Biologia Serbica
2013, Vol. 35 No. 1-2 15-23

Review

Cellular responses to endoplasmic reticulum stress

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Recieved for Review: 10 December 2013 / Accepted: 04 March 2014.

Summary. The Endoplasmic reticulum (ER) is a highly dynamic organelle that provides high fidelity quality control in protein synthesis, maturation and transport. The complex function of the ER can be significantly influenced by various factors both inside the cell and in its microenvironment. Disturbances in ER protein folding capacity result in accumulation of misfolded proteins in the ER lumen and in activation of ER stress. The unfolded protein response (UPR) normally has prosurvival functions and protects cells by providing the reestablishment of protein processing and cellular homeostasis. However, prolonged and excessive ER stress results in activation of apoptotic pathways. Dysregulation of ER function has been recognized as a cause of numerous pathophysiological conditions. Therefore, detailed investigation of ER stress signaling during disease may provide promising approaches in the development of UPR-modifying therapeutic strategies.

Keywords: atherosclerosis, cancer, diabetes, neurodegenerative disease, unfolded protein response.

INTRODUCTION

The endoplasmic reticulum (ER), which is present in all eukaryotic cells, represents a membranous labyrinth of branching interlinked tubules and flattened sacs extending from the perinuclear space throughout the cytoplasm, and is responsible for an assortment of critical cellular housekeeping functions. The rough ER, assembled with ribosomes, plays a key role in protein synthesis, folding, posttranslational modification, and transport. The smooth ER has a central role in the biosynthesis of lipids and steroids, assembly of lipid bilayers, metabolism of carbohydrates, metabolism of drugs and xenobiotics and regulation of calcium intracellular homeostasis. The morphology and the extent of the ER network organization very much depends on the predominant function of specific cells and tissues. All eukaryotic cells have significant amounts of rough ER that is essential for the synthesis of plasma membrane proteins and proteins of the extracellular matrix. Rough ER is particularly abundant in secretory cells, where a large fraction of the cytosol is occupied by rough ER, oftentimes more than 10% of the total cell volume (Schönthal 2012a).

Quality control mechanisms of the cell ensure that newly synthesized proteins are folded into their correct configuration according to their function and destination in the cell. Therefore, protein folding in particular represents an exquisitely orchestrated aspect of protein synthesis in the ER that involves pathways for folding, assembly, modification, quality control, and recycling. In addition to an oxidizing environment, protein folding requires the participation of chaperone proteins, glycosylating enzymes and adequately high calcium (Ca^{2+}) levels (Schönthal 2013). Appropriate folding of the nascent polypeptide chain is achieved through the actions of a series of molecular chaperones and foldases, which keep the polypeptide in soluble form and facilitate folding into a thermodynamically favored structure (Luoma 2013).

The lumen of the ER is rich in Ca^{2+}-dependent molecular chaperones, such as glucose-regulated protein 78 (GRP78, also called BiP: immunoglobulin heavy chain-binding protein, HSPA5), GRP94, calnexin, calreticulin. Moreover, enzymes involved in posttranslational protein modifications, such as protein disulphide isomerase (PDI), oxidoreductases, enzymes involved in protein glycosylation and lipidation,

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and numerous other proteins involved in lipid and membrane biosynthesis, are located in ER lumen (Braakman and Bulleid 2011).

Since the ER provides high fidelity quality control in protein synthesis, maturation and transport, it is a highly dynamic organelle, whose complex function can be significantly influenced by various factors both inside the cell and in its microenvironment. N-linked glycosylation of proteins is impaired under conditions of low glucose supply (Csala et al. 2012). The imbalance in cellular redox homeostasis caused by hypoxia and prooxidant or reducing agents interferes with disulfide bonding of proteins (Hagiwara and Nagata 2012). Depletion of calcium levels has an effect on the activity of Ca^{2+}-dependent chaperones (Krebs et al. 2011). Viral infections may overload the ER lumen with virus-encoded proteins (von dem Bussche 2010). A diet rich in sugars and fats (chronic hyperglycemia and hyperlipidemia) has also been linked to increased ER stress, particularly in the liver and in insulin secreting pancreatic β-cells (Dara and Kaplowitz 2011; Back and Kaufman 2012; Fu et al. 2012). Other factors include protein mutations, environmental toxins, hyperthermia, acidosis, metabolic starvation and aging. Failures in control mechanisms lead to accumulation of unfolded, misfolded, insoluble or otherwise damaged proteins in the lumen of the ER resulting in a state known as ER stress.

