Role of p58IPK in Endoplasmic Reticulum Stress-associated Apoptosis and Inflammation

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INTRODUCTION

The endoplasmic reticulum (ER) is an essential site responsible for protein synthesis and maturation. Newly synthesized polypeptide chains enter the ER through a peptide translocon, and undergo maturation processes such as cleavage, glycosylation, disulfide bond formation, folding and assembly. Perturbation of the protein maturation processes, caused by hypoxia, viral and bacterial infections, inhibition of protein glycosylation or disulfide bond formation, results in accumulation of unfolded and misfolded proteins in the lumen of the ER. This condition is known as ER stress. Increased rate of protein synthesis and unbalanced protein folding capacity of the ER can also lead to ER stress. Studies over the past decade have demonstrated that ER stress can be induced in a variety of cells and tissues when exposed to physiological and pathological stresses, and plays an important role in cell injury and disease development in cancer, diabetes, and neurodegenerative and vascular diseases (for review, please see[1, 2]).

To protect ER function and ensure the fidelity of protein folding, eukaryotic cells have evolved an adaptive mechanism named unfolded protein response (UPR). The UPR consists of three branches, which can be activated by any given ER stress event, but timing and sequence of the activation may differ.[3,4] Activation of the UPR enhances protein folding capacity and reduces ER stress by inducing ER chaperones. These ER resident molecular chaperones are important for post-translational modification, assembly and quality control of newly synthesized proteins.[5-7] In this review, we summarize recent research progress on p58IPK, an ER stress-regulated chaperone, and discuss its role in signaling pathways of ER stress, apoptosis and inflammation.

ACTIVATION OF THE UPR

The UPR is mediated by activation of three ER membrane-associated proteins, PKR-like eukaryotic initiation factor 2α kinase (PERK)[8], inositol requiring enzyme 1 (IRE1)[9,10] and activating transcription factor-6 (ATF6)[11] (Figure 1). Normally, these transmembrane proteins are bound to the ER-resident chaperone, glucose-regulated protein 78 (GRP78), blocking its activation. However, the increased level of misfolded proteins in the ER lumen results in dissociation of GRP78 away from these transmembrane proteins, which are subsequently activated by autophosphorylation and oligomerization (PERK and IRE1) or cleavage in the Golgi apparatus (ATF6). Activation of these signaling proteins causes transient global translation inhibition, degradation of unfolded or misfolded proteins and the induction of molecular chaperones and folding enzymes to increase ER capacity for protein folding.

Upon activation, the cleaved (active) form of ATF6 is transported to the nucleus and functions as a major transcription factor for ER stress-inducible genes.[1,12,13] These genes include ER chaperones, such as GRP78, other UPR proteins, such as x-box binding protein 1 (XBP1)[14], anti-apoptotic proteins, such as regulator of calcineurin 1 (RCAN1)[15] and proteins involved in ER-associated degradation (ERAD).[16,17] Activation of ATF6 leads to an increase in chaperone activity and degradation of unfolded

