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Review

New insights into the roles of CHOP-induced apoptosis in ER stress

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Endoplasmic reticulum stress (ER stress) is triggered due to a loss of homeostasis in the ER, resulting in accumulation of misfolded proteins in the ER lumen. ER stress activates a series of adaptive mechanisms known as the unfolded protein response. Perturbation of the ER is a powerful inducer of the transcription factor C/EBP homologous protein (CHOP). Although it has been proved that excessive or adverse stress to the ER triggers apoptosis, the specific mechanisms underlying these processes induced by CHOP remain unclear. By now, CHOP-induced apoptosis in ER stress has been implicated in numerous human diseases, such as neurodegenerative diseases, diabetes, ischemic diseases, tumor, and so on. In this review, we summarized the current understanding of the roles of CHOP in the development of several diseases from the laboratory to the clinic.

Keywords CHOP; ER stress; apoptosis; transcriptional factor; gene expression regulation; signal transduction; diseases

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Introduction

Endoplasmic reticulum (ER) widely exists in eukaryotes and functions as an essential synthesis and folding manufactory of secretory proteins. Perturbations of ER homeostasis affect protein folding and cause ER stress when the cell suffers from the adverse environmental challenges such as toxics, ischemia, virus infection, pH changes, and so on. All the above factors can result in an accumulation of unfolded or misfolded protein in the ER lumen, namely ER stress. To adapt to the occurrence of ER stress, cells can sense these stresses and activate highly conversed stress responses to them through translational attenuation, up-regulation of the genes for ER chaperones and related proteins, and degradation of unfolded proteins by quality-control system. However, when the ER function is severely impaired, the organelle elicits apoptotic signals. ER stress has been implicated in various diseases such as neurodegenerative disorders, metabolic diseases, ischemia diseases, and tumors. One of the components of the ER stress-mediated apoptosis pathway is CHOP (C/EBP homologous protein), also known as growth arrest and DNA damage-inducible gene 153 (GADD153). According to the current research progress of CHOP-mediated apoptosis in ER stress and in diseases including neurodegenerative disorders, metabolic diseases, ischemia diseases, and tumors, we summarized the current understanding of the roles of CHOP/GADD153 in the following aspects.

Structure and Properties of C/EBP Homologous Protein

C/EBP homologous protein (CHOP), also known as GADD153, belongs to the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors that have been implicated in the regulation of processes relevant to cellular proliferation, differentiation, and expression, and energy metabolism of cell type-specific genes [1,2]. CHOP is a 29 kDa protein with 169 (human) or 168 (rodents) amino acid residues. CHOP is generally induced by genotoxic stress and growth arrest signals. CHOP is consisted of two functional domains, an N-terminal transcriptional activation domain and a C-terminal basic-leucine zipper (bZIP) domain composed of a basic amino acid-rich DNA-binding region followed by a leucine zipper dimerization motif [3] (**Fig. 1**). Deletion mutant analysis of CHOP revealed that bZIP domain is important for CHOP-induced apoptosis [4].

CHOP and Endoplasmic Reticulum Stress

ER is the site of synthesis and folding of secretory proteins. However, under various physiological and pathological conditions, such as toxicity, ischemia, virus infection, and pH changes, the normal progress of protein synthesis is impaired, which is collectively called ER stress. Under the

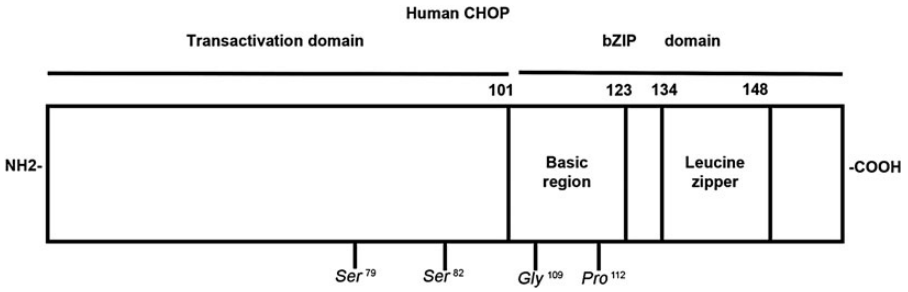

Figure 1. Domain structure of human CHOP CHOP is composed of two functional domains, an N-terminal transcriptional activation domain and a C-terminal bZIP domain consisting of a basic amino acid-rich DNA-binding region followed by a leucine zipper dimerization motif. The basic region contains conserved glycine (109) and proline (112) residues [5].

circumstances of ER stress, cells mainly elicit two responses, one leading to survival and the other leading to apoptosis.

As for the anti-apoptosis effect, in order to conquer these disadvantageous effects and maintain its homeostasis, this organelle mainly has three classical specific responses involving three ER stress sensors: PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) [5]. The first response is translational attenuation. Activated PERK phosphorylates its target protein eukaryotic initiation factor 2 (eIF2) during ER stress and represses protein synthesis, which prevents further influx of ER client proteins [6]. PERK/eIF2P/ATF4 pathway is required not only for translational control, but also for activation of ATF6 and its target genes. The PERK pathway facilitates both the synthesis of ATF6 and trafficking of ATF6 from the ER to the Golgi for intramembrane proteolysis and activation of ATF6 [7]. The second response is the up-regulation of the expression of molecular chaperones by ATF6, such as immunoglobulin-binding protein (Bip)/glucose-regulated protein 78 (GRP78) and glucose-regulated protein 94 (GRP94), enzymes including protein disulfide isomerase and peptidyl-prolyl isomerase, and structural components of the ER including sarcoplasmic ER Ca^{2+}-ATPase2 (SERCA2), to increase the protein-folding capacity in ER [8,9]. In addition, components of ER-associated degradation (ERAD), including IRE1 and ER degradation-enhancing α-mannosidase-like protein (EDEM), are transcriptionally induced to eliminate misfolded proteins in the ER by the ubiquitin-proteasome system [10,11]. The third response is the association with Ca^{2+}-NF-κB pathway which is triggered by accumulation of membrane proteins in the ER, thereby also called ER overload response (EOR). NF-κB is activated by degradation of IκB, which calls for the release of Ca^{2+} stored in the ER and subsequent effects of reactive oxygen intermediates.