DISCUSSION

The unfolded protein response

Continued accumulation of incorrectly folded proteins can irreversibly and irreparably damage cellular functions leading to cytotoxicity. Therefore, several cellular sensors and pathways have evolved to respond to this threat and to reduce this risk. Prime among these is the unfolded protein response (UPR), a signaling pathway primarily aiming at protecting cellular integrity by restoring proper ER folding capacity and overall protein processing (Chakrabarti et al. 2011). However, terminally misfolded proteins that cannot be repaired may be removed from the cell by one of two separate processes. One process is ER-associated degradation (ERAD), which involves the retro-translocation of irreparably misfolded proteins from the ER back into the cytosol, where they are ubiquitinated and subsequently subjected to degradation *via* the proteasome. Furthermore, insoluble misfolded proteins may be assembled together with other cellular debris into aggresomes (juxtanuclear complexes that occur as a cell culture phenomenon, for sequestration of toxic, aggregated proteins) and then recycled *via* autophagy (Nakatsukasa and Brodsky 2008; Clarke et al. 2012).

ER stress response or the UPR, involves a set of adaptive pathways, signaling across the ER membrane and through the cytoplasm into the nucleus, resulting in altered gene expression patterns with the ultimate goal of alleviating ER stress and re-establishing cell homeostasis, or, if necessary, to stimulate apoptosis (Tabas and Ron 2011; Jäger et al. 2012). Therefore UPR has dichotomic characteristics. Mild or short-term stress triggers activation of a response that either leads to neutralization of the initial stress trigger or adaption. However, if excessive damage is induced by severe or persisting stress, the initial prosurvival efforts are replaced by activation of a concerted proapoptotic mechanisms resulting in elimination of the damaged cell. These two opposing forces of cell survival and cell death ensure survival and protection of the organism's integrity.

The primary goal of UPR is to eliminate inappropriately folded proteins and reduce the load of newly synthesized unfolded proteins within the ER. This can be accomplished through three mechanisms: (a) translational attenuation to arrest the influx of newly synthesized proteins into the ER lumen, (b) transcriptional activation of genes encoding proteins involved in protein folding to assist the maturation of proteins that can be salvaged, and (c) transcriptional activation of genes coding for components of the ERAD system to reduce the amount of misfolded proteins (Kimata and Kohno 2011). However, if the stress is prolonged or severe, UPR initiates programmed cell death (Hetz 2012).

Key players in the UPR

Accumulation of unfolded or misfolded proteins is detected by ER transmembrane receptors. ER stress engages the ER molecular chaperone GRP78 and three ER transmembrane proteins: protein kinase activated by double-stranded RNA (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) - to mobilize the UPR (Fig. 1). These sensors initiate ER-to-nucleus signaling cascades aimed at maintaining ER function (Kraskiewicz and Fitzgerald 2012). They have an ER-luminal part that senses the protein-folding environment, and a cytoplasmic part that interacts with the transcriptional and/or translational apparatus (Ron and Walter 2007). These three arms of the UPR are tightly regulated, with respect to timing and response amplitude. The activation of particular arms of the UPR is specific to the source of ER stress and governs cell fate, supporting either an adaptive response (cell survival) or a maladaptive response (cell death) (Rutkowski et al. 2006).