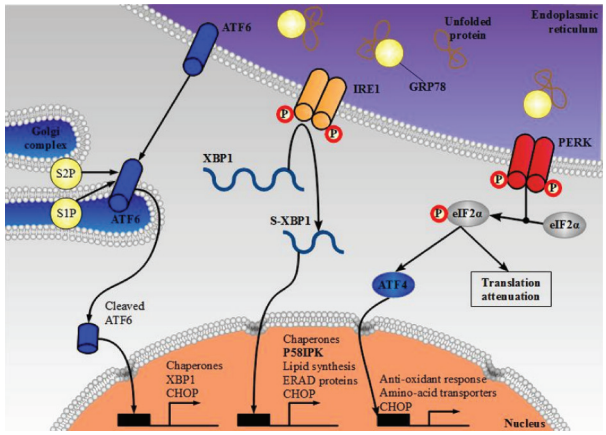

Figure 1. Activation of the URP and p58IPK. There are three branches of the UPR through which eukaryotic cells react to ER stress. In response to an increased level of unfolded proteins in the ER, PERK and IRE1 are activated by oligomerization and autophosphorylation. Activated PERK phosphorylates eIF2α leading to global protein attenuation. Lower global protein synthesis reduces ER unfolded protein load but increases production of ATF4 and CHOP. Activated IRE1 removes introns from the XBP1mRNA resulting in activation of XBP1 and induction of ER chaperones such as p58IPK. Upon ER stress, ATF6 translocates to the Golgi complex, where it undergoes two-step cleavage by site-1 and site-2 proteases. Cleaved ATF6 then translocates to the nucleus and activates a subset of UPR genes encoded chaperones, XBP1 and CHOP.

proteins, and is thus important for protein quality control and maintaining ER homeostasis and cell survival. While activation of the ATF6 pathway is generally considered to be cytoprotective, activation of the IRE1 and PERK branches of the UPR initiates signal pathways with both pro-survival and pro-apoptotic directions. Activation of PERK mediates phosphorylation of the α-subunit of eukaryotic initiation factor 2 (eIF-2α) to inhibit mRNA translation.[8,18,19] The main aim of this signaling pathway is an attenuation of global protein synthesis in the presence of misfolded and unfolded proteins. Long-term activation of PERK will increase ATF4 protein translation resulting in activation of the pro-apoptotic gene C/EBP homologous protein (CHOP).[20] Activation of IRE1 induces the activation of the transcription factor XBP1 through unconventional splicing of the XBP1 mRNA.[21] In turn, XBP1 up-regulates ER chaperones and co-chaperone proteins such as DNAJ homolog subfamily C member 3 (p58IPK).[22] Additionally, IRE1 interacts with an adaptor protein named tumor necrosis factor receptor (TNFR)-associated factor 2 (TRAF2) to activate signal-regulating kinase (ASK1) and activates c-Jun N-terminal kinase (JNK) and p38 MAPK.[23] This pathway is one of many modes of crosstalk between ER stress and inflammatory response.

STRUCTURE AND FUNCTION OF P58IPK

p58IPK is a member of the heat shock protein (HSP) 40 family and contains three types of protein interaction sites: an N-terminal ER-targeting signal domain, a tetratricopeptide repeat (TPR) domain and a C-terminal J domain. The N-terminal ER-targeting signal is responsible for translocation of p58IPK from the cytosol to the ER. In resting cells, p58IPK localizes in the cytosol, and translocates to the ER during ER stress. Deletion of the N-terminal domain blocks the translocation of p58IPK into ER lumen, suggesting that this domain is required for its ER localization.[24] p58IPK also consists of nine TPR motifs, which are known to mediate protein–protein interactions. Of the nine TPR motifs, TPR1–TPR3 are the binding sites for unfolded proteins in the ER lumen.[23] The TPR6 motif is the site that inhibits double-stranded RNA-dependent protein kinase (PKR) and PERK in the cytosol[2-4], and TRP7 is the binding site with P52rIPK, an inhibitor of p58IPK.[24] As a member of HSP40 family, p58IPK has a C-terminal J domain, which is responsible for interaction with HSP70 proteins. Thus, p58IPK appears to have diverse functional domains, which determine the protein's multifaceted activities depending on its location in the cell.

p58IPK was originally discovered as a cytosolic protein and functions as an inhibitor of double-stranded RNA-dependent protein kinase (PKR).[25, 26] During a viral infection, the presence of viral double-stranded RNA activates PKR, which attenuates protein synthesis by phosphorylation of eIF2α. This is a crucial protective response to prevent production of viral proteins. However, viral infection may restore global protein synthesis in host cells by influencing p58IPK.[25] p58IPK is released from

its own inhibitor P52rIPK and suppresses the kinase activity of PKR resulting in recovery of protein synthesis.[27-29] Recent studies have also revealed that p58IPK inhibits other kinases such as PERK, which also phosphorylates eIF2α.[30,31] PERK is an important sensor of ER stress and can regulate global protein translation. Thus, in the cytoplasm, p58IPK can inhibit PKR and PERK and is considered the moderator of global protein translation by removing translational attenuation.