As for the apoptosis effect, it occurs when functions of the ER are severely impaired for the sake of the normal functional mechanisms by activating relevant caspases [12]. The essential downstream proteins include CHOP, cJUN NH2-terminal kinase (JNK), and ER-associated caspase-12 (**Fig. 2**). CHOP knock-down prevents perturbations in the AKT (protein kinase B)/FOXO3a (forkhead box, class O, 3a) pathway in response to ER stress [13]. Inorganic arsenic induces reactive oxygen species (ROS) causing neuronal cell death via both JNK/ERK (extracellular signal-regulated kinase)-mediated mitochondria-dependent and GRP78/CHOP-triggered apoptosis pathways [14].

Roles of CHOP in ER Stress-Mediated Apoptosis

Expression profile of CHOP

Cells have evolved elaborate mechanisms to ensure that proteins are folded and assembled accurately before being transported to other organelles. Only correctly folded proteins are allowed to leave the ER. Normally, the expression of CHOP is extremely low. However, when self-protective mechanisms are overwhelmed, apoptosis can be initiated. Expression of CHOP and its accumulation in the nucleus are canonically up-regulated during apoptosis induced by ER stress [15]. The *CHOP* gene was initially identified in the search for genes induced by genotoxic stress such as UV irradiation and alkylating agents methyl methanesulfonate (MMS), and was thus named as GADD153 [16]. MMS may affect ER protein folding by alkylating cysteine residues of ER proteins rather than by damaging DNA. Other strong inducers of CHOP, such as tunicamycin which blocks protein glycosylation, thapsigargin which promotes ER stress by depletion of ER calcium stores, and dithiothreitol which disrupts disulfide bond formation, strongly perturb ER functions.

CHOP was also noted to be induced by nutrient depletion such as glucose deprivation and amino acid starvation. Glucose deprivation induces ER stress by inhibiting N-linked protein glycosylation. It has also been well established that amino acids are involved in the control of gene expression of CHOP. Amino acid response element of CHOP (AARE) is related to C/EBP and ATF/CRE binding sites and binds to ATF2 *in vitro* under leucine starved and unstarved conditions [17]. ATF2 is essential for the transcriptional activation of CHOP by leucine starvation and

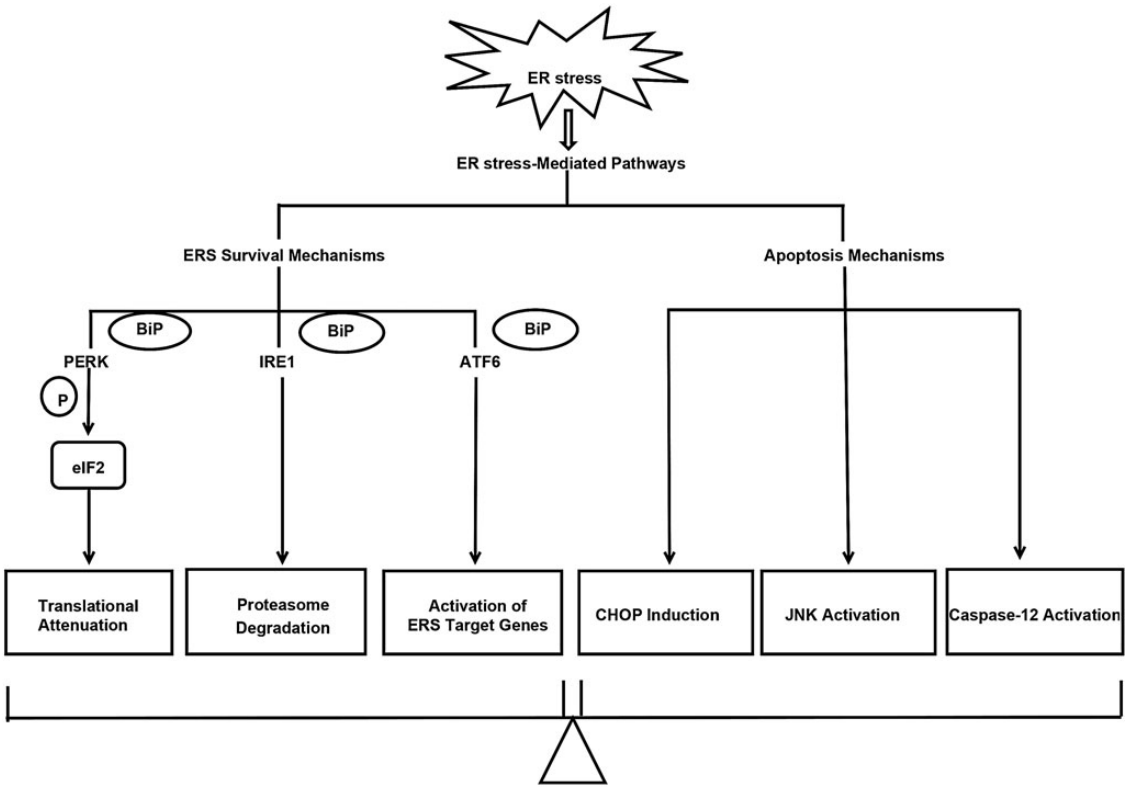

Figure 2. Proposed profile of ERSR [5]

may be a member of a cascade of molecular events by which the cellular concentration of amino acids regulates mammalian gene expression. Furthermore, in response to leucine starvation, ATF4 expression and ATF2 phosphorylation are increased. However, induction of ATF4 expression by the ER stress pathway does not fully activate the AARE-dependent transcription [18]. Thus, at least two pathways, one leading to ATF4 induction and one leading to ATF2 phosphorylation, are necessary to induce CHOP expression by amino acid starvation. A recent study has shown that CREBZF protein (also called ZF or Zhangfei), a basic region-leucine zipper transcription factor which has been implicated in the herpes virus infection cycle and related cellular processes, is induced by amino acid deprivation in the canine MDCK cells and may be an amino acid stress sensor. CREBZF and CHOP may be two sensors that regulate different yet related signaling pathways governing the AARE [19].