GRP78 is a member of the heat shock proteins-70 family of chaperones, present in different cellular compartments (Ni et al. 2011). Within the ER, it acts as a chaperone and participates in protein folding and assembly. Also, GRP78 negatively regulates the UPR signaling pathways by physically interacting with luminal parts of the three UPR transducers: PERK, IRE1 and ATF6 (Fig.1). In the absence of stress, each is maintained in an inactive state through its association with GRP78 (Bertolotti et al. 2000). As unfolded proteins accumulate, GRP78 dissociates from the molecular sensors

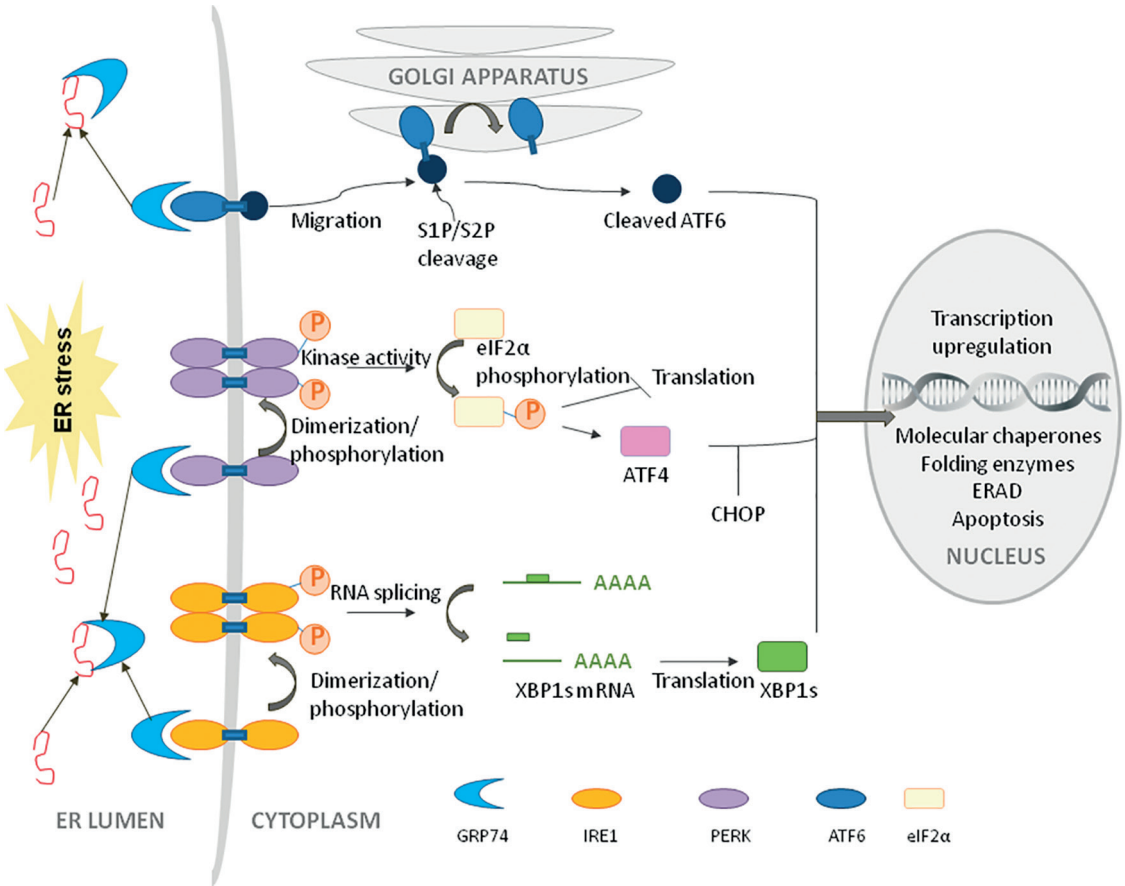

Figure 1. The unfolded protein response pathways.

and binds to hydrophobic domains on the surface of these unfolded proteins in an attempt to affect their repair (Clarke et al. 2012). As a result, dissociation from GRP78 leads to the activation of all three of these transmembrane proteins, thereby activating three distinct branches of the ER stress response/UPR (Parmar and Schroder 2012). The significantly increased amount of GRP78 protein over baseline expression has become an established indicator and marker for the presence of cellular ER stress (Zhang LH and Zhang X 2010).

