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Review

Endoplasmic reticulum stress and the unfolded protein responses in retinal degeneration

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ABSTRACT

The endoplasmic reticulum (ER) is the primary intracellular organelle responsible for protein and lipid biosynthesis, protein folding and trafficking, calcium homeostasis, and several other vital processes in cell physiology. Disturbance in ER function results in ER stress and subsequent activation of the unfolded protein response (UPR). The UPR up-regulates ER chaperones, reduces protein translation, and promotes clearance of cytotoxic misfolded proteins to restore ER homeostasis. If this vital process fails, the cell will be signaled to enter apoptosis, resulting in cell death. Sustained ER stress also can trigger an inflammatory response and exacerbate oxidative stress, both of which contribute synergistically to tissue damage. Studies performed over the past decade have implicated ER stress in a broad range of human diseases, including neurodegenerative diseases, cancer, diabetes, and vascular disorders. Several of these diseases also entail retinal dysfunction and degeneration caused by injury to retinal neurons and/or to the blood vessels that supply retinal cells with nutrients, trophic and homeostatic factors, oxygen, and other essential molecules, as well as serving as a conduit for removal of waste products and potentially toxic substances from the retina. Collectively, such injuries represent the leading cause of blindness world-wide in all age groups. Herein, we summarize recent progress on the study of ER stress and UPR signaling in retinal biology and discuss the molecular mechanisms and the potential clinical applications of targeting ER stress as a new therapeutic approach to prevent and treat neuronal degeneration in the retina.

1. Introduction

The endoplasmic reticulum (ER) has long been recognized as the cell's protein factory, engaging in the biosynthesis, post-translational modification, folding, and trafficking of proteins (Alberts et al., 2002). Only properly folded proteins with native configurations can be released from the ER and transported successfully to the Golgi apparatus. Similar to its function in protein production, the ER serves as the primary site for the *de novo* synthesis of phospholipids and sterols, which constitute the major lipid components of the plasma membrane and the membranes of subcellular organelles. In addition, the ER is the central reservoir for storage of intracellular calcium and actively modulates calcium homeostasis (Timmins et al., 2009). Activation of the calcium channels on the ER membrane leads to calcium release from the ER into cytoplasm, which in turn activates calcium-dependent kinases

Abbreviations: AMD, age-related macular degeneration; ASK1, apoptosis signal-regulating kinase 1; ATF, activating transcription factor; Bip, immunoglobulin heavy chain-binding protein; CHOP, C/EBP homologous protein-10; CNV, choroidal neovascularization; DR, diabetic retinopathy; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GRP78, glucose-regulated protein 78; IRE1, inositol-requiring enzyme 1; LCA, Leber congenital amaurosis; LRAT, lecithin-retinol acyltransferase; PBA, 4-phenylbutyric acid; PEDF, pigment epithelium-derived factor; PERK, PKR-like endoplasmic reticulum kinase; RIDD, regulated Ire1-dependent decay; ROS, reactive oxygen species; RP, retinitis pigmentosa; RPE, retinal pigment epithelium; RPE65, retinal pigment epithelium protein of 65 kDa; TMAO, trimethylamine-N-oxide; TRAF2, tumor necrosis factor-associated factor 2; TUDCA, tauroursodeoxycholic acid; UPR, unfolded protein response; XBP1, X-box binding protein 1.

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and phosphatases, resulting in a diverse variety of cellular responses as well as detrimental events such as apoptosis.

Apart from its traditional roles in protein, lipid and calcium homeostasis, emerging evidence demonstrates that the ER is centrally involved in sensing of subtle metabolic changes within the cell and transmittal of the signal to the nucleus for gene regulation (Ron and Walter, 2007; Todd et al., 2008). This novel role of the ER is mediated by three major signal transducers: PKR-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). These proteins are activated in response to increased concentrations of misfolded or unfolded proteins in the ER lumen, a condition known as ER stress. In turn, IRE1, PERK and ATF6 initiate their downstream signaling pathways, collectively comprising the unfolded protein response (UPR), to combat ER stress through three complementary strategies: 1) up-regulating chaperones and folding enzymes to facilitate recovery of the damaged protein's original three dimensional structure; 2) attenuating global protein translation to reduce the influx of client proteins to the ER; and 3) enhancing ER-associated degradation (ERAD) to facilitate clearance of misfolded proteins from the ER (Schroder and Kaufman, 2005) (Fig. 1). However, if the duration and intensity of ER stress overwhelms the capacity of the UPR to restore ER homeostasis, the apoptotic cascade will be activated to eliminate stressed cells, leading to cell death and dropout (Paschen and Frandsen, 2001; Rao et al., 2004). Unresolved ER stress also activates pathological signaling pathways of oxidative stress, inflammation and immune responses, and dysregulated angiogenesis, and is implicated in a plethora of human diseases, such as neurodegenerative diseases (*e.g.*, Alzheimer's disease), cardiovascular and peripheral vascular diseases, and diabetes.