Transcriptional regulation of CHOP

The ER consists of a network of folded membranes in which secretory and most membrane proteins are synthesized, post-translationally modified, and folded into their correct three-dimensional conformations. Secretory and transmembrane proteins enter the ER as unfolded proteins and exit as either folded proteins in transit to their target organelles or as misfolded proteins targeted for degradation. The general output of the unfolded protein response (UPR) is to up-regulate genes involved in ER function, thus restoring and/or increasing the capacity of the ER to fold and process proteins. Activation of the UPR depends on three ER stress sensor proteins, IRE1, PERK, and ATF6. In non-stressed cells, all three ER stress transducers are kept in an inactive state through binding to the ER chaperone Bip [20]. Upon ER stress, excessive unfolded proteins accumulate in the ER lumen, resulting in the dissociation of Bip from the ER stress transducers, which triggers activation of the UPR branches and promotes cell survival [21]. However, during prolonged or overwhelming ER stress, UPR fails to restore the normal function of the ER, and apoptotic cascade will be activated [22].

IRE1. IRE1 dissociates with Bip dimeride and becomes activated. Activated IRE1 functions as endogenous ribonuclease (RNase) and splices a 26-nucleotide intron from the mRNA of *X-box-binding protein-1* (*XBP1*). The non-conventional splicing results in a shift in the translational frame of the *XBP1* gene, encoding a transcription activator XBP1 that drives the transcription of genes such as ER chaperones [23,24] and proteins involved in ERAD [25] (**Fig. 3**).

PERK. Like IRE1, PERK is activated by ER stress via dimerization and autophosphorylation upon the dissociation with Bip. Activated PERK phosphorylates its downstream target protein, eIF2, resulting in the inhibition of global

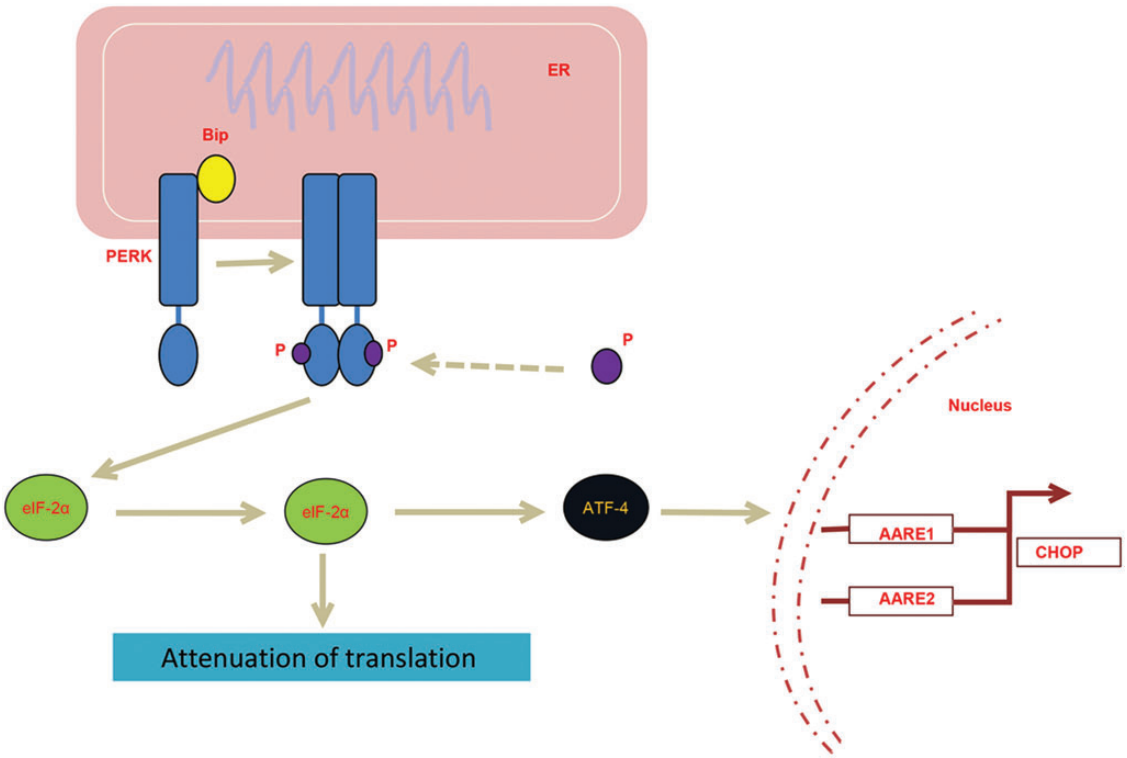

Figure 3. Mechanism of transcriptional induction of CHOP by IRE1 in ER stress Activated IRE1 cleaves XBP-1 mRNA precursor to mature *XBP-1* mRNA by unique splicing and produces an active transcription factor named spliced XBP1 (XBP1-S), which up-regulates ER chaperones and proteins implicated in the ERAD.

protein translation [26]. This phosphorylation event decreases cap- or eIF2-dependent translation, which shut off global mRNA translation and reduces the protein load on the ER [27,28] (**Fig. 4**). However, certain mRNAs encoded by ER stress response (ERSR) genes gain structural features and as elective advantage for translation that allow them to escape PERK-mediated translational inhibition.