PERK is a transmembrane serine/threonine kinase. It has a luminal ER-stress sensing domain and is activated through its homodimerization and transauto-phosphorylation (Fig. 1). The activation PERK is followed by phosphorylation of eukaryotic translation initiation factor-2 alpha (eIF2α), which results in global translational attenuation and, therefore, entry of proteins into the ER, which serves to reduce the risk of further accumulation of misfolded proteins in the ER (Harding et al. 2000). However, phosphorylated eIF2α promotes the translation of an activating transcription factor-4 (ATF4), that stimulates a set of genes involved in recovery and adaptation (Fels and Koumenis 2006). Among numerous genes, ATF4 induces the UPR effector, a pro-apoptotic CHOP (C/EBPα-homologous protein, GADD153). In unstressed cells, CHOP protein levels generally are below detection levels, but are substantially increased upon acute ER stress. Its proapoptotic capacity fully emerges only if misfolded protein levels remain elevated for extended periods of time, as is the case when ER stress cannot be suppressed by the action of GRP78 and other proteins. Prolonged CHOP expression triggers apoptotic program through a variety of mechanisms, including downregulation of the antiapoptotic factor B-cell lymphoma-2 (Bcl-2) and induction of a pro-apoptotic BH3-only members of the Bcl-2 family (particularly Bim, Puma, and Noxa), ER oxidoreductin-1α and tibbles-related protein-3 (Nishitoh 2012; Logue et al. 2013). The combination of increased BH3 - only protein expression and the expression of antiapoptotic proteins shifts the balance in favor of apoptosis, permitting Bax-Bak homo-oligomerization and mitochondrial outer membrane permeabilization, causing cytochrome c release and subsequent apoptosome formation. CHOP induces death receptor 5 (DR5), which further sensitizes cells to apoptotic stimulation by a variety of conditions that cause ER stress (Yamaguchi and Wang 2004). Besides its well-established proapoptotic function, CHOP also participates in alleviating the general block on translation *via* stimulation of GADD34 (growth arrest and DNA damage inducible protein-34). GADD34 upregulates an enzyme named protein phosphatase type-1 (PP1), which

dephosphorylates phospho-eIF2α (Kojima et al. 2003). Unphosphorylated eIF2α resumes its function in order to restart general translation. PERK also increases levels of tumor suppressor p53. The increase in p53 during UPR response leads to cell-cycle inhibition, suggesting another adaptive mechanisms for UPR-mediated cell survival (Zhang et al. 2006).

Another target of PERK phosphorylation is nuclear factor-erythroid 2-related factor 2 (Nrf2), a transcriptional factor that migrates into the nucleus where it activates genes encoding for antioxidant proteins and detoxifying enzymes. Because ER stress may involve the accumulation of reactive oxygen species (ROS), thereby promoting a state of oxidative stress, Nrf2 plays a critical role in preventing such perturbations in redox homeostasis (Cullinan and Diehl 2006).

IRE1 is a bifunctional molecule with both serine/threonine protein kinase and endoribonuclease (RNase) activity in its cytosolic domain. Release from GRP78 triggers its activation by homodimerization and autophosphorylation (Fig. 1). The main homeostatic signaling output of IRE1 emanates from its RNase domain. Thanks to its RNase activity, IRE la can induce the splicing of a 26 nucleotide intron from X-box binding protein-1 (XBP1) mRNA, generating a transcription factor called spliced XBP1 (XBP1s) (Ron and Hubbard 2008). XBP1s is a highly active transcription factor that regulates ER folding capacity by binding ER stress-response elements. XBP1s upregulates chaperones for ER protein folding and quality control, proteins involved in ERAD and in autophagy. XBP1s also upregulates synthesis of phospholipids that are required for the expansion of the ER membrane surface area needed for maintenance of ER function during ER stress (Sriburi et al. 2007). IRE1 signaling and XBP1 splicing are particularly important in highly secretory cells (such as pancreatic β-cells and plasma cells), where the protein folding machinery is continuously engaged in producing a high amount of nascent proteins (Iwawaki et al. 2010).

