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)5, 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 proteins.
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 cytosol2–4, 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. Recent studies have also revealed that p58IPK inhibits other kinases such as PERK, which also phosphorylates eIF2α. 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 and regulator of GRP78. GRP78 is a major ER-resident chaperone, which promotes protein folding and prevents misfolded protein aggregation by binding to the exposed hydrophobic residues. 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.

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, IRE1-TRAF2-ASK1-MAP kinase pathway, and activation caspase-12 (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.

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...
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.\textsuperscript{37,38,48} All three branches of UPR induce transcription of CHOP, but the PERK/eIF2\(\alpha\) signaling pathway plays a key role in the induction of CHOP in ER stress.\textsuperscript{49,50} However, maximal induction of CHOP is achieved by the presence of all these signaling pathways.\textsuperscript{31} Upregulation of p58IPK occurs several hours after phosphorylation of PERK and eIF2\(\alpha\), suggesting p58IPK may mark the end of UPR adaptation and restore protein translation.\textsuperscript{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.\textsuperscript{24} Moreover, in vitro experiments have shown that deletion of p58IPK does not change global protein synthesis, suggesting that inhibition of the PERK/eIF2\(\alpha\) branch by p58IPK may have a more profound effect on its downstream effectors such as ATF4 and CHOP.\textsuperscript{20}

PKR is another member of the family eIF2\(\alpha\) kinases inhibited by p58IPK. Viral infection of PKR and leads to apoptosis via FADD and caspase-8.\textsuperscript{51,52} Activation of caspase-8 ultimately activates apoptosis through activation of caspase-3.\textsuperscript{53} Studies with p58IPK knockout (KO) mice have shown increased levels of caspase-3 and caspase-8 during influenza virus infection.\textsuperscript{54} Deficiency of p58IPK also leads to increased and prolonged eIF2\(\alpha\) phosphorylation resulting in apoptosis of infected cells. Indeed, p58IPK KO mice demonstrate up-regulated pro-apoptotic genes in pancreatic islets and increased \(\beta\) 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 \(\beta\)-cells.\textsuperscript{55}

**ROLE OF P58IPK IN INFLAMMATORY RESPONSES**

Recent studies have shown that ER stress pathways are involved in the induction of not only apoptosis, but also inflammation.\textsuperscript{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.\textsuperscript{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-\(\kappa\)B and AP-1.\textsuperscript{62-64} NF-\(\kappa\)B is a family of structurally related eukaryotic transcription factors involved with immune and inflammatory responses,\textsuperscript{57,64-67} developmental processes, cellular growth and apoptosis.\textsuperscript{68,69} NF-\(\kappa\)B belongs to the category of “rapid-acting” primary transcription factors and is kept in an inactive form within the cytoplasm by the I\(\kappa\)B proteins (inhibitor of NF-\(\kappa\)B). In the classical NF-\(\kappa\)B signaling pathway, the key event is activation of I\(\kappa\)K\(\beta\). Activated I\(\kappa\)K\(\beta\) catalyzes the phosphorylation of I\(\kappa\)B on two N-terminal serine residues, which is subsequently polyubiquinated and degraded.\textsuperscript{70} Dissociation and degradation of I\(\kappa\)B proteins activates NF-\(\kappa\)B, allowing it to translocate to the nucleus and activate transcription of inflammatory genes.\textsuperscript{66,71,72} It has been shown that all three branches of the UPR can regulate NF-\(\kappa\)B activation during ER stress (Figure 4). The assembly and activation of the IRE1\(\alpha\)–TRAF2 complex activates I\(\kappa\)K\(\beta\), which degrades I\(\kappa\)B leading to NF-\(\kappa\)B activation and inflammation.\textsuperscript{23,69} Furthermore, the IRE1 branch upregulates inflammatory genes through activation of the transcription factor AP-1.\textsuperscript{73,74} Like the IRE pathway, the PERK and ATF6 branches can also regulate NF-\(\kappa\)B activity.\textsuperscript{56,64,65,75,76}

