

11. Fonseca AC, Resende R, Cardoso SM, et al. The role of proteotoxic stress in vascular dysfunction in the pathogenesis of Alzheimer's disease. Endoplasm Reticul Stress Dis, 2015; 2, 67-81.

12. Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest, 2005; 115, 2656-64.

13. Di M, Wang L, Li M, et al. Dickkopf1 destabilizes atherosclerotic plaques and promotes plaque formation by inducing apoptosis of endothelial cells through activation of ER stress. Cell Death Dis, 2017; 8, e2917.

14. Smedlund K, Dube P, Vazquez G. Early steatohepatitis in hyperlipidemic mice with endothelial-specific gain of TRPC3 function precedes changes in aortic atherosclerosis. Physiol Genomics, 2016; 48, 644-9.

15. Szeto HH, Liu S, Soong Y, et al. Protection of mitochondria prevents high-fat diet-induced glomerulopathy and proximal tubular injury. Kidney Int, 2016; 90, 997-1011.

16. Tao YK, Yu PL, Bai YP, et al. Role of PERK/eIF2alpha/CHOP Endoplasmic Reticulum Stress Pathway in Oxidized Low-density Lipoprotein Mediated Induction of Endothelial Apoptosis. Biomed Environ Sci, 2016; 29, 868-76.

17. Maamoun H, Zachariah M, McVey JH, et al. Heme oxygenase (HO)-1 induction prevents Endoplasmic Reticulum stress-mediated endothelial cell death and impaired angiogenic capacity. Biochem Pharmacol, 2017; 127, 46-59.

18. Fiorentino TV, Procopio T, Mancuso E, et al. SRT1720 counteracts glucosamine-induced endoplasmic reticulum stress and endothelial dysfunction. Cardiovasc Res, 2015; 107, 295-306.

19. Zhang C, Cai Y, Adachi MT, et al. Homocysteine induces programmed cell death in human vascular endothelial cells through activation of the unfolded protein response. J Biol Chem, 2001; 276, 35867-74.

20. Sim WC, Han I, Lee W, et al. Inhibition of homocysteine-induced endoplasmic reticulum stress and endothelial cell damage by l-serine and glycine. Toxicol In Vitro, 2016; 34, 138-45.

21. Kil JS, Jeong SO, Chung HT, et al. Piceatannol attenuates homocysteine-induced endoplasmic reticulum stress and endothelial cell damage via heme oxygenase-1 expression. Amino Acids, 2017; 49, 735-45.

22. Lau YS, Mustafa MR, Choy KW, et al. 3',4'-dihydroxyflavonol ameliorates endoplasmic reticulum stress-induced apoptosis and endothelial dysfunction in mice. Sci Rep, 2018; 8, 1818.

23. Morikawa Y, Shibata A, Okumura N, et al. Sibutramine provokes apoptosis of aortic endothelial cells through altered production of reactive oxygen and nitrogen species. Toxicol Appl Pharmacol, 2017; 314, 1-11.

24. Hu HJ, Jiang ZS, Qiu J, et al. Protective effects of hydrogen sulfide against angiotensin II-induced endoplasmic reticulum stress in HUVECs. Mol Med Rep, 2017; 15, 2213-22.

25. Pan L, Hong Z, Yu L, et al. Shear stress induces human aortic endothelial cell apoptosis via interleukin1 receptorassociated kinase 2induced endoplasmic reticulum stress. Mol Med Rep, 2017; 16, 7205-12.

26. Zeng L, Zampetaki A, Margariti A, et al. Sustained activation of XBP1 splicing leads to endothelial apoptosis and atherosclerosis development in response to disturbed flow. Proc Natl Acad Sci USA, 2009; 106, 8326-31.