The second function of IRE1 is to activate a signaling cascade involved in controlling cell fate with particular emphasis on cell death. Therefore, IRE1 has an intrinsic kinase activity that appears to be involved in the regulation of its nuclease function. Upon its activation, IRE1 binds the adaptor protein, TNF receptor–associated factor–2 (TRAF2), which then promotes the activation of c-Jun N-terminal kinase (JNK) *via* apoptosis signal–regulating kinase–1 (ASK1) (Urano et al. 2000). Sustained JNK activity during prolonged ER stress inhibits antiapoptotic members of the Bcl-2 family of proteins. On the other hand, JNK phosphorylates and activates proapoptotic BH3-only proteins, such as Bid (BH3 interacting domain death agonist) and Bim (Bcl-2-interacting mediator of cell death). When combined, these events lead to oligomerization of Bax and Bak, resulting in permeabilization of the outer mitochondrial membrane and execution of the intrinsic apoptotic process (Dhanasekaran and Reddy 2008; Jäger et al. 2012). In addition to its endoribonuclease and kinase activities, in some cell types IRE1 activation has been reported to trigger the recruitment of the proapoptotic ER-resident cysteine protease, caspase 12 (Nakagawa et al. 2000).

ATF6 is a transcription factor that contains a DNA-binding domain with a basic leucine zipper (bZIP) motif. Once GRP78 has dissociated from the luminal domain, ATF6 is translocated to the Golgi apparatus where it becomes proteolytically cleaved from its membrane anchor by site 1 (S1P) and site 2 (S2P) proteases, releasing the DNA-binding domain. The resultant transcription factor then migrates to the nucleus and binds to promoters containing ER stress response elements, increasing the expression of ER chaperones such as Grp78 and GRP94, PDI, XBP1, and CHOP (Adachi et al. 2008). ATF6 stimulates expression of a number of genes coding for protein products involved in protein folding, secretion and ERAD, thereby supporting the cells efforts to cope with accumulated misfolded or unfolded proteins.

The ER has essential roles in physiologic regulation of many processes including development, differentiation, maintenance of homeostasis and apoptosis (Walter and Ron 2011). Accumulating evidence indicates that malfunction of the ER with chronic activation of UPR contributes to the pathogenesis of many human diseases, including metabolic diseases such as obesity and diabetes mellitus, atherosclerosis, liver disease, neurodegenerative diseases and cancer (Park and Ozcan 2013).

ER Stress in Obesity and Diabetes

An imbalance in energy intake and expenditure leads to obesity, a major health threat that increases the risk of type 2 diabetes (T2D), cardiovascular disease (CVD) and cancer (Luoma 2013).

Obese subjects show activation of UPR in metabolic tissues including adipose tissue, liver, and the pancreas (Boden et al. 2008; Gregor et al. 2009). Obesity is also associated with both hepatic and peripheral insulin resistance, together with elevated levels of proinflammatory cytokines (Gregor and Hotamisligil 2011). Studies with genetically obese or diet-induced obese mice revealed elevated levels of PERK and eIF2*α* phosphorylation, IRE1-mediated JNK activation, and higher amounts of GRP78 in the liver and adipose tissue (Flamment et al. 2012).

ER stress in obesity is thought to be induced by an augmented demand for protein synthesis under nutrient excess and by elevated levels of saturated free fatty acids (FFA). Excess in saturated FFA, especially palmitate has been shown to cause ER stress and to activate the UPR in pancreatic β-cells and hepatocytes by altering the integrity of ER membrane (Pfaffenbach et al. 2010). It is well-recognized that hyperglycemia, high plasma levels of saturated FFAs and obesity in general are key risk factors for the development of T2D. These same conditions are recognized as triggers of ER stress, particularly in organs such as the liver and pancreas (Cnop et

al. 2012). In addition, leptin resistance, a condition that has been documented in the majority of the obese population, has been shown to contribute to obesity-linked disorders *via* ER stress (Konner and Bruning 2012).

Obesity induces T2D, a metabolic disorder characterized by a combination of insulin resistance, dysregulated hepatic glucose production, and inadequate insulin secretion by pancreatic β-cells. At the molecular level, it involves perturbations in insulin signaling, such as reduced insulin receptor function and reduced post–insulin receptor phosphorylation steps. ER stress parameters such as phosphorylation of PERK and IRE1, are increased in the liver and adipose tissues of T2D animals (Boden 2009; Stankov 2010). The three branches of the UPR: IRE1, PERK, and ATF6, have been implicated in the cellular inflammatory processes. Increased activation of IRE1, XBP1 and JNK results in decreased insulin receptor signaling and insulin resistance (Park and Ozcan 2013). Moreover, ER stress parameters including Grp78, XBP1s, phospho-eIF2α, and phospho-JNK, are increased in the liver and adipose tissues of obese insulin-resistant nondiabetic humans and these parameters are significantly reduced after weight loss (Gregor et al. 2009).

