

Review Article

Integration of ER stress, oxidative stress and the inflammatory response in health and disease

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Abstract: There has been much effort to define the molecular basis by which pathophysiological stimuli initiate and/or propagate the inflammatory response. Recent research endeavors on stress response from a cellular organelle called the endoplasmic reticulum (ER) shed new light on the understating of the molecular basis of the inflammatory response and its interaction with other intracellular stress signaling pathways. As a protein folding compartment and dynamic calcium store, the ER plays major roles in sensing cellular stress and mediating highly-specific signaling pathways termed Unfolded Protein Response (UPR). The UPR signaling emanating from the ER has been identified as one of the avenues leading to the inflammatory response. The integration of ER stress, oxidative stress, and the inflammatory response is critical to the pathogenesis of a variety of diseases. In this brief review, we discuss some representative evidence for the integration of ER stress, oxidative stress, and inflammation in health and disease.

Keywords: ER stress, oxidative stress, inflammation, unfolded protein response, metabolic factors, inflammatory disease

Introduction

In eukaryotic cells, the ER is an organelle responsible for folding and assembly of membrane and secreted proteins, synthesis of lipids and sterols, and storage of free calcium [1, 2]. As a protein folding compartment, the ER provides a unique oxidizing environment in which molecular chaperones and folding enzymes facilitate and promote protein post-translational modification, folding, and oligomerization [1]. The ER has evolved a high-fidelity quality control system to ensure that only correctly folded proteins are transported to the Golgi compartment and then delivered to the extracellular environment. However, physiological states that increase protein-folding demand, or stimuli that disrupt protein folding reactions, create an imbalance between the protein-folding load and the capacity of the ER. This can cause the accumulation of unfolded or misfolded proteins in the ER lumen - the condition referred to "ER stress" [2-7]. To deal with the accumulation of unfolded or misfolded protein in the ER, the cells activate a group of signal transduction

pathways collectively termed Unfolded Protein Response (UPR) to alter transcriptional and translational programs. In mammalian cells, the basic UPR pathways are mediated through three ER-transmembrane protein factors, including IRE1 α (inositol-requiring 1 alpha), PERK (double-strand RNA-activated protein kinase-like ER kinase), and ATF6 (activating transcription factor 6). IRE1 α is a protein kinase and endoribonuclease that can splice the mRNA encoding a basic leucine zipper (bZIP) transcription factor XBP1 under the ER stress, leading to expression of the activated form of XBP1 [6, 8, 9]. The IRE1 α /XBP1 arm of the UPR facilitates cell adaptation to ER stress by activating expression of a group of genes that are required for protein refolding, secretion, and degradation of misfolded proteins [10]. PERK is a protein kinase that phosphorylates alpha-subunit of eukaryotic translation initiation factor (eIF2 α), leading to translational attenuation in general [11, 12]. The translational attenuation can reduce the workload of the ER by preventing production of newly-synthesized proteins. ATF6 is a bZIP transcription factor of CREB/ATF family that is acti-