In the ER, p58IPK functions as a co-chaperone[24,32] and regulator of GRP78.[33] GRP78 is a major ER-resident chaperone, which promotes protein folding and prevents misfolded protein aggregation by binding to the exposed hydrophobic residues.[34,35] In the ER lumen, p58IPK binds unfolded proteins and this complex associates with molecular chaperon GRP78 in an ATP-dependent manner. In the next step, p58IPK stimulates hydrolysis of ATP in the GRP78, causing a conformational change, tight binding of GRP78 to its substrate and releasing p58IPK from GRP78 (Figure 2). During ER stress, p58IPK is unregulated, like GRP78, and the upregulation of these chaperones play a pivotal role in misfolded protein refolding and restoring ER homeostasis. Additionally, recent studies have shown that p58IPK can function as a molecular chaperone by interacting with unfolded proteins such as luciferase, rhodanese and insulin, and prevent protein aggregation.[36]

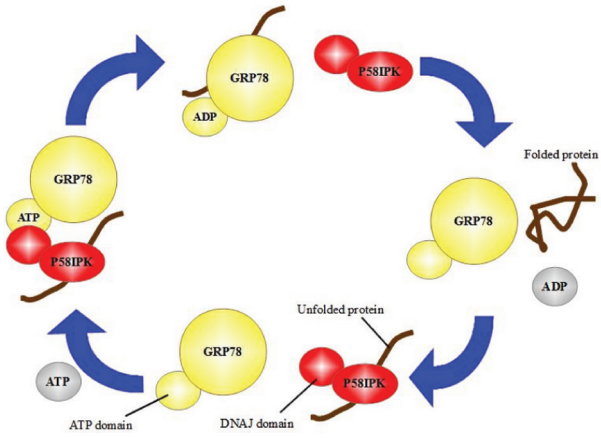

Figure 2. The interaction between p58IPK and GRP78. p58IPK TRP domains associate with unfolded proteins in the ER lumen. The complex then binds GRP78 via the DNAJ domain and ATPase domain. This interaction is ATP-dependent; P58IPK associates with GRP78 only in the presence of ATP and their dissociation is dependent on the hydrolyzation of ATP to ADP, resulting in a conformal change of GRP78.

ROLE OF P58IPK IN ER STRESS-ASSOCIATED APOPTOTIC SIGNALING

When ER homeostasis is severely impaired and ER stress cannot be resolved, signaling switches from pro-survival to pro-apoptotic to eliminate the damaged cell from the organism. To date, PERK, ATF6 and IRE1 cannot directly cause apoptosis but are involved in at least three apoptotic pathways: the transcriptional activation of the gene for CHOP,[37-39] IRE1-TRAF2-ASK1-MAP kinase pathway,[23, 40-42] and activation caspase-12[44-47] (Figure 3). These three pathways finally activate caspase-3 and suggest that ER stress pathways are likely linked with mitochondrial dysfunction and ultimately converge on apoptotic cascades leading to cell death.[39,47]

The classical apoptotic pathway induced by ER stress is CHOP-mediated. CHOP is a member of the CCAAT/enhancer binding proteins (C/EBPs) that serves as a dominant negative inhibitor

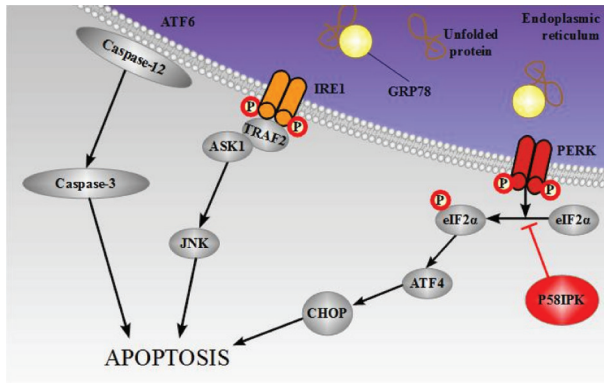

Figure 3. P58IPK and ER stress-mediated apoptosis. During ER stress, activated PERK phosphorylates eIF2α resulting in an increased production of ATF4 and upregulation of CHOP. Active IRE1 forms a complex with adaptor molecule TRAF2. The complex recruits apoptosis-signal-regulating kinase (ASK1), which phosphorylates and activates JNK. Both CHOP and JNK function as pro-apoptotic molecules. Caspase-12, which is localized on the cytoplasmic side of the ER in resting cells, is also activated by ER stress and implicated in promoting apoptosis. P58IPK suppresses CHOP activation likely through inhibition of PERK activation, while its effect on other ER stress-related apoptotic pathways remains unclear.