Additionally, the PERK/eIF2\(\alpha\) pathway modulates expression of immune regulatory
genes via global protein synthesis attenuation and CHOP. Activation of the UPR through eIF2α kinases results in phosphorylation of eIF2α 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. 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. However, recent studies have shown that ER stress-induced CHOP activation can negatively regulate the inflammatory responses by modulating NF-κB and JNK. More research is needed to decipher how these two opposing pathways are operating in regulation of inflammatory responses 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. 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), transcription factors required for the expression of genes which encode pro-inflammatory cytokines: NF-κB, the signal transducer and activator factor-1 (STAT-1), interferon regulatory factor 1 (IRF-1) and activating transcription factor-3 and -4 (ATF3 and ATF4). In addition, Lu et al. 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. 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. Recent studies have revealed that ER stress is critically implicated in retinal neural and vascular injury in various disease models, including glaucoma, retinitis pigmentosa,
and diabetic retinopathy. 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. 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 and decreases pro-apoptotic and proinflammatory factors in retinal endothelial cells under high glucose condition. 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.

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**Conflicts of Interest**

None.

**REFERENCES**

1. Yoshida H. ER stress and diseases. *FEBS J* 2007;274:630-658.
2. Wu J, Kaufman RJ. From acute ER stress to physiological roles of the unfolded protein response. *Cell Death Differ* 2006;13:374-384.
3. DuRose JB, Tam AB, Niwa M. Intrinsic capacities of molecular sensors of the unfolded protein response to sense alternate forms of endoplasmic reticulum stress. Mol Biol Cell 2006;17:3095-3107.

4. Lin JH, Li H, Yasumura D, Cohen HR, Zhang C, Panning B, et al. IRE1 signaling affects cell fate during the unfolded protein response. Science 2007;318:944-949.

5. Calnexin, calreticulin and the folding of glycoproteins. Trends Cell Biol 1997;7:193-200.

6. Hellman R, Vanhove M, Lejeune A, Stevens FJ, Alberini CM. Quality control of ER synthesized proteins: an exposed thioul group as a three-way switch mediating assembly, retention and degradation. EMBO J 1993;12:4755-4761.

7. Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic- reticulum-resident kinase. Nature 1999;397:271-274.

8. Cox JS, Shamu CE, Walter P. Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell 1993;73:1197-1206.

9. Mori K, Ma W, Gething MJ, Sambrook J. A transmembrane protein with a cdc2+/cdc28-related kinase activity is required for signaling from the ER to the nucleus. Cell 1993;74:743-756.

10. Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell 1999;10:3787-3799.

11. Chen X, Shen J, Prywes R. The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the ER to the Golgi. J Biol Chem 2002;277:13045-13052.

12. Adachi Y, Yamamoto K, Okada T, Yoshida H, Harada A, Mori K. ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum. Cell Struct Funct 2008;33:75-89.

13. Yoshida H, Matsu T, Yamamoto A, Okada T, Mori K. XBPF1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 2001;107:881-891.

14. Belmont PJ, Tadimalla A, Chen WJ, Martindale JJ, Thuerauf DJ, Marcinko M, et al. Coordination of growth and endoplasmic reticulum stress signaling by regulator of calcineurin 1 (RCAN1), a novel ATF6-inducible gene. J Biol Chem 2008;283:14012-14021.

15. Yamamoto K, Sato T, Matsui T, Sato M, Okada T, Yoshida H, et al. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBPF1. Dev Cell 2007;13:365-376.

16. Wu J, Rutkowski DT, Dubois M, Swathi R, Saunders T, Wang J, et al. ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. Dev Cell 2007;13:351-364.

17. Wek RC, Cavener DR. Translational control and the unfolded protein response. Antioxid Redox Signal 2007;9:2357-2371.

18. Kimball SR. Eukaryotic initiation factor eIF2. Int J Biochem Cell Biol 1999;31:25-29.

19. Huber AL, Lebeau J, Guillaumot P, Pétrilli V, Malek M, Chilloux J, et al. p58(IPK)-mediated attenuation of the proapoptotic PERK-CHOP pathway allows malignant progression upon low glucose. Mol Cell 2013;49:1049-1059.