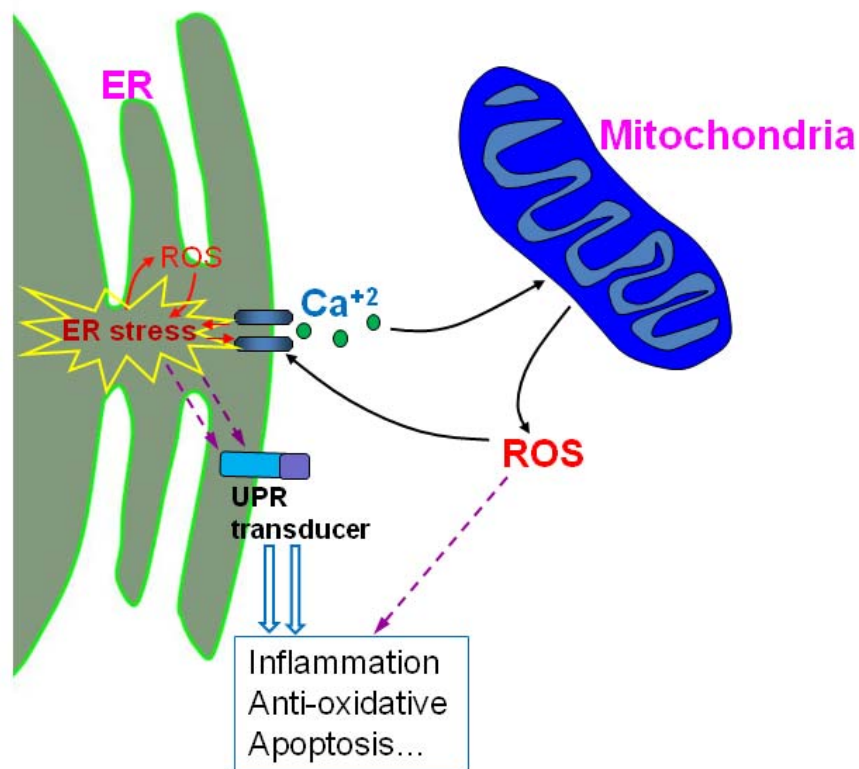


Figure 1. Crosstalk between ER stress, oxidative stress, and the inflammatory response. Many physiologic or pathologic conditions can stimulate the production of ROS. ROS can target ER-based calcium channels and chaperones, leading to release of calcium from the ER to the cytosol. Increased cytosolic calcium can stimulate mitochondria metabolism to produce more ROS. Mitochondrial ROS can further accentuate calcium release from the ER, leading to the accumulation of a toxic level of ROS. Meanwhile, perturbation of ER calcium homeostasis can disrupt protein folding process, causing ER stress and the activation of the UPR. The UPR can subsequently promote the inflammatory response, anti-oxidative stress response, apoptosis, and other stress response pathways.

vated through a regulated-intramembrane proteolysis (RIP) under ER stress [13]. Activated ATF6 activates expression of UPR target genes encoding ER chaperones and folding enzymes to help protein folding, secretion, and degradation [14]. The initial phase of the UPR is to facilitate the cell adaption to ER stress. However, if the attempt to recover from ER stress fails or the ER stress gets prolonged, the UPR will induce cell death programs to eliminate the stressed cells [15].

In recent years, the scope and consequence of ER stress and the UPR have been significantly expanded. A growing body of evidence suggests that ER stress, oxidative stress, and the inflammatory response are cross-linked and that limiting of either one will affect the others [7, 16]. Inflammation can be triggered by chronic excess

of metabolic factors, such as cytokines, hormones, cholesterol, lipid, and glucose. Interestingly, in many physiological or pathological systems, those metabolic factors can also trigger ER stress and oxidative stress, which can further disrupt metabolic function, leading to more inflammation. Such vicious cycles could be accounted for many pathophysiological events leading to metabolic deterioration in specialized cell types, such as macrophage, adipocytes, and pancreatic β -cells [7]. Importantly, intracellular calcium signals and free radicals, such as reactive oxygen species (ROS) and nitric oxide (NO), are key messengers for the interaction between ER stress, oxidative stress, and inflammation. In these interactive signal transduction processes, the functions of the ER and mitochondria are closely linked. These two organelles build up a dynamic network where they generate calcium

signals and ROS to stimulate ER stress, oxidative stress, and inflammation (**Figure 1**).

Metabolic factors that trigger ER stress, oxidative stress, and the inflammatory response