Pancreatic β-cells play an essential role in the synthesis of insulin. Augmented maturation of insulin which entails processing of proinsulin to insulin in the ER, combined with the increased presence of glucose and FFAs, triggers chronic ER stress. If these conditions are maintained for extended periods of time, particularly in obese patients and people consuming the Western high-fat and high-sugar diet, chronic ER stress condition may lead to the CHOP-mediated apoptosis of β-cells and absolute insulin deficiency (Su et al. 2013). In addition, pancreatic islets from patients with T2D have elevated levels of Grp78 and CHOP proteins.

Tauro-ursodeoxycholic acid (TUDCA), a hydrophilic bile acid, is an agent with known capacity to reduce ER stress (Özcan et al. 2006; Stanimirov et al. 2012). Administration of TUDCA resulted in normalization of hyperglycemia and restoration of hepatic and muscle insulin sensitivity in obese humans (Kars et al. 2010). TUDCA has also been shown to act as leptin-sensitizing agent. Therefore TUDCA represents an ER-stress modifying agent with therapeutic potential in ER stress-induced complications of obesity.

ER stress in cardiovascular system disorders

ER stress has been implicated in many cardiovascular diseases, from atherosclerosis to myocardial infarction, one of the most severe consequences of atherosclerosis in cardiovascular system.

Atherosclerosis is a multi-factorial disease characterized by the accumulation of apo B-containing lipoproteins and inflammatory factors (monocytes and other immune cells) in subendothelial stratum of the arterial intima (Hansson and Hermansson 2011). Numerous evidence recently emphasized the significance of ER stress in the pathogenesis of atherosclerotic disease (Hotamisligil 2010). ER stress promotes hepatic lipogenesis, lipid accumulation, and dyslipidemia, as well as dysregulation of glucose homeostasis through insulin resistance, stimulation of gluconeogenesis and suppression of glucose utilization. The combination of these systemic ER stress-inducing stimuli promotes lipid accumulation, inflammation and apoptosis, processes that accelerate atherosclerosis (McAlpine et al. 2010).

Accumulation of cholesterol in macrophages (so called lipid-loaded "foam cells") causes ER stress (Thorpe et al. 2011). Chronic ER stress in these cells that form the atherosclerotic plaque causes activation of CHOP-mediated apoptotic pathways in foam cells, subsequent inflammation and progression of the disease (Gotoh et al. 2011). Overexpression of oxidized lipids and phospholipids deposited in the arterial wall recruits inflammatory cells such as neutrophils and monocytes, triggering production of inflammatory cytokines and generation of ROS. ER stress is a common characteristic of many chronic inflammatory diseases including atherosclerosis. Induction of UPR, ROS production, Ca^{2+} release from the ER as well as activation of nuclear factor κ light-chain enhancer of activated B cells (NF-κB) and of JNK can additionally trigger the inflammatory response observed during atherosclerosis (Groenendyk et al. 2013). NF-κB is involved in the regulation of genes that are responsible for stress and growth and can regulate both proapoptotic and antiapoptotic genes, depending on the stimulus. Under prolonged ER stress, NF-κB initiates apoptosis, thereby shifting the outcome of the compensatory mechanism from an adaptive one to a maladaptive one (Madonna and De Caterina 2012). These events cause an enlargement of lesions containing necrotic macrophages and lipids that can potentially occlude the lumen of blood vessels.