ATF6. In comparison with PERK and IRE-1, the luminal domain of ATF6 is associated with Bip in an inactive form in normal cells. In contrast to PERK and IRE-1, ATF6 released from Bip during ERS is not thought to be uncompetitive in binding to Bip in comparison to other proteins [29]. Moreover, ATF6 exists in the ER as a dimer linked by intermolecular disulfide bonds in the luminal domain. Under ER stress, ATF6 translocates to the Golgi where it undergoes cleavage by serine protease site-1 protease (S1P) and metalloprotease site-2 protease (S2P), yielding an active transcription factor N-ATF6 which is subsequently translocated to the nucleus and induces target ER genes [30,31]. The active ATF6 then moves to the nucleus and activates ER stress response element (ERSE) related genes through binding their promoters (**Fig. 5**).

ATF6 also regulates other URP genes, such as *XBP-1* and *CHOP* [32]. Although both the IRE-1 and ATF6 pathways can up-regulate CHOP, the PERK pathway predominates through selective up-regulation of translation of ATF4, which subsequently induces transcription of *CHOP* and other genes involved in amino acid metabolism and transport, and oxidation–reduction reactions. The downstream CHOP AARE is identified to be an essential motif for amino acid activation of the *CHOP* promoter [33]. This sequence has weak homology with C/EBP- and ATF-binding sites. ATF2 and ATF4 are shown to be involved in the amino acid regulation of CHOP. ATF4 binds to AARE1 and AARE2. ATF2 and ATF3 can bind to the CHOP AARE. However, the role of ATF2 and ATF3 in the induction of CHOP during ER stress is still unclear. Both pATF6(N) and XBP-1 bind to the CACG part of ERSE1 and ERSE2 [34]. NF-Y constitutively binds to the CCAAR part of ERSE1 and ERSE2.

Roles of CHOP in the Apoptotic Pathways

Over-expression of CHOP has been reported to lead to cell cycle arrest and/or apoptosis. Concerning the downstream pathway of CHOP-mediated apoptosis, cells lacking CHOP's major dimerization partner C/EBβ are also resistant to ER stress-induced apoptosis, suggesting that CHOP works as a transcriptional factor that regulates genes involved in either survival or death [35]. As a transcriptional factor, CHOP has been shown to regulate numerous pro- and anti-

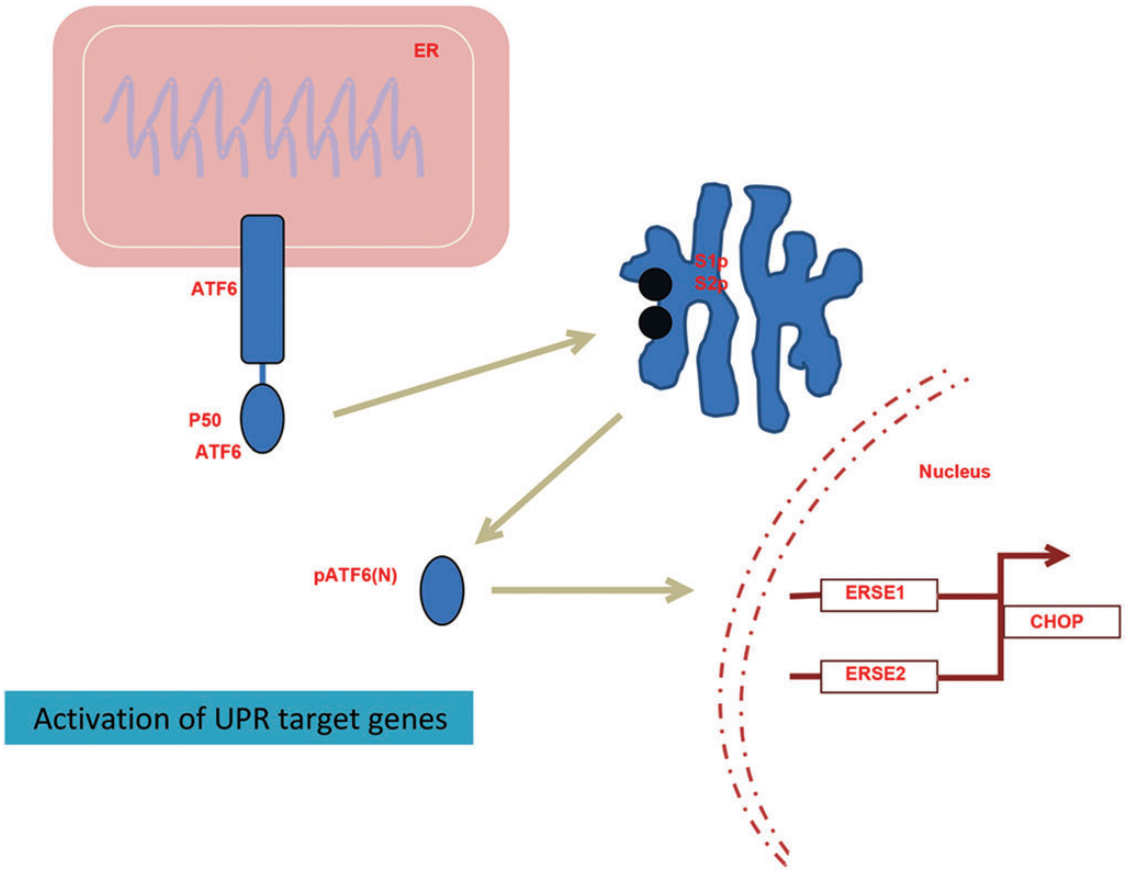

Figure 4. Mechanism of transcriptional induction of CHOP by PERK in ER stress The activation of PERK increases phosphorylation of eIF2, leading to a global attenuation of protein synthesis and a concomitant increase in ATF4 translation. In turn, ATF4 binds to AARE1 and AARE2 and induces the expression of CHOP.

apoptotic genes, including *DOCs* (for *down-stream of CHOP*), *Bcl-2* (*B-cell lymphoma-2*), *TRB3* (*tribbles-related protein3*), and *GADD34* (**Fig. 6**).

