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REVIEW

Role of endoplasmic reticulum stress in endothelial dysfunction

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Abstract *Aim:* Endoplasmic reticulum (ER) stress is implicated in the pathogenesis of several human disorders, including cardiovascular disease (CVD). CVD recognizes endothelial dysfunction (ED) as its pathogenetic *primum movens*; interestingly a large body of evidence has identified the unchecked ER stress response as a main actor in vascular damage elicited by various cardio-metabolic risk factors. In the present Review, we summarize findings from experimental studies on the ER stress-related ED, focusing on the mechanisms underlying this association.
Data synthesis: Different noxious agents, such as hyperhomocysteinemia, hyperlipidemia, hyperglycemia and chronic inflammation, induce ED promoting an amplified ER stress response as demonstrated by several studies in animal models, as well as in human primary and immortalized endothelial cells. ER stress represents therefore a key mediator of vascular damage, operating in a setting of increased inflammatory burden and oxidative stress, thus contributing to foster a vicious pathogenic cycle.
Conclusions: Experimental studies summarized in this Review strongly suggest that an unchecked ER stress response plays a central role in the pathogenesis of ED and, consequently, CVD. Counteracting ER stress may thus represent a promising, even if largely unexplored as-yet, therapeutic approach aimed to prevent vascular damage, slowing the progression from ED to cardiovascular events.

Acronyms list: AP, activator protein; ASK1, apoptosis-signaling-kinase 1; ATF, activating transcription factor; b-ZIP, basic leucine zipper; CHOP, C/EBP homologous protein; CV, cardiovascular; ED, endothelial dysfunction; e-NOS, endothelial nitric oxide synthase; EGFR, epidermal growth factor receptor; eIF2, eukaryotic initiation factor 2; ER, endoplasmic reticulum; LDL-G, glycated low-density lipoproteins; GLP-1, glucagon-like peptide 1; GlcN, glucosamine; HBP, hexosamine biosynthetic pathway; IκB, inhibitor of κB; IKK, IκB kinase; IL, interleukin; IRE, inositol-requiring enzyme; JNK, Jun-N-terminal kinase; LDL, low-density lipoproteins; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; NFkB, nuclear factor κB; Nfr2, nuclear factor (erythroid-derived-2) related transcription factor-2; NO, nitric oxide; LDL-ox, oxidized LDL; HOG-LDL, oxidized and glycated LDL; PERK, PKR-like ER kinase; ROS, reactive oxygen species; SERCA, Sarcoplasmic/Endoplasmic Reticulum CalciumATPase; STAT, Signal Transducer and Activator of Transcription; TDAG51, T cell death–associated gene 51; TLR, toll-like receptor; TNF, tumor necrosis factor; TRAF-2, TNF-receptor-associated factor 2; XBP-1, X-box binding protein 1; UPR, unfolded protein response.

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Introduction

Over the last decades the vascular endothelium has been widely investigated as an active organ with paracrine, endocrine and autocrine activity, essential for the maintenance of vascular homeostasis, rather than as a simple barrier between the blood and the vascular wall [1,2]. The endothelium produces several vasoactive substances: including nitric oxide (NO), bradykinin and prostacyclin, that act synergistically inhibiting platelet aggregation, while stimulating fibrinolysis; and vasoconstrictor and/or pro-oxidant molecules, such as endothelin, reactive oxygen species (ROS), prostaglandin H_2, thromboxane A_2, and angiotensin II [3]. Given its central role in the maintenance of vascular homeostasis, the endothelium has been recognized as a crucial site for the pathophysiological changes occurring in many cardio-metabolic diseases, especially atherosclerosis. An imbalance of the vascular protective effects of the healthy endothelium (i.e.: vasodilation, antioxidant activity, inhibition of pro-inflammatory cytokines), in response to a variety of noxious stimuli, leads to a condition known as endothelial dysfunction (ED). The main features of ED are the reduction of NO bioavailability, with the consequent lowering of endothelium-dependent vasodilation, and the predominance of pro-inflammatory and pro-coagulant properties of the endothelium (platelet aggregation, leukocyte adhesion, and pro-inflammatory cytokines production) [4]. ED is the *primum movens* of a multifactorial cascade of events that leads to vascular wall damage and consequently to atherosclerotic plaque formation, on a soil of mild inflammation and enhanced oxidative stress, that reduces NO bioavailability and promotes vascular injury. Both traditional (i.e.: age, hypertension, dyslipidemia, diabetes mellitus, smoking) and emerging (i.e.: uric acid, alkaline phosphatase, serum phosphorus, etc.) cardiovascular (CV) risk factors are associated with ED, that occurs early during the process leading to atherosclerosis [1,5]. Several evidence demonstrates that ED is the trigger of the pathogenic pathway ensuing in atherosclerotic plaque formation [1]. In addition ED plays a key role in the development and progression of subclinical organ damage [6,7]; furthermore ED is an independent predictor of CV events [1], and of type 2 diabetes in hypertensive patients [8].