20. Kaufman RJ, Scheuner D, Schroder M, Shen X, Lee K, Liu CY, et al. The unfolded protein response in nutrient sensing and differentiation. Nat Rev Mol Cell Biol 2002;3:411-421.

21. Lee AH, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol 2003;23:7448-7459.

22. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science 2000;287:664-666.

23. Rutkowski DT, Kang SW, Goodman AG, Garrison Jl, Taunton J, Katze MG, et al. The role of p58IPK in protecting the stressed endoplasmic reticulum. Mol Biol Cell 2007;18:3681-3691.

24. Melville MW, Hansen WJ, Freeman BC, Welch WJ, Katze MG. The molecular chaperone hsp40 regulates the activity of p58IPK, the cellular inhibitor of PKR. Proc Nat Acad Sci U S A 1997;94:97-102.

25. Polyak SJ, Tang N, Wambach M, Barber GN, Katze MG. The F58 cellular inhibitor complexes with the interferon-induced, double-stranded RNA-dependent protein kinase, PKR, to regulate its autophosphorylation and activity. J Biol Chem 1996;271:1702-1707.

26. Lee TG, Tang N, Thompson S, Miller J, Katze MG. The 58,000-dalton cellular inhibitor of the interferon-induced double-stranded RNA-activated protein kinase (PKR) is a member of the tetratricopeptide
repeat family of proteins. Mol Cell Biol 1994;14:2331-2342.

28. Lee TG, Tomita J, Hovanessian AG, Katze MG. Purification and partial characterization of a cellular inhibitor of the interferon-induced protein kinase of MR 68,000 from influenza virus-infected cells. Proc Natl Acad Sci U S A 1990;87:6208-6212.

29. Lee TG, Tomita J, Hovanessian AG, Katze MG. Characterization and regulation of the 58,000-dalton cellular inhibitor of the interferon-induced, dsRNA-activated protein kinase. J Biol Chem 1992;267:14238-14243.

30. van Huizen R, Martindale JL, Gorospe M, Holbrook NJ, p58IPK, a novel endoplasmic reticulum stress-inducible protein and potential negative regulator of eIF2alpha signaling. J Biol Chem 2003;278:15558-15564.

31. Yaw N, Frank CL, Korth MJ, Sopher BL, Novoa I, Ron D, et al. Control of PERK eIF2alpha kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. Proc Natl Acad Sci U S A 2002;99:15920-15925.

32. Tao J, Wu Y, Ron D, Sha B. Preliminary X-ray crystallographic studies of mouse UPR responsive protein p58(IPK) TPR fragment. Acta Crystallogr Sect F Struct Biol Cryst Commun 2008;64:108-110.

33. Petrova K, Oyadomari S, Hendershot LM, Ron D. Regulated association of misfolded endoplasmic reticulum lumenal proteins with P58/DNAJc3. EMBO J 2008;27:2862-2872.

34. Flynn GC, Pohl J, Flocco MT, Rothman JE. Peptide-binding specificity of the molecular chaperone BiP. Nature 1991;353:726-730.

35. Gething MJ. Role and regulation of the ER chaperone BiP. Semin Cell Dev Biol 1999;10:465-472.

36. Tao J, Sha B. Structural insight into the protective role of P58(IPK) during unfolded protein response. Methods Enzymol 2011;490:259-270.

37. Gotoh T, Mori M. Nitric oxide and endoplasmic reticulum stress. Arterioscler Thromb Vasc Biol 2006;26:1439-1446.

38. Oyadomari S, Mori M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ 2004;11:381-389.

39. Matsumoto M, Minami M, Takeda K, Sakao Y, Akira S. Ectopic expression of CHOP (GADD153) induces apoptosis in M1 myeloblastic leukemia cells. FEBS Let 1996;395:143-147.

40. Nishitoh H, Matsuzawa A, Tobiume K, Saegusa K, Takeda K, Inoue K, et al. ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. Genes Dev 2002;16:1345-1355.

41. Ventura JJ, Hubner A, Zhang C, Flavell RA, Shokat KM, Davis RJ. Chemical genetic analysis of the time course of signal transduction by JNK. Mol Cell 2006;21:701-710.

42. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995;270:1326-1331.

43. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, et al. Caspase-12 mediates endoplasmic reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 2000;403:98-103.

44. Fischer H, Koenig U, Eckhart L, Tsachler E. Human caspase 12 has acquired deleterious mutations. Biochem Biophys Res Commun 2002;293:722-726.

45. Nakagawa T, Yuan J. Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. J Cell Biol 2000;150:887-894.

46. Szegedi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 2006;7:880-885.

47. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, et al. Proapoptotic BAX and BAK: A requisite gateway to mitochondrial dysfunction and death. Science 2001;292:727-730.

48. Zinzsnher H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, et al. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 1998;12:982-995.

49. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 2000;6:1099-1108.

50. Scheuner D, Song B, McEwen E, Liu C, Layburt R, Gillespie P, et al. Translational control is required for the unfolded protein response and in vivo glucose homeostasis. Mol Cell 2001;7:1165-1176.

51. Gil J, Alcamij J, Esteban M. Activation of NF-kappa B by the dsRNA-dependent protein kinase, PKR involves the I kappa B kinase complex. Oncogene 2000;19:1369-1378.

52. Balachandran S, Kim CN, Yeh WC, Mak TW, Bhalia K, Barber GN. Activation of the dsRNA-dependent protein kinase, PKR, induces apoptosis through FADD-mediated death signaling. EMBO J 1998;17:6888-6902.

53. Gil J, Esteban M. The interferon-induced protein kinase (PKR), triggers apoptosis through FADD-mediated activation of caspase 8 in a manner.
61. Martinon F, Glimcher LH. Regulation of innate immunity by signaling pathways emerging from the endoplasmic reticulum molecular chaperone gene P58IPK. *Diabetes* 2005;54:1074-1081.

62. Verfaillie T, Garg AD, Agostinis P. Targeting ER stress and the unfolded protein response. *PLoS Pathog* 2009;5:e1000348.

63. Hotamisligil GS, Erbay E. Nutrient sensing and regulation of autophagy and resistance to drugs and hypoxia. *Cell Cycle* 2009;8:3838-3847.

64. Goodman AG, Fornek JL, Medigeshi GR, Perrone LA, Peng X, Dyer MD, et al. P58(IPK): A novel “CIHD” member of the host innate defense response against pathogenic virus infection. *PLoS Pathog* 2009;5:e1000438.

65. Ladiges WC, Knoblaugh SE, Morton JF, Korth MJ, Sopher BL, Baskin CR, et al. Pancreatic beta-cell failure and diabetes in mice with a deletion mutation of the endoplasmic reticulum molecular chaperone gene P58IPK. *Diabetes* 2005;54:1074-1081.

66. Deng J, Lu PD, Zhang Y, Scheuner D, Kaufman RJ, Sonenberg N, et al. Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. *Mol Cell Biol* 2004;24:10161-10168.

67. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* 1999;18:6853-6866.

68. Ward C, Walker A, Dransfield I, Haslett C, Rossi AG. Regulation of granulocyte apoptosis by NF-kappaB. *Biochem Soc Trans* 2004;32:465-467.

69. Hu P, Han Z, Couvillon AD, Kaufman RJ, Exton JH. Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. *Mol Cell Biol* 2006;26:3071-3084.

70. Bonizzi G, Karin M. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004;25:280-288.

71. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002;109 Suppl:S81-96.

72. Tak PP, Firestein GS. NF-kappaB: a key role in inflammatory diseases. *J Clin Invest* 2001;107:7-11.

73. Angel P, Szabowski A, Schorpp-Kistner M. Function and regulation of AP-1 subunits in skin physiology and pathology. *Nature* 2001;20:2413-2423.

74. Davis RJ. Signal transduction by the JNK group of MAP kinases. *Cell* 2000;103:239-252.

75. Yamazaki H, Hiramatsu N, Hayakawa K, Tagawa Y, Okamura M, Ogata R, et al. Activation of the Akt-NF-kappaB pathway by subtilase cytotoxin through the ATF6 branch of the unfolded protein response. *J Immunol* 2009;183:1480-1487.

76. Zhang K, Shen X, Wu J, Sakaki K, Saunders T, Rutkowski DT, et al. Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* 2006;124:587-599.