of C/EBPs. CHOP is expressed at low levels under normal conditions, but is strongly induced at the transcriptional level in response to ER stress.[37,38,48] All three branches of UPR induce transcription of CHOP, but the PERK/eIF2a signaling pathway plays a key role in the induction of CHOP in ER stress.[49,50] However, maximal induction of CHOP is achieved by the presence of all these signaling pathways. As mentioned above, p58IPK can inhibit the activity of PERK and finally relieves the PERK-mediated translational attenuation.[31] Upregulation of p58IPK occurs several hours after phosphorylation of PERK and eIF2α, suggesting p58IPK may mark the end of UPR adaptation and restore protein translation.[46] Countered data showed upregulation of p58IPK and translocation into the ER, where it functions as a molecular chaperone and promotes protein folding during ER stress.[24] Moreover, *in vitro* experiments have shown that deletion of p58IPK does not change global protein synthesis, suggesting that inhibition of the PERK/eIF2α branch by p58IPK may have a more profound effect on its downstream effectors such as ATF4 and CHOP.[20]

PKR is another member of the family eIF2α kinases inhibited by p58IPK. Viral infection of eukaryotic cells results in activation of PKR and leads to apoptosis via FADD and caspase-8.[51,52] Activation of caspase-8 ultimately activates apoptosis through activation of caspase-3.[53] Studies with p58IPK knockout (KO) mice have shown increased levels of caspase-3 and caspase-8 during influenza virus infection.[54] Deficiency of p58IPK also leads to increased and prolonged eIF2α phosphorylation resulting in apoptosis of infected cells. Indeed, p58IPK KO mice demonstrate up-regulated pro-apoptotic genes in pancreatic islets and increased β cell failure and apoptosis. Male p58IPK KO mice develop moderate hyperglycemia and hypoinsulinemia, which may be partially attributed to disturbances in insulin protein folding in p58IPK-deficient β-cells.[55]

ROLE OF P58IPK IN INFLAMMATORY RESPONSES

Recent studies have showed that ER stress pathways are involved in the induction of not only apoptosis, but also inflammation.[56-61] Studies suggest that ER stress and inflammation are closely related and interdependent. As inflammation induces ER stress and activates the UPR, UPR signaling is also likely to be involved in the induction and exacerbation of inflammatory responses. The main aim of inflammation in the ER stress response is to moderate tissue damage and assist in tissue repair. However, some pathological conditions such as diabetes, obesity, atherosclerosis, and cancer potentiate ER stress-related inflammation resulting in undesired pathological consequence and worsened tissue injury.[57]

The UPR has been shown to induce production of a number of pro-inflammatory and anti-inflammatory molecules. All three UPR pathways are involved in pro-inflammatory responses, which are mainly governed by two transcriptional factors: NF-κB and AP-1.[62-64] NF-κB is in a family of structurally related eukaryotic transcription factors involved with immune and inflammatory responses,[57,64-67] developmental processes, cellular growth and apoptosis.[68,69] NF-κB belongs to the category of "rapid-acting" primary transcription factors and is kept in an inactive form within the cytoplasm by the IκB proteins (inhibitor of NF-κB). In the classical NF-κB signaling pathway, the key event is activation of IKKβ. Activated IKKβ catalyzes the phosphorylation of IκB on two N-terminal serine residues, which is subsequently polyubiquinated and degraded.[70] Dissociation and degradation of IκB proteins activates NF-κB, allowing it to translocate to the nucleus and activate transcription of inflammatory genes.[66,71,72] It has been shown that all three branches of the UPR can regulate NF-κB activation during ER stress (Figure 4). The assembly and activation of the IRE1α–TRAF2 complex activates IKKβ, which degrades IκB leading to NF-κB activation and inflammation.[23,69] Furthermore, the IRE1 branch upregulates inflammatory genes through activation of the transcription factor AP-1.[73,74] Like the IRE pathway, the PERK and ATF6 branches can also regulate NF-κB activity.[56,64,65,75,76]