The molecular mechanisms underlying ED have not been completely elucidated yet; however several studies suggest that a disproportionate activation of the endoplasmic reticulum (ER) stress response may play a central role in this phenomenon (Fig. 1); herein we will review recent experimental evidence sustaining this hypothesis.

Molecular mechanisms of the ER stress response

The ER intraluminal oxidative environment and high calcium concentration are critical for the proper folding of secretory and transmembrane proteins. Several disturbances, such as viral infections, energy deprivation, oxidative stress, depletion of calcium, gene mutations, and elevated protein traffic may alter the ER environment and consequently promote the accumulation of unfolded proteins in the ER lumen, a condition known as ER stress [9]. Accumulation of unfolded proteins then triggers the activation of an adaptive signaling cascade called the unfolded protein response (UPR), whose initial intent is to restore ER homeostasis by counteracting protein synthesis, increasing ER chaperone levels, and facilitating degradation of irreversibly misfolded proteins. However, in addition to its cytoprotective function, when noxious stimuli are prolonged or excessive and compensatory mechanisms fail to reestablish normal ER function, the UPR triggers intracellular signals that culminate in the activation of inflammatory pathways and finally in apoptotic cell death [10], as summarized in Fig. 2.

The UPR is driven by the activation of three ER-localized transmembrane signal transducers: PKR-like ER kinase (PERK), inositol-requiring enzyme (IRE)-1, and activating transcription factor (ATF)-6, that, in unstressed conditions, are maintained in an inactive status by the binding with the ER chaperone BiP (Grp78) [11]. Upon ER stress, BiP dissociates from the ER stress sensors to bind misfolded proteins in an attempt to facilitate their proper folding.

BiP dissociation results in the activation of the ER stress sensors: PERK dimerizes, autophosphorylates and consequently activates the α subunit of eukaryotic initiation factor 2 (eIF2α). Phosphorylated eIF2α, in turn, prevents mRNA translation thus counteracting protein load to the ER. Furthermore, the phosphorylation of eIF2α leads to a selective advantage in the translation of several mRNAs such as the mRNA encoding for ATF-4. ATF-4 belongs to the basic leucine zipper (b-ZIP) family of transcription factors and promotes the expression of several genes implicated in the UPR, including those encoding for promoters of ER stress-mediated apoptosis such as C/EBP homologous protein (CHOP) [11].

Similarly to PERK, IRE-1 oligomerizes and autophosphorylates when released by BiP. As a consequence its site-specific endoribonuclease (RNase) activity is induced. Alternatively, it has been suggested that unfolded proteins may directly interact with ER-luminal domain of IRE-1 thus promoting its oligomerization and activation [12,13]. The activated RNase domain of IRE-1 cleaves a 26-base intron from X-box binding protein 1 (XBP-1) mRNA. This unconventional splicing reaction leads to the translation of transcriptionally active XBP-1. Spliced XBP-1 is a b-ZIP transcription factor able to promote the expression of UPR genes implicated in the ER-associated protein degradation. In addition to XBP-1, the RNase activity of IRE-1 controls the splicing of several RNAs, including its own, and microRNAs that modulate the expression of caspase proteins. Furthermore, upon conditions of high levels of chronic ER stress, IRE-1 is able to interact with tumor necrosis factor (TNF)-receptor-associated factor 2 (TRAF-2) and, therefore, activate cell death and inflammatory pathways. The complex IRE-1/TRAF-2 has been shown to recruit the inhibitor of κB (IκB) kinase (IKK) and consequently promote the activation of nuclear factor κB (NFkB), a transcriptional factor known to upregulate the