Acute myocardial infarction (AMI) occurs as the result of complications associated with compromised supply of oxygen and glucose to the cardiac muscle, disrupted ER homeostasis and increased amounts of misfolded protein, followed by triggering the UPR three branches, in order to protect myocytes from ischemic damage (Glembotski 2008). Because one of the primary consequences of ischemia is hypoxia, several ER luminal folding enzymes, such as PDI, are ineffective in disulfide bond formation, leading to the accumulation of misfolded proteins and ER stress. During myocardial infarction, two major ER-resident stress proteins, GRP94 and GRP78, are upregulated upon glucose starvation to cope with cellular damage. GRP78 protein expression is increased near the infarction site, but in tissue distant from the damage, no increase is observed (Thuerauf et al. 2006). Ischemia directly activates the ER stress response in the heart by inducing the ATF6 branch and triggering the upregulation of chaperones such as GRP78 to target the damage produced by nutrient and oxygen starvation (Doroudgar et al. 2009). However, upon reperfusion, ATF6 activation and

GRP78 promoter activity are attenuated.

Ischemia and hypoxia during an AMI induce oxidative stress, when the lack of oxygen leads to ROS production that overcomes the detoxification capacity of cardiomyocytes, causing ATP depletion, DNA damage and the initiation of apoptosis. The generation of ROS promotes hypoxia-inducible factor 1α (HIF1α) accumulation and activation through the inhibition of prolyl hydroxylase, a regulator of HIF1α that depends on molecular oxygen (Natarajan et al. 2009). The use of prolyl hydroxylase inhibitors preserves oxygen for cellular respiration, allows stabilization and activation of PERK and HIF1α, significantly reducing ischemic damage and the size of infarction. In addition, PDI overexpression has been shown to prevent cardiac remodeling and apoptosis during myocardial infarction (Toldo et al. 2011). PDI is important in preventing the accumulation of misfolded proteins in the ER, as well as in enhancing the activity of ROS scavenger enzyme, superoxide dismutase-1 (SOD1), and therefore may be used to restore the redox homeostasis after myocardial infarction (Groenendyk et al. 2013). Modulation of ER stress may, therefore, offer unique opportunity for the regulation of dysregulated metabolic pathways in CVD.

ER stress in neurodegenerative diseases

Neurodegenerative diseases may be classified as "protein misfolding" diseases, considering that the accumulation of misfolded proteins in the brain is a common feature. The aggregation of abnormal proteins can perturb cellular structure and function, leading to neuronal cell loss (Matus et al. 2011).

Alzheimer's disease (AD) is the most common neurodegenerative disease characterized by a progressive decline in cognitive processes, eventually leading to dementia. The hallmark of AD is accumulation of insoluble aggregated proteins, extracellular amyloid-β peptide (Aβ) and intracellular aggregates of phosphorylated Tau protein (Ittner and Götz 2011). The accumulation of Aβ has been considered to be the main factor in the pathogenesis of AD. The two most common Aβ peptides are: Aβ40, formed in Golgi apparatus, and Aβ42 that is formed in the neuronal ER. The generation of Aβ42 may be an initial event in AD development. Recent reports have indicated that UPR is activated in AD neurons. Increased expression of the ER chaperone Grp78, which is a marker of UPR activation, is found in the temporal cortex and the hippocampus of AD patients. Autopsy studies have shown increased immunohistochemical staining of phosphorylated PERK, eIF2α, and IRE1 in the in AD neurons (Cornejo and Hetz 2013).

The second most common neurodegenerative disease is Parkinson's disease (PD), characterized by loss of dopaminergic neurons and accumulation of protein aggregates (Lewy bodies). A major component of Lewy bodies is α-synuclein (αSyn) that is overexpressed and triggers chronic ER stress and cell death. In accordance with this, phosphorylated PERK and eIF2α have been found to be increased in the neurons of PD patients (Matus et al. 2011). Studies from juvenile-onset autosomal form of PD have revealed mutations in the Parkin gene, which encodes an enzyme involved in the degradation of unfolded proteins. Overexpression of Parkin gene in dopaminergic neurons suppresses UPR induced cell death. The loss of activity of this protein results in the accumulation of a substrate of Parkin in the ER, leading to ER stress and apoptosis (Mercado et al. 2013).