DOCs are a family composed of three members: DOC1, DOC4, and DOC6 [36]. DOC1 is a stress-inducible form of carbonic anhydrase VI which is predicted to increase the proton concentration and to decrease intracellular pH. DOC4 is a homolog of Tenm/Odz which might function in signaling at compartment boundaries. DOC6 is a homolog of the actin-binding proteins villin and gelsolin, and is implicated in changes in the actin cytoskeleton during apoptosis. However, none is directly linked to either survival or death [37].

Another widely accepted mechanism of CHOP-induced apoptosis is the suppression of the pro-survival protein Bcl-2. Known to date, the Bcl-2 family consists of 25 members that share up to four conserved motifs known as Bcl-2 homology domain (BH1–4). These family members can be either pro-apoptotic (BAX, Bak, Bok) or anti-apoptotic (Bcl-2, Bcl-X, Bcl-W). Under ER stress, CHOP down-regulates the expression of Bcl-2 but not Bcl-X, sensitizing cells to apoptosis [38]. A correlation between CHOP-mediated apoptosis and down-regulation of Bcl-2 *in vivo* was shown in a mouse model of cardiomyocyte apoptosis, where there was a small but statistically significant decrease in cardiomyocyte Bcl-2 in ER-stressed wild-type mice but not in CHOP$^{-/-}$ mice [39]. BAX is also up-regulated during ER stress in cardiomyocyte models, showing that CHOP-deficient mice have less apoptotic cell death and lower caspase-3 activation related to a decrease of BAX levels [40]. Recent studies have shown that Bcl-2-associated athanogene 5 (Bag5) is over-expressed in prostate cancer and inhibits ER stress-induced apoptosis. Bag5 over-expression resulted not only in a decreased *CHOP* but also in a decreased *BAX* and an increased *Bcl-2* gene expression [41]. CHOP has also been reported to regulate the expression of BH3-only proteins by interacting with FOXO3A (in neuronal cells treated with tunicamycin) and AP-1 complex protein cJUN leading to its phosphorylation (in saturated fatty acid-treated hepatocytes) [14,42]. In neuroblastoma cells, Bcl-2-related protein Bak is up-regulated in response to fenretinide. Fenretinide induces apoptosis via retinoic acid receptor (RAR)-dependent and -independent pathways in which the RAR-independent pathway is characterized by the ROS-dependent induction of CHOP and Bak. Bax–Bak through mitochondria permeabilization is a major pro-apoptotic action, which is also a key pro-apoptotic end

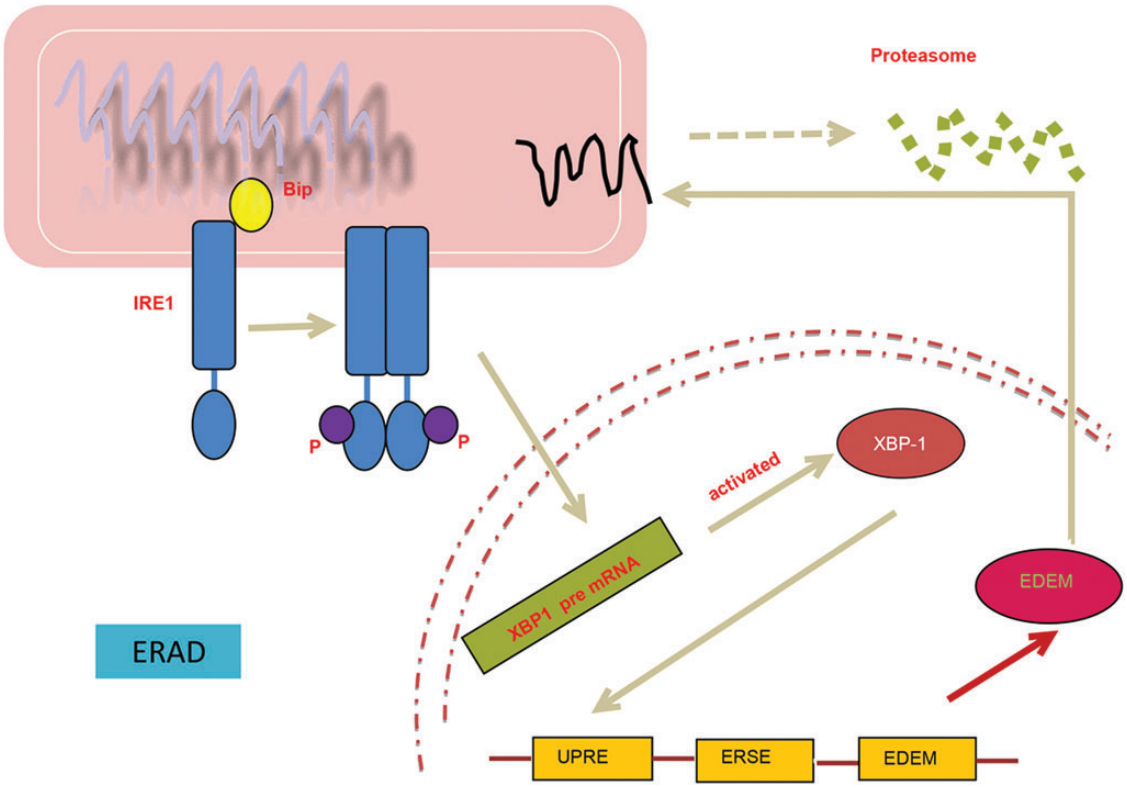

Figure 5. Mechanism of transcriptional induction of CHOP by ATF6 in ER stress After the dissociation of Bip, ATF6 translocates to Golgi apparatus, where it is activated by proteolysis. Activated ATF6 transcriptionally induces ERAD genes and up-regulates CHOP expression.