Additionally, the PERK/eIF2α pathway modulates expression of immune regulatory

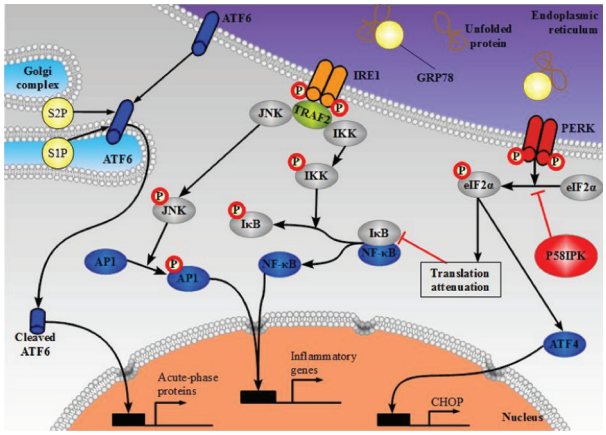

Figure 4. p58IPK and UPR-associated inflammatory signaling pathways. During ER stress, activated IRE1 forms a complex with TRAF2 and activates IKK. Activated IKK leads to IκB degradation and subsequent activation of NF-κB, which transcribes pro-inflammatory genes. Furthermore, the IRE1-TRAF2 complex activates JNK and consequently activates other pro-inflammatory transcription factors. Activated PERK promotes NF-κB activation via translational attenuation of IκB. p58IPK attenuates the PERK/CHOP pathway through inhibition of PERK activity.

genes via global protein synthesis attenuation and CHOP. Activation of the UPR through eIF2α kinases results in phosphorylation of eIF2a and translational attenuation. Due to the short half-life of IκB, translational attenuation reduces IκB levels and increases the NF-κB to IκB ratio resulting in activation of NF-κB.[65] Apart from regulating protein translation, PERK as an eIF2α kinase can modulate immune response through CHOP. CHOP activates transcription of the IL-23 gene, which produces a pro-inflammatory cytokine.[77] However, recent studies have shown that ER stress-induced CHOP activation can negatively regulate the inflammatory responses by modulating NF-κB and JNK .[78] More research is needed to decipher how these two opposing pathways are operating in regulation of inflammatory responses during ER stress. p58IPK as an inhibitor of PERK attenuates CHOP activation [20], and is therefore expected to modulate NF-κB activation during ER stress. The potential inhibitory effect of p58IPK on the pro-inflammatory NF-κB-dependent pathway may be useful for developing new treatments for chronic inflammatory diseases in medicine.

Another kinase involved in inflammatory signaling during ER stress is PKR.[79] PKR, in response to ER stress, coordinates other inflammatory kinases such as JNK to regulate cellular metabolism. In addition, PKR is classically activated by double-stranded RNA viruses and is a critical mediator of the anti-proliferative and antiviral effects exerted by interferon. PKR activation results in inflammation and immune regulation through several signaling pathways. These pathways include activity mitogen-activated protein kinases (MAPK),[79,80] transcription factors required for the expression of genes which encode pro-inflammatory cytokines: NF-κB,[81,82] the signal transducer and activator factor-1 (STAT-1),[83,84] interferon regulatory factor 1 (IRF-1)[85] and activating transcription factor-3 and -4 (ATF3 and ATF4).[86,78] In addition, Lu et al.[88] showed that autophosphorylated PKR physically interacts with inflammasome NLRP3, NLRP1, NLRC4, and AIM2, and is necessary for their activation.