Cholesterol

Cholesterol is an essential nutrient component for life but also associated with inflammation and the pathogenesis of metabolic disease if it is in pathological excess. Cholesterol, either synthesized in the ER or derived from the diet, is an essential component required to build and maintain all cell membranes. The cholesterol metabolism is closely associated with the ER function. This is well illustrated by the macrophage loaded by excessive unesterified, or "free" cholesterol [17, 18]. Upon elevation of lipids in the bloodstream, a metabolic condition referred as "hyperlipidemia", macrophages take up excessive amounts of cholesterol, resulting in formation of foam cells or inflammatory macrophages. Notably, the accumulation of cholesterol in macrophages induced synthesis of pro-inflammatory cytokines, including TNF α and IL6, by activating the nuclear factor kappa B (NF- κ B) and the mitogen-activated protein kinases-mediated inflammatory pathways [19, 20]. Importantly, the activation of all these inflammatory pathways required cholesterol trafficking to ER and were modulated by PERK- and IRE1 α -mediated UPR signaling. It has been demonstrated that the accumulation of cholesterol in macrophage leads to overload of unesterified, or "free" cholesterol in the ER membranes and subsequent depletion of ER calcium stores [17]. This condition causes ER stress and elicits the UPR in the macrophages as shown by the activation of three ER stress sensors, including PERK, IRE1 α and ATF6, and their downstream effector molecules. Interestingly, CHOP, the target of PERK-mediated UPR pathway, is required for IL6 induction and full activation of Erk kinase induced by overload of cholesterol [19]. However, one or more other ER stress response pathways, possibly the ER-overload response, might also contribute to NF- κ B activation induced by cholesterol accumulation, as the ER calcium release and ROS generation occurred with cholesterol overloading in the macrophage [19, 21]. Eventually, if the hyperlipidemia condition becomes chronic, the ER stress-induced pro-apoptotic factor CHOP would be activated to induce macrophage death that

possibly contributes to necrosis and plaque disruption in atherosclerotic lesion [17, 22].

Homocysteine

Homocysteine is a sulfur-containing amino acid that is generated inside the body and required for biosynthesis of methionine and cysteine. The absence of vitamin B6, B12 or folic acid in food, or chronic alcohol consumption can affect activities of enzymes required for methionine, homocysteine and cysteine metabolisms and subsequently lead to accumulation of homocysteine in the body. Homocysteine has been considered as an independent risk factor for cardiovascular diseases. The highly reactive thiol group of homocysteine undergoes oxidation to form ROS, inducing oxidative stress and the inflammatory response [23, 24]. Homocysteine can also interrupt disulfide bond formation during protein folding, and thus causing ER stress and activation of the UPR [25, 26]. In fact, multiple cellular stress pathways including ER stress, oxidative stress, and inflammation are associated with accelerated atherosclerosis in animal models fed with high-homocysteine diets. Markers of ER stress (GRP78/94 and phospho-PERK), oxidative stress (HSP70), and inflammation (phospho-I κ B) were simultaneously detected in advanced atherosclerotic lesions fed with high-homocysteine diets. ER stress-induced apoptosis, which is synergized by oxidative stress and inflammatory signaling, likely accounts for the cell injury and dysfunction during atherosclerosis induced by homocysteine [27].

Cytokines

Pro-inflammatory cytokines including TNF α , IL6, and IL1 β can induce ER stress in liver hepatocytes, leading to the activation of the hepatocyte-specific stress sensor CREBH to mediate a liver acute phase response [28]. In mouse cancer fibrosarcoma cells, TNF α appears to be a strong ER stress inducer that activates three major UPR pathways as shown by PERK-mediated eIF2 α phosphorylation, Xbp1 mRNA splicing and ATF6 cleavage [29]. In the central nervous system, the presence of T cell-derived cytokine interferon-gamma (IFN- γ) was associated with the activation of the PERK branch of the UPR and ER stress-induced apoptosis in the oligodendrocyte, a cell type that produces vast amounts of myelin [30-32]. Myelin is a unique, lipid-rich, multilamellar sheath that wraps axons

of neurons. Thus, IFN- γ is believed to contribute to immune-mediated demyelinating disorders by inducing ER stress-mediated oligodendrocyte death. It has been documented that those inflammatory cytokines induce ER stress by triggering calcium signals and accumulation of ROS associated with the ER protein folding process and mitochondrial metabolisms [30]. ER stress can also arise in the inflammatory state of obese adipose tissue. In obesity, elevated levels of cytokine and hormones, such as leptin, insulin, TNF α , IL6, and IL1 β , stimulate metabolisms and cell differentiation programs in the liver, adipose, and pancreas, leading to increased protein folding demands to the ER [33]. This situation would result in a physiological UPR and chronic inflammation in the specialized cell types, such as macrophages, hepatocytes, adipocytes, and pancreatic β cells [34]. Additionally, the bacterial toxin lipopolysaccharide (LPS), a strong inducer of the inflammatory response, has been shown to induce ER stress and ER stress-associated apoptosis in the liver and lung tissues [28, 35].