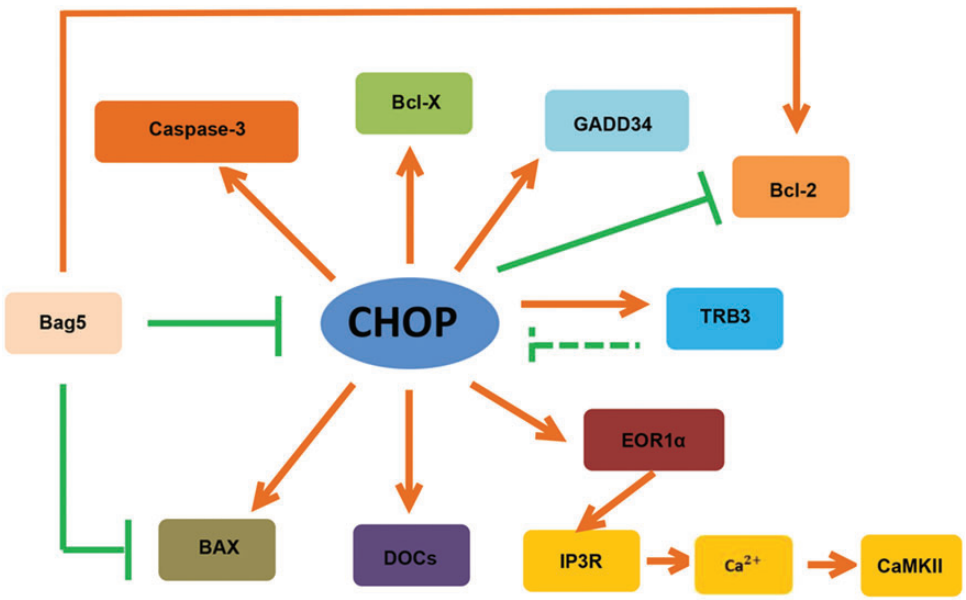

Figure 6. Downstream of CHOP in ER stress-induced apoptosis Over-expression of CHOP leads to decrease in Bcl-2 but increase in caspase-3, Bcl-X, BAX, GADD34, DOCs, EOR1α, TRB3. EOR1α initiates the IP3R-Ca^{2+}-CaMKII pathway. In turn, over-induction of TRB3 gives a negative feedback to the expression of CHOP. In addition, Bag5 inhibits the expression of CHOP and BAX, but induces Bcl-2 expression.

effect of the CHOP-ERO1α-IP3R-calcium-calcium/calmodulin-dependent protein kinase II (CaMKII) pathway [43].

TRB3 is an intracellular pseudokinase that modulates the activity of several signal transduction cascades. *TRB3* gene expression is highly regulated in many cell types. Amino acid starvation, hypoxia, and ER stress promote TRB3 expression in non-cardiac cells. During ER stress, TRB3 is up-regulated by an ER stress-inducible transcriptional factor,

ATF4/CHOP [44]. Excess expression of TRB3 down-regulates its own expression by negative feedback via the re-pression of ATF4/CHOP transcriptional activity [45]. CHOP expression increases in diabetic mouse kidneys and in podo-cytes treated with ROS and free fatty acid (FFA). In podo-cytes, transfection of CHOP increases *TRB3* expression, and ROS augments the recruitment of CHOP to the proximal *TRB3* promoter [46].

GADD34, representing another pro-apoptotic mechanism of prolonged CHOP expression, can promote the depho-sphorylation of phosphorylated eIF2 and thus restore protein translation. Impaired GADD34 expression reduces client protein load and ER stress in $CHOP^{-/-}$ cells exposed to per-turbations that impair ER function. GADD34 mutant cells accumulate less high molecular weight protein complexes in their stressed ER than wild-type cells [47].

Jauhiainen A *et al*. [48] analyzed cells carrying tamoxifen-inducible CHOP expression constructs and showed distinct gene expression profiles for cells with cytoplasmic and nuclear localized CHOP. Only 3 target genes of 175 identi-fied by now were regulated by CHOP in both cytoplasmic and nuclear localizations. The fact that more than two-third of the genes were down-regulated indicated that CHOP mainly functions as a dominant negative factor in either cytoplasmic or nuclear sequestration of dimer forming tran-scription factor partners. Characterization of CHOP-regulated functions will help to understand its role in stress response and involvement in cancer and degenerative disorders.

Roles of CHOP-Mediated Apoptosis in Disease

The ability to respond to perturbations in ER function is a fundamentally important property of all cells, but ER stress can also lead to apoptosis. As mentioned above, when ER stress conditions become too severe and excessive, this cel-lular stress response system turns on its pro-apoptotic module, which then gains dominance and triggers cell death. Accumulated evidence suggested that dysregulation of apop-tosis is involved in the pathogenesis of a number of human diseases. Here, we briefly summarize what is known of the roles of CHOP in the development of several diseases, with emphases on relevance to pathophysiology and integration and complementation among the various apoptotic pathways induced by CHOP.

Neurodegenerative disease

Neurodegenerative diseases have an underlying similarity in progressive neuronal dysfunction and neuronal cell death. Protein misfolding has been implicated in the pathogenesis of neurodegenerative diseases. Many mutations of the key functional proteins are related to the up-regulation of CHOP. The most classical example may be the Alzheimer's disease (AD). ER stress may play a role in the pathophysiology of many diseases including AD.