During viral infection, p58IPK modulates the inflammatory response and apoptosis via inhibition of PKR.[54,89] Influenza virus infection in p58IPK knockout mice results in increased lung pathology, inflammation and was more lethal. Mechanistic studies showed that p58IPK binds PKR with the TRP6 motif, inhibits autophosphorylation and dimerization of PKR, and ultimately decreases NF-κB activation. Although PKR activation has been implicated in inflammatory responses through different signaling pathways, the role of p58IPK in association with PKR and its function in regulation of inflammation and tissue injury remain to be investigated.

P58IPK AND RETINAL DISEASES

Retinal diseases are the leading cause of blindness in patients of all ages. Vascular and neuronal degeneration observed in these diseases are the key histological markers that contribute to retinal dysfunction and vision loss.[85, 86] Recent studies have revealed that ER stress is critically implicated in retinal neural and vascular injury in various disease models, including glaucoma,[87] retinitis pigmentosa,[17]

and diabetic retinopathy.[88-90] In relation to ER stress, the role of molecular chaperones in retinal cell damage is being actively investigated. In an earlier study, Datta and colleagues demonstrated that mutations in the human carbonic anhydrase IV (hCAIV), the primary cause of retinal degeneration in autosomal-dominant retinitis pigmentosa (RP17), induced ER stress-associated renal cell injury.[90] Expression of the mutants in mice with haploinsufficiency of p58IPK resulted in markedly exacerbated and accelerated kidney damage, suggesting a potential protective role for p58IPK in ER stress. Unfortunately, the study did not evaluate the impact of p58IPK haploinsufficiency on retinal injury in this RP model. Thus future investigation of p58IPK in ER stress-related retinal diseases, such as retinal degeneration, will be of great interest. In addition, studies have shown that overexpression of p58IPK may provide a potential salutary effect on vascular damage in diabetic retinopathy. Forced expression of p58IPK by adeno-associated viruses reduces retinal vascular leakage in diabetic rats[91] and decreases pro-apoptotic and proinflammatory factors in retinal endothelial cells under high glucose condition.[91,92] The mechanisms and potential effect of p58IPK on retinal vascular protection and angiogenesis warrant further intensive investigation.

SUMMARY AND PERSPECTIVE

Conditions of ER stress have been observed in numerous neurodegenerative, cardiovascular and infectious diseases, and the processes of protein synthesis and maturation is crucial for cell survival and function. Recent studies have demonstrated that p58IPK is an important component of the protein folding system in the ER and can regulate protein translation through inhibition of the eIF2α kinases PERK and PKR. Regulation of these kinases was thought to be related not only to regulation of protein translation, but also to a number of important processes in cellular metabolism and cell fate. For instance, emerging evidence suggests that p58IPK can modulate apoptosis and inflammatory responses in ER-stressed and infected cells likely through inhibition of PERK and PKR. However, the underlying mechanisms remain poorly understood. Furthermore, p58IPK appears to be a multifaceted protein and its functions are closely associated with the protein's intracellular localization. It is unclear but yet of great interest what molecular signaling regulates p58IPK expression and localization during ER stress and whether the cellular localization of the protein can be an indicator of the severity of ER stress. Additionally, the interaction and regulation between p58IPK and PKR suggests a potentially important role for p58IPK in inflammatory modulation. It has been shown that PKR regulates not only NF-κB, but other pro-inflammatory factors, MAP kinases and inflammasome. Researching the influence of p58IPK on these cues may help us understand the mechanisms through which PKR signaling regulates inflammation. Given the current findings that suggest a protective role for p58IPK on apoptosis and inflammation in various tissues, future studies as to how to modulate the p58IPK activity may lead to the discovery of novel therapeutics for relief of neurodegenerative, cardiovascular, malignant and infectious diseases.

Acknowledgments

This work was supported in part by National Institutes of Health grant EY019949, Research Award 7-11-BS-182 from the American Diabetes Association, and Unrestricted Grants from Research to Prevent Blindness to the Department of Ophthalmology of University at Buffalo (SXZ).

Conflicts of Interest

None.

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