Glucose

Glucose is a primary source of energy and metabolic intermediate in most organisms from bacteria to humans. The metabolism of glucose is tightly controlled at the levels of synthesis and utilization through hormonal regulation [36]. The ER is exquisitely sensitive to glucose availability. The blood glucose levels influence the energy supply for the protein folding process in the ER, and thus are associated with the activation of the UPR and inflammation by stimulating ER stress and the production of free radicals. UPR signaling has been proposed to be essential to maintain glucose homeostasis [37]. This is well exemplified in pancreatic β cells, the specialized insulin-producing cells. As blood glucose declines, energy may become limiting for protein folding in the ER and therefore PERK-mediated UPR pathway may be activated, leading to translational attenuation to reduce the ER workload. As blood glucose levels rise, the UPR pathways are turned off so that translation would accelerate, allowing entry of new proinsulin into the ER. However, continual elevation of blood glucose could prolong elevated proinsulin translation and eventually activate the UPR as the secretion capacity of the ER is overwhelmed [38]. This condition could also induce inflammation as characterized by increased expression of

pro-inflammatory cytokines [39, 40]. Therefore, a delicate balance between glucose levels and the UPR needs to be maintained. Disturbances in either direction may lead to β cell dysfunction.

Fatty acids

Accumulating evidence suggested that saturated fatty acids, such as palmitate and oleate, can induce ER stress and ER stress-associated apoptosis in various metabolic cells by triggering calcium signals and free radicals [34, 41]. Palmitate, the major saturated fatty acid in the "Western" style diet, was shown to induce ER stress-associated apoptosis mediated through CHOP in liver cells [41]. The presence of increased circulating and/or hepatic saturated fatty acids (palmitate and stearate), but not polyunsaturated fatty acids (oleate and linoleate) may exacerbate steatohepatitis and lipotoxicity through activating ER stress-associated apoptosis [41, 42]. These observations implicated a crucial role for the signaling pathways from the ER in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Moreover, inhibition of ER calcium release by calcium chelators can prevent saturated fatty acid-induced ER stress and apoptosis in H4IIE liver cells and in primary rat hepatocytes, supporting that saturated fatty acids induce ER stress through disrupting ER calcium pool [43]. More recently, palmitate was shown to induce ER stress and apoptosis in hypothalamic neurons through a c-Jun N-terminal kinase (JNK)-dependent pathway [44]. Palmitate can also induce lipotoxic ER stress in pancreatic β cells via the effect on intracellular calcium homeostasis [45, 46].

In addition to those described above, many other metabolic factors can also induce both ER stress and inflammatory pathways in a variety of cell types when they are in pathological excess. For example, neurotransmitters including 6-hydroxydopamine and glutamine can induce the UPR and inflammation in cellular models of Parkinson's disease by disrupting calcium homeostasis and/or stimulating oxidative stress in the neuron cells [47] [48]. Notably, most of those effects are generated by the similar mechanism involving production of calcium signals and reactive oxygen intermediates.

Calcium and free radicals as the messengers triggering ER stress, oxidative stress, and the inflammatory response

Calcium and free radicals including ROS and NO are intracellular signaling messengers that play critical roles in cell physiology and disease pathogenesis. In fact, calcium signals and free radicals are major inflammatory mediators. Over the past few years, it has become increasingly apparent that calcium and free radicals are essential mediators that link ER stress to inflammation during metabolic processes. As an intracellular calcium store, the ER possesses calcium at a concentration thousands of times greater than that in the cytosol. The calcium levels inside the ER are modulated by the ER-located calcium release channels, including inositol-1,4,5-trisphosphate receptor (IP₃R) and the ryanodine receptor, the calcium uptake mechanism mediated through Sarco/Endoplasmic Reticulum Calcium ATPase (SERCA), and numerous ER-associated enzymatic cascades [49-52]. ER stress-inducing pharmaceutical agents and many metabolic factors, such as cytokines, hormones, neurotransmitters, and lipids, can target on ER-based calcium channels and affect the activities of calcium-dependent protein folding enzymes, leading to the release of Ca²⁺ from the ER lumen [7, 16, 53]. This will increase the concentration of cytosolic Ca²⁺ influx, which subsequently stimulates the mitochondria metabolism to generate more ROS. ROS can further target the ER calcium channels and protein folding enzymes to exacerbate ER calcium release and ER stress. As a consequence, unfolded or misfolded proteins accumulate in the ER, followed by the activation of the UPR pathways to promote the inflammatory response, antioxidant response, apoptosis, and other stress response pathways [6, 7] (**Figure 1**). This forward vicious cycle may account for many pathological processes that are triggered by inflammatory stimuli, oxidative stress, and/or ER stress. Notably, recent studies suggested that mitochondria can form junctions with the sarco/ER, which provides a platform for the local calcium-ROS interaction [52, 54].