The etiology of AD is unknown, but it is widely accepted that increased production and accumulation of β-amyloid is an instigator of the neurodegenerative processes observed in AD. Reduction in β-amyloid production and accumulation is an appealing strategy to reduce the progression of AD. β-Amyloid induces CHOP expression both in cells and animal brains, whereas treatment of cells with CHOP anti-sense RNA improved neuronal survival after exposure to β-amyloid [49]. Mutation in presenilin-1, which increases the production of β-amyloid, was shown to increase CHOP protein in presenilin-1 mutant knock-in mice [50]. Another study showed that mutations in presenilin which result in early onset AD cause both increased calcium release from intracellular stores, primarily ER and changes in NF-κB acti-vation. The enhanced release of calcium from inositol tri-phosphate (IP3)-mediated ER stores in mPS1 neurons stimulates increased NF-κB compared with normal neurons, which inhibits CHOP expression [51]. β-Amyloid is derived from the amyloid precursor protein (APP) through an initial cleavage by aspartyl protease BACE1 (β-site amyloid pre-cursor protein-cleaving enzyme 1) and subsequent cleavage by the γ-secretase enzyme complex [52]. The initial cleav-age of APP by BACE1 is the rate-limiting step in β-amyloid production. Increased levels of BACE1 in the triple trans-genic mouse model for AD are preceded by CHOP and NF-κB activation [53]. Activated CHOP can generate oxida-tive damage and ROS, increase β-amyloid levels, disturb iron homeostasis, and induce inflammation as well as cell death, which are all pathological hallmarks of AD. CHOP siRNA can reduce 27-hydroxycholesterol (27-OHC)-induced Aβ production by mechanisms involving reduction in levels of APP and β-secretase (BACE1), the enzyme that initiates cleavage of APP to yield Aβ peptides. In addition, 27-OHC-induced tau phosphorylation, ROS generation, tumor necro-sis factor α activation, and iron and apoptosis-regulatory protein levels alteration are also markedly reduced by CHOP siRNA [54].

Both PUMA (p53 up-regulated modulator of apoptosis) and BIM are induced in response to ER stress in neuronal cells, and the transcriptional induction of PUMA regulates ER stress-induced cell death, independent of p53. CHOP co-immunoprecipitated with FOXO3a in tunicamycin-treated cells, suggesting that CHOP may also regulate other pro-apoptotic signaling cascades culminating in PUMA and BIM activation and cell death [55]. Histopathological ana-lyses of brains of Alzheimer's patients have hinted that ER stress may be a component of the pathogenesis of this neuro-degenerative disease. For instance, both spliced XBP-1 and phosphorylated IRE1 were found in the brains of individuals with AD [14]. In Parkinson's disease, dopaminergic neurons are filled with intracytoplasmic inclusions called Lewis

Bodies, in dopaminergic neurons of Parkinson's disease patients [56]. In addition, Parkinson mimetic drugs (such as 6-hydroxydopamine) increase ER stress in dopaminergic neurons whereas a null mutation in CHOP reduce the 6-hydroxydoamine-induced apoptosis *in vivo* [57].

Metabolic disease

ER stress is a highly promising therapeutic target for metabolic disease. The emergence of compounds that target specific UPR signaling pathways will provide more options for this purpose. Special emphasis is placed on the use of novel small molecules in animal disease models and human pathologies, including diabetes, obesity, fatty liver disease, and atherosclerosis.

Apoptotic cell death in pancreatic β-cells is involved in the pathogenesis of diabetes. Pancreatic β-cells are strongly engaged in protein secretion and have highly developed ER. Recent studies have revealed that β-cell is one of the most susceptible cells for ER stress, and ER stress-mediated apoptosis in β-cells can be a cause of diabetes. In type 2 diabetes, due to high insulin demand, β-cells greatly rely on the ER to ensure synthesis and proper folding of pro-insulin [58]. Prolonged ER stress triggers apoptosis at least in part through the UPR-activated CHOP. CHOP has elevated as a critical mediator connecting accumulation and aggregation of unfolded proteins in the ER and oxidative stress and also contributes to the induction of apoptosis in β-cell under conditions of increased insulin demand. Type 2 diabetes up-regulate systemic and local ERSR by activating CHOP and pro-inflammatory mechanisms, thereby contributing to renal injury [59]. Prolonged ER stress can both hyperoxidize the ER lumen, which may result in H_2O_2 leakage into the cytoplasm, and directly induce cytotoxic ROS in the cytoplasm. Oxidation of the ER lumen is induced by the CHOP transcriptional target ER oxidase 1α (ERO1α). Partial silencing of ero-1 in *Caenorhabditis elegans* can protect the organism from tunicamycin-induced death [60]. A recent study showed that CHOP expression is increased in diabetic mouse kidneys and in podocytes treated with ROS such as H_2O_2 and superoxide anion (via the xanthine/xanthine oxidase reaction) as well as the circulating FFAs [61]. Enhanced ROS and/or FFA associated with the diabetic milieu induce podocyte CHOP and TRB3 expression [62]. Evidenced by recent work, CHOP-induced ERO1α activates the ER calcium-release channel IP3R1, and cytoplasmic calcium triggers apoptosis by activating the calcium-sensing kinase CaMKII, which in turn triggers a number of downstream apoptosis. Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) activities are modulated in a manner that reflects the secretory demand on β-cells to integrate long- and short-term nutrient sensing information. Lawrence M *et al*. found that *CHOP* gene expression, especially that is regulated by nutrients, is also ERK1/2-dependent in β-cells [63]. Liew CW *et al*. found that an increase in pancreatic β-cell apoptosis in mice over-expressing a hyper-stable form of TRB3 is associated with decreased β-cell function in humans [64].

Atherosclerosis

Significant progress has been made on elucidating the roles of CHOP-induced ERS in the macrophages. Prolonged ER stress leads to CHOP-mediated macrophage apoptosis through a pathway involving release of ER calcium and activation of the death receptor Fas [65]. Release of ER Ca^{2+} stores activates CaMKII, leading to activation of pro-apoptotic STAT1 (signal transducers and activators of transcription 1), induction of Fas, and stimulation of the mitochondria-cytochrome c pathway of apoptosis [66]. CHOP expression in macrophages also correlates with a decrease in the anti-apoptotic protein BCL-2, which correlates with macrophage apoptosis *in vivo* [67]. A recent study has shown that ATF6 mediates oxidized LDL-induced cholesterol accumulation and apoptosis in macrophages by up-regulating CHOP expression [68]. Several inducers of ER stress have also been identified in smooth muscle cells (SMCs) in atherosclerosis. Increased CHOP expression in SMCs follows mechanical stretch or treatment with 7-ketocholesterol, homocysteine, and glucosamine [69–71].