The retina is a very thin, stratified neural tissue lining the posterior pole of the eye, composed of a highly organized network of neurons (photoreceptors, interneurons and ganglion cells), glia and astrocytes, and blood vessels. Irreversible damage to retinal neurons causes retinal dysfunction, degeneration, and cell death, resulting in acute or chronic loss of visual function, such as is manifest in age-related macular degeneration (AMD), retinitis pigmentosa (RP), retinal detachment, and glaucomatous retinopathy. In diabetic retinopathy (DR), injury of retinal vascular cells resulting in retinal edema and ischemia also causes retinal neuronal death and blindness. Although many advances and breakthroughs in the basic and clinical research of retinal diseases have been made over the past several years, the mechanisms underlying key pathophysiological events that cause retinal degeneration are not fully understood. Intriguingly, a number of studies have demonstrated that ER stress plays a role in neuronal cell injury in the retina, and these findings have raised the possibility that inhibition of ER stress may provide a novel and effective therapeutic approach in the treatment of retinal diseases (Adachi et al., 2011; Gorbatyuk et al., 2010; Inokuchi et al., 2009; Li et al., 2009, 2011; Zhong et al., 2012a,c). In this article, we review recent progress on the study of ER stress in the retina, discuss the mechanisms and therapeutic promise of targeting ER stress in retinal degeneration, and highlight the importance of the adaptive UPR in protecting retinal cells in pathological and stress conditions.

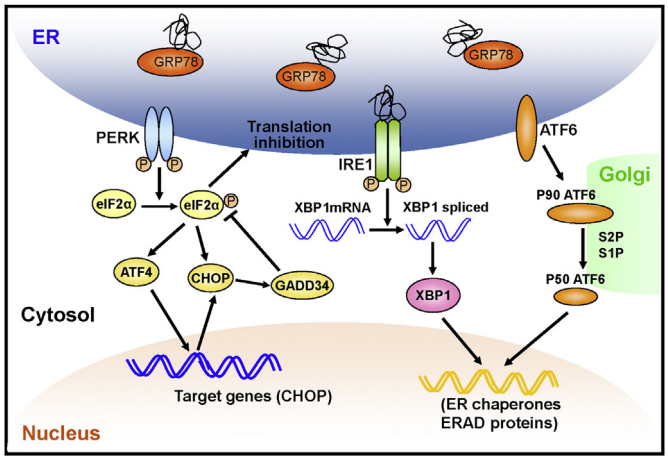

Fig. 1. Activation of the UPR during ER stress. Upon accumulation of unfolded or misfolded proteins in the ER lumen, GRP78 dissociates from ER stress transducers including IRE1, PERK and ATF6. Loss of GRP78 binding allows oligomerization and autophosphorylation of IRE1. In addition, unfolded proteins can directly bind to IRE1 resulting in its activation. Activated IRE1 splices XBP1 mRNA through its RNase activity, generating spliced XBP1 encoding a transcription factor that upregulates ER chaperones and UPR genes involved in the ER-associated protein degradation (ERAD). PERK is activated in a similar manner to IRE. Activated PERK phosphorylates eIF2α, leading to a global attenuation of protein synthesis and a concomitant increase in ATF4 translation. ATF4 binds to the UPR response element (UPRE) and induces its target genes such as CHOP. Enhanced eIF2α phosphorylation further increases CHOP protein level by facilitating its translation. CHOP, in turn, suppresses eIF2α phosphorylation by upregulating GADD34 resulting in the recovery of protein synthesis. After the dissociation of GRP78, ATF6 translocates to Golgi apparatus, where it is activated by proteolysis. Activated ATF6 transcriptionally induces ERAD and other UPR target genes.

2. The unfolded protein response (UPR)

At present it remains unclear how these ER membrane proteins sense ER stress and activate the UPR. One widely accepted model implicates the ER chaperone GRP78 (glucose-regulated protein 78), also known as Bip (immunoglobulin heavy chain-binding protein) (reviewed in Ron and Walter, 2007). It has been shown that each of these three ER membrane proteins contains an ER luminal domain that sequesters GRP78 when concentrations of misfolded proteins are low in the ER lumen. Binding of GRP78 to ER stress transducers keeps these proteins in an inactive state in unstressed cells (Bertolotti et al., 2000; Ng et al., 1992). During ER stress, GRP78 dissociates from the ER membrane proteins and binds to misfolded or unfolded proteins and facilitates their ATP-dependent protein folding. Loss of GRP78 binding allows oligomerization and autophosphorylation of PERK and IRE1, resulting in activation of the corresponding UPR pathways (Haze et al., 2001). However, this model is challenged by the evidence that dissociation of GRP78 from IRE1 is not sufficient to activate IRE1 (Kimata et al., 2004). Further, recent work by Gardner and Walter shows that unfolded proteins *per se* may be the activating ligands for IRE1, whereas the GRP78/BiP association only plays a role in fine-tuning of IRE1-mediated signaling (Gardner and Walter, 2011). Thus, more complex mechanisms may be involved in the initiation of ER stress response or UPR in mammalian cells under distinct stress conditions. Nevertheless, activation of the UPR pathways by the three major ER stress sensors, IRE1, PERK and ATF6, plays a pivotal role in remaining the function and homeostasis of the ER and has also been implicated in a vast variety of cellular processes. Major molecular components of the UPR are summarized in Table 1.

2.1. The IRE1 pathway

The IRE1 (inositol-requiring enzyme 1) pathway is the most evolutionarily conserved UPR branch, from yeast to humans, and has been shown to play a critical role in protecting stressed cells from injury and cell death (Lin et al., 2007). In mammalian cells, there are two functional homologs of IRE1p: IRE1α and IRE1β. IRE1α is ubiquitously expressed, whereas IRE1β expression is restricted primarily to intestinal epithelial cells (Tirasophon et al., 1998a; Wang et al., 1998). Both proteins localize to the ER and are capable of transducing the signal across the ER membrane and