77. Goodall JC, Wu C, Zhang Y, McNeill L, Ellis L, Saudek V, et al. Endoplasmic reticulum stress-induced transcription factor, CHOP, is crucial for dendritic cell IL-23 expression. *Proc Natl Acad Sci U S A* 2010;107:17698-17703.

78. Jin J, Jeong SI, Shin YM, Lim KS, Shin H, Lee YM, et al. Transplantation of mesenchymal stem cells within a poly (lactide-co-epsilon-caprolactone) scaffold improves cardiac function in a rat myocardial infarction model. *Eur J Heart Fail* 2009;11:147-153.
with stress and metabolic homeostasis. *Cell* 2010;140:338-348.

80. Goh KC, deVeer MJ, Williams BR. The protein kinase PKR is required for p38 MAPK activation and the innate immune response to bacterial endotoxin. *EMBO J* 2000;19:4292-4297.

81. Zamanian-Daryoush M, Mogensen TH, DiDonato JA, Williams BR. NF-kappaB activation by double-stranded-RNA-activated protein kinase (PKR) is mediated through NF-kappaB-inducing kinase and IkappaB kinase. *Mol Cell Biol* 2000;20:1278-1290.

82. Bonnet MC, Weil R, Dam E, Hovanessian AG, Meurs EF. PKR stimulates NF-kappaB irrespective of its kinase function by interacting with the IkappaB kinase complex. *Mol Cell Biol* 2000;20:4532-4542.

83. Deb A, Haque SJ, Mogensen T, Silverman RH, Williams BR. RNA-dependent protein kinase PKR is required for activation of NF-kappaB by IFN-gamma in a STAT1-independent pathway. *J Immunol* 2001;166:6170-6180.

84. Deb A, Zamanian-Daryoush M, Xu Z, Kadereit S, Williams BR. Protein kinase PKR is required for platelet-derived growth factor signaling of c-fos gene expression via Erks and Stat3. *EMBO J* 2001;20:2487-2496.

85. Zhang P, Samuel CE. Induction of protein kinase PKR-dependent activation of interferon regulatory factor 3 by vaccinia virus occurs through adapter IPS-1 signaling. *J Biol Chem* 2008;283:34580-34587.

86. Lee ES, Yoon CH, Kim YS, Bae YS. The double-strand RNA-dependent protein kinase PKR plays a significant role in a sustained ER stress-induced apoptosis. *FEBS Lett* 2007;581:4325-4332.

87. Guerra S, Lopez-Fernandez LA, Garcia MA, Zaballos A, Esteban M. Human gene profiling in response to the active protein kinase, interferon-induced serine/threonine protein kinase (PKR), in infected cells. Involvement of the transcription factor ATF-3 in PKR-induced apoptosis. *J Biol Chem* 2006;281:18734-18745.

88. Lu B, Nakamura T, Inouye K, Li J, Tang Y, Lundback P, et al. Novel role of PKR in inflammasome activation and HMGB1 release. *Nature* 2012;488:670-674.

89. Goodman AG, Smith JA, Balachandran S, Perwitasari O, Proll SC, Thomas MJ, et al. The cellular protein P58IPK regulates influenza virus mRNA translation and replication through a PKR-mediated mechanism. *J Virol* 2007;81:2221-2230.

90. Datta R, Shah GN, Rubbelke TS, Waheed A, Rauchman M, Goodman AG, et al. Progressive renal injury from transgenic expression of human carbonic anhydrase IV folding mutants is enhanced by deficiency of p58IPK. *Proc Natl Acad Sci U S A* 2010;107:6448-6452.

91. Yang H, Liu R, Cui Z, Chen Z-q, Yan S, Pei H, et al. Functional characterization of 58-kilodalton inhibitor of protein kinase in protecting against diabetic retinopathy via the endoplasmic reticulum stress pathway. *Mol Vis* 2011;17:78-84.

92. Li B, Li D, Li GG, Wang HW, Yu AX. P58(IPK) inhibition of endoplasmic reticulum stress in human retinal capillary endothelial cells in vitro. *Mol Vis* 2008;14:1122-1128.