The pathologic feature of Amyotrophic lateral sclerosis (ALS) is the selective degeneration of brain and spinal cord motoneurons that leads to muscle atrophy and paralysis. Accumulating evidence suggests that ER stress contributes to ALS pathogenesis. Mutations in the SOD1 gene, which have been linked to the familial form of the disease, lead to misfolding of SOD1 that forms the aggregates and induce the UPR activation (Saxena et al. 2009). Moreover, mutation in ER-resident vesicle-associated membrane protein-associated protein B (VAPB), a protein involved in lipid transport, causes familial ALS *via* interacting and inhibiting ATF6 and XBP1 and increases ER-stress-induced motoneuron vulnerability and death (Suzuki et al. 2009).

ER stress and cancer

Under regular homeostatic conditions, the majority of normal cells do not experience ER stress and therefore express only very limited amounts of GRP78 and insignificant levels of CHOP. During tumorigenesis, the high proliferation index of cancer cells requires increased activities of ER protein folding, assembly and transport, a condition that can induce physiological ER stress (Stankov 2010). Following initiation of malignancy, poor vascularization in tumors results in hypoxia, hypoglycemia and acidosis. All of these processes are strong inducers of UPR pathways. In addition, some cancer cells express mutant proteins that cannot be correctly folded and activate UPR. Unlike normal cells, most cancer cells express chronically elevated baseline ER stress levels, as indicated by permanently increased expression of GRP78 (Schonthal 2012b). Overexpression of this protein is a protective and a prosurvival mechanism, which enables tumor cell growth and survival within sub-optimal microenvironmental conditions. One of the prosurvival functions of GRP78 is to alleviate the transcription of proapoptotic CHOP-mediated pathways, which is achieved *via* binding of GRP78 and subsequent inactivation of the ER transmembrane signaling components PERK, IRE1, and ATF6. However, during conditions of prolonged stress, GRP78 remains bound to misfolded proteins in the lumen of the ER in order to repair them, and therefore permanently dissociated from the UPR proteins that continue to stimulate expression of CHOP. As a consequence, CHOP expression remains increased under these conditions, thus leading to

apoptosis. However, despite chronic ER stress conditions, the prosurvival module remains dominant and expression levels of proapoptotic CHOP in cancer cells are negligible (Boyce and Yuan 2006). In addition, ER stress leads to degradation of tumor suppressor p53 in cancer cells (Pluquet et al. 2005).

Increased expression of Grp78 has been detected in breast, colon, and hepatocarcinoma cell lines. Elevated Grp78 level has been reported to correlate well with higher pathologic grade, recurrence rate, and poor prognosis in patients with breast, liver, prostate, colon, and gastric cancers; and suppression of GRP78 inhibited the proliferation of cancer cells (Luo and Lee 2013). In addition, overexpression of GRP78 has been shown to provide resistance towards various chemotherapy agents including doxorubicin and paclitaxel (Lee et al. 2006; Wang et al. 2009). The presence of chronic ER stress may constitute an Achilles' heel of tumor cells, providing a therapeutic target for pharmacological intervention. Due to the fact that defensive pathways of ER stress are already activated in malignant cells, cancer cells lack the extended capacity to survive additional ER stress. Therefore, tumor specific aggravation of ER stress in cancer cells may be a strategy for the development of novel antitumor agents and/or chemotherapy-sensitizing agents.

CONCLUSIONS

During the past decade, a significant increase in our understanding of the role of ER stress in the regulation of cell homeostasis has been achieved. ER stress, considered as both a cause and consequence of metabolic disturbances, results in UPR activation. This sophisticated signaling pathway attempts to maintain cell homeostasis or induce cell death, in order to ensure survival of the organism as a whole. However, both dysregulation of ER signaling networks and protein folding capacity have been recognized as causes of numerous human diseases. Therefore, more detailed knowledge of ER stress mechanisms will open up promising avenues for the development of UPR-manipulating therapeutic strategies. Therapeutic agents aimed at ameliorating ER stress by promoting proper protein processing and generally supporting proper ER maintenance may have useful effects in the therapy of obesity, T2D, CVDs and neurodegenerative diseases. On the other hand, chronic ER stress in cancer cells may be exploited therapeutically *via* the opposite approach: the selective pharmacological aggravation of pre-existing ER stress and tumor-specific induction of apoptosis in cancer cells.

ACKNOWLEDGMENTS

This work is supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Grant № III41012.

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