Ischemia disease

Tissue ischemia with many characteristic features such as depletion of oxygen, glucose, and ER Ca^{2+} is strongly associated with ER stress. Induction of *CHOP* mRNA is observed in the rat hippocampus subjected to global cerebral ischemia [72]. $CHOP^{-/-}$ mice have smaller infarcts than wild-type animals subjected to bilateral carotid artery occlusion [73]. Nitric oxide (NO) plays a critical role in the pathophysiology of brain ischemia. NO functions by diminishing ER Ca^{2+} and promotes the transcription of *CHOP* gene in primary neuronal cultures [74]. In addition, comorbid type 2 diabetes exacerbates brain ischemia/reperfusion (I/R) injury which may be mediated through enhanced ER stress and cell death involving CHOP and caspase-12 activation [75]. ERS-associated apoptosis is also involved in the pathogenesis of chronic myocardial ischemia and heart failure induced by pressure overload.

Pieces of evidence have demonstrated that CHOP-dependent cell death pathway may be involved in the transition from cardiac hypertrophy to heart failure in mice [76]. Expressions of CHOP, caspase-12, and GRP78 are down-regulated in myocardial cells in rat heart failure after myocardial infarction [77]. A recent study has shown that calnexin (CNX) silenced cardiomyocytes exhibit ER stress as evidenced by increased GRP78 and ATF6 protein levels, increased levels of spliced *XBP1* mRNA, *ERO1a*, and *CHOP* mRNA levels [78]. Evidenced by other studies, sulfur dioxide has been found to be produced endogenously in the cardiovascular

system and has adverse effects prior to and during rat myocardial I/R injury by inducing ERS [79]. Prior to myocardial I/R, sulfur dioxide pre-conditioning induces expression of myocardial GRP78 and p-eIF2a. However, during myocardial I/R, sulfur dioxide suppresses the expressions of myocardial GRP78, CHOP, and p-eIF2a in association with improved myocardial injury *in vivo* and *in vitro* [80]. ERS induced by ischemic pre-conditioning protects against subsequent lethal ischemia injury effect on myocardial I/R and alleviates myocardial injury.

Tumor

The tumor microenvironment is characterized by low oxygen supply, poor vascularization, nutrient deprivation, and acidic pH, all of which are activators of ER stress. In tumor cells, the cell-protective features of the ERSR appear to be chronically activated and thus provide support for continuous proliferation and survival even under adverse microenvironmental conditions, which may include chemotherapy. However, persistent activity of these pro-survival pathways primarily in tumor cells may provide a window of opportunity for therapeutic intervention that is principally aimed at these tumor-specific conditions.

CHOP has been shown to trigger tumor cell death in ER stress. Hypoxia is a physiologically important stress that induces cell death in the context of the ER, especially in solid tumors. Jeong K *et al*. finds that cyclophilin B (CypB) physically interacts with the N-terminal α-helix domain of CHOP under hypoxia and cooperates with p300 to modulate the ubiquitination of CHOP [81]. CypB and CHOP control hypoxia-induced cell death on a linear pathway [82]. In leukemia, treatment of Na^+/H^+ exchanger 1 (NHE1) inhibitor Cariporide leads to ER stress-induced up-regulation of the Death Receptor 5 (DR5) receptor which is mediated by CHOP at transcriptional level [83]. Up-regulation of CHOP is also involved in the ER stress-induced apoptosis in B-chronic lymphocytic leukemia cells [84]. As for lung cancer, a recent study showed that *lipocalin 2* is a new CHOP target gene as an apoptosis inducer of ER stress in lung cancer A549 cells. ER stress increases *CHOP* DNA binding to *lipocalin 2* promoter. Furthermore, silencing of lipocalin 2 mitigates ER stress-mediated apoptosis in A549 cells [85]. In several human tumors including lung, breast, colon, ovary, and prostate, CHOP was reported to act as a key mediator in the cytotoxic effect induced by Choline kinase alpha (ChoKα) inhibition. Silencing of CHOP expression leads to a reduction in C/EBPβ, ATF3, and GRP78 protein levels and abrogates apoptosis in tumor cells after treatment with ChoKα inhibitors [86]. In addition, in pancreatic cancer cells, capsaicin significantly increases the mRNA and protein expression of CHOP and down-regulation of CHOP induced by specific siRNA significantly diminishes capsaicin-induced apoptosis [87,88].

Conclusions and Future Perspectives

CHOP is a non-ER localized transcription factor that is induced by a variety of adverse physiological conditions including ER stress. Emerging evidence suggested that CHOP-induced apoptosis plays a pivotal role in ER stress. Research in this area has provided rich mechanistic insights into the critical downstream molecules of CHOP leading to apoptosis and has suggested promising and new areas of potential clinical application. Nevertheless, although several potential regulators that may control apoptosis induced by CHOP have been identified, the critical mechanisms underlying the CHOP-mediated cell death, which seems different between species and cell types, are far from clear, and none of these regulators is directly involved in CHOP-induced apoptosis. For instance, as is mentioned, CHOP is responsible for the induction of DR5 by ER stress and treatment of NHE1 inhibitor Cariporide leads to CHOP-induced up-regulation of the DR5 receptor. Moreover, JNK activation is also involved in the modulation of CHOP. Thus, NHE1 activity correlates with CHOP-induced apoptosis in ER stress and confers pharmaceutical potential to NHE1 inhibitor as anti-tumor agent. Although increasing evidence has suggested that numerous diseases are relevant to the abnormal expression of CHOP in ER stress, many questions remain to be answered regarding the roles of CHOP in various physiological and pathological conditions. To what extent do the cells have the tendency for apoptosis rather than maintain their intra-environment homeostasis? How do these regulatory molecules interact with each other? Which one plays the ultimate and decisive role in the ER stress-mediated apoptosis? A better understanding and integrative approaches are needed to uncover the actual mechanisms governing this essential process and to put forward new critical therapeutic targets for CHOP-induced apoptosis in various diseases.

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