NO is a free radical gas produced during a variety of metabolic processes, such as regulation of blood vessel dilatation, immune response, and neurotransmissions. Accumulation of a large amount of NO is toxic to the host and

known to cause many deteriorious diseases including hypotension, septic shock, neurodegenerative disease, and diabetes. Excessive NO can target on ER calcium stores and mitochondrial electron transfer chains, causing ER stress and production of ROS. This mechanism accounts for most NO-induced inflammation and metabolic disorders. In addition, recent findings suggest that NO can inhibit the activity of protein disulfide isomerase (PDI) that is required for protein disulfide bond formation, thereby hampering proper protein folding and aggravating ER stress in neuronal cells [55].

Specialized cell types in which ER stress, oxidative stress, and the inflammatory response are integrated.

The cross-talks between ER stress, oxidative stress and the inflammatory response are well demonstrated at the functional interface of professional cells of primarily immune or metabolic nature. Those cells, including liver hepatocytes, macrophages, adipocytes, pancreatic β cells, and neuronal oligodendrocytes, are specialized for a high secretory capacity in cope with the increased demand of protein synthesis [7, 56, 57]. Therefore, they are exquisitely sensitive to ER stress or changes of metabolic status. In those cells, nutrients such as glucose, lipids, and cytokines can activate both the UPR and the inflammatory pathways by triggering calcium signals, ROS, and/or NO. The inflammatory stress signaling regulates the production of various cytokines including TNF α and IL6, and promotes production of other metabolic factors classified as hormones, growth factors, and neurotransmitters to mount a broader inflammatory response. For example, adipocyte is the most abundant cell type composing the adipose tissue. In addition to its inherent properties of fat cells in energy storage, adipocytes play a crucial role in the generation of a variety of pro-inflammatory cytokines, chemokines and hormones [57]. Metabolic conditions, such as lipid accumulation, elevated glucose or excessive cytokines, can stimulate increased demands for protein synthesis and production of ROS in adipocytes, which subsequently induce the UPR and inflammatory response to accelerate production of cytokines and hormones [57, 58]. Integrated UPR and inflammatory signaling in adipocytes may significantly contribute to the inflammatory state of obesity that is associated with insulin resistance. A similar "stress-

inflammation” interactive loop also occur to macrophages and pancreatic β cells where ER stress, oxidative stress, and inflammation interact and amplify by attacking the ER and mitochondria as well as various other metabolic pathways [7, 19, 33, 59].

Concluding remark

We discussed recent evidence of the integration of ER stress, oxidative stress, and inflammation in health and disease. Depending on the cell type and physiological process, either ER stress, or oxidative stress, or the inflammatory response may be more prominent or upstream of the others. However, these signaling pathways interact and are ultimately integrated in the pathogenesis of a variety of diseases. The related information is particularly important for the design of effective therapeutics for the inflammatory diseases by modulating ER stress, oxidative stress, and/or the inflammatory response. Given the complexity of stress signaling network, targeting a signal inflammatory stress mediator may not be effective or beneficial in controlling disease pathogenesis. Therefore, the research work in delineating the interface of stress signaling and the inflammatory response will definitely contribute to our better understanding of the pathogenesis of inflammatory diseases as well as pharmaceutical interventions toward controlling inflammation and stress.

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