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# Endoplasmic Reticulum Stress and Autophagy

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## Abstract

In eukaryotic cells, the aggregation of the endoplasmic reticulum (ER)-mediated unfolded or misfolded proteins leads to disruption of the ER homeostasis, which can trigger ER stress. To restore the ER homeostasis, the ER stress activates the intracellular signaling cascade from the ER to the nucleus, referred to as the unfolded protein response (UPR). Autophagy primitively portrayed as an evolutionarily conserved process is involved in cellular homeostasis by facilitating the lysosomal degradation pathway for the recycling and elimination of intracellular defective macromolecules and organelles. Autophagy is tightly regulated by the protective mechanism of UPR. The UPR and autophagy are interlinked, which indicates that the ER stress can not only induce autophagy but also suppress it. Here, we discuss the molecular mechanism of ER stress and autophagy and their induction and inhibition signaling network.

**Keywords:** ER stress, autophagy, calcium, lysosome

## 1. Endoplasmic reticulum

The endoplasmic reticulum (ER) is a central membrane-bound organelle constructed from a dynamic network of tubules involved in cellular processes such as protein synthesis, gluconeogenesis, lipid synthesis and processing, and calcium storage and release in the cell and contributes to the generation of autophagosomes and peroxisomes [1]. The extension of ER morphology depends on the cell's activity and lineage; it is organized into subcompartments of different shapes, such as cisternae and tubules. ER appears as two main interconnected compartments, namely, the smooth endoplasmic reticulum (SER) and the rough endoplasmic reticulum (RER), which are abundant in different proportions in different cell lineages [2]. RER is less tubular than the SER, which forms an interrelated network of subdomains of ER; the RER is illuminated with ribosomes on their membranes, which are absent in the SER. RER has appeared in all cells and its density is higher, similar to that of the Golgi apparatus and nucleus because in all cells the nascent polypeptide is cotranslationally inserted into their membranes from the ER membrane. However, SER is present in only certain cell types, such as the liver cells, steroid-synthesizing cells, neurons, and muscle cells. SER is involved in the generation of steroid hormones within the adrenal cortex and endocrine glands and acts as a center for detoxification and protein transportation [3, 4]. A remarkable number of proteins are  $\text{Ca}^{2+}$ -dependent and need a completely oxidizing environment [5]. In the lumen, the

abundant molecular chaperones bind to the proteins and prevent them from aggregation, which makes the ER an ideal and unique milieu for proper protein folding. In fact, the ER quality control checkpoints allow the existence of only the precisely folded proteins. In addition, the ER facilitates the formation of three-dimensional structures by cotranslational and posttranslational modifications of the proteins [6].

## **2. ER stress**

The ER is a subcellular organelle predominantly known as a protein-folding checkpoint, which has an important role to ensure the proper folding and maturation of newly secreted proteins and transmembrane proteins. Several pathological and physiological conditions, such as perturbation in the cellular ATP level, calcium fluctuation, hypoxia, viral infection, inflammatory cytokines, nutrient deprivation, and environmental toxins, result in the loss of ER homeostasis and a reduction in the protein-folding potential of ER, eventually leading to the accumulation and aggregation of unfolded proteins in the ER lumen, acknowledged as the ER stress [7]. In experimental settings, the ER stress and protein misfolding or aggregation is instigated by treating cells with ER stress-inducing toxic chemicals. Versatile mechanisms of the UPR, under these nonphysiological conditions, are unable to maintain the homeostasis in the ER and the cells finally undergo apoptosis [8].

## **3. The unfolded protein response (UPR)**

The UPR can be viewed as a process that is involved in the sensing of the ER stress and transduces this signal to the regulation of downstream transcription factors that are involved in stress reduction or the induction of proapoptotic programs [9]. The ER stress enacts the UPR as an adaptive response for maintaining protein homeostasis [10, 11]. The UPR is initiated by three ER transmembrane proteins: the inositol-requiring 1 $\alpha$  (IRE1 $\alpha$ ), PKR-like ER kinase (PERK), and activating transcription factor 6 $\alpha$  (ATF6 $\alpha$ ). Under normal conditions, the ER chaperone, luminal domain binding immunoglobulin protein (BiP), binds to these proteins and keeps them inactive; but, when ER stress occurs, the BiP dissociates from these three proteins, UPR arms are activated [12]. This activates the UPR, which has three noteworthy functions: (a) adaptive feedback, which encompasses decreasing the ER workload, in anticipation of further augmentation of unfolded proteins, by the upregulation of molecular chaperones and protein-processing enzymes that maximize the folding efficiency, and an accompanying increase in the ER-associated protein degradation (ERAD) and the upregulation of the autophagy components to aid in the removal of misfolded proteins; (b) feedback control, which includes prevention of the hyperactivation of UPR, when the ER homeostasis is retrieved; (c) cell fate regulation, by the coordination of apoptotic and antiapoptotic signals, in the form of a switch between life and death of ER-stressed cells [12, 13]. The gene targets of the UPR change depending on the type of tissue and the nature of the physiological trigger that induces the ER stress. In distinctive hereditary backgrounds like the mouse and human cells, the different gene expression patterns triggered by the ER stress have been reported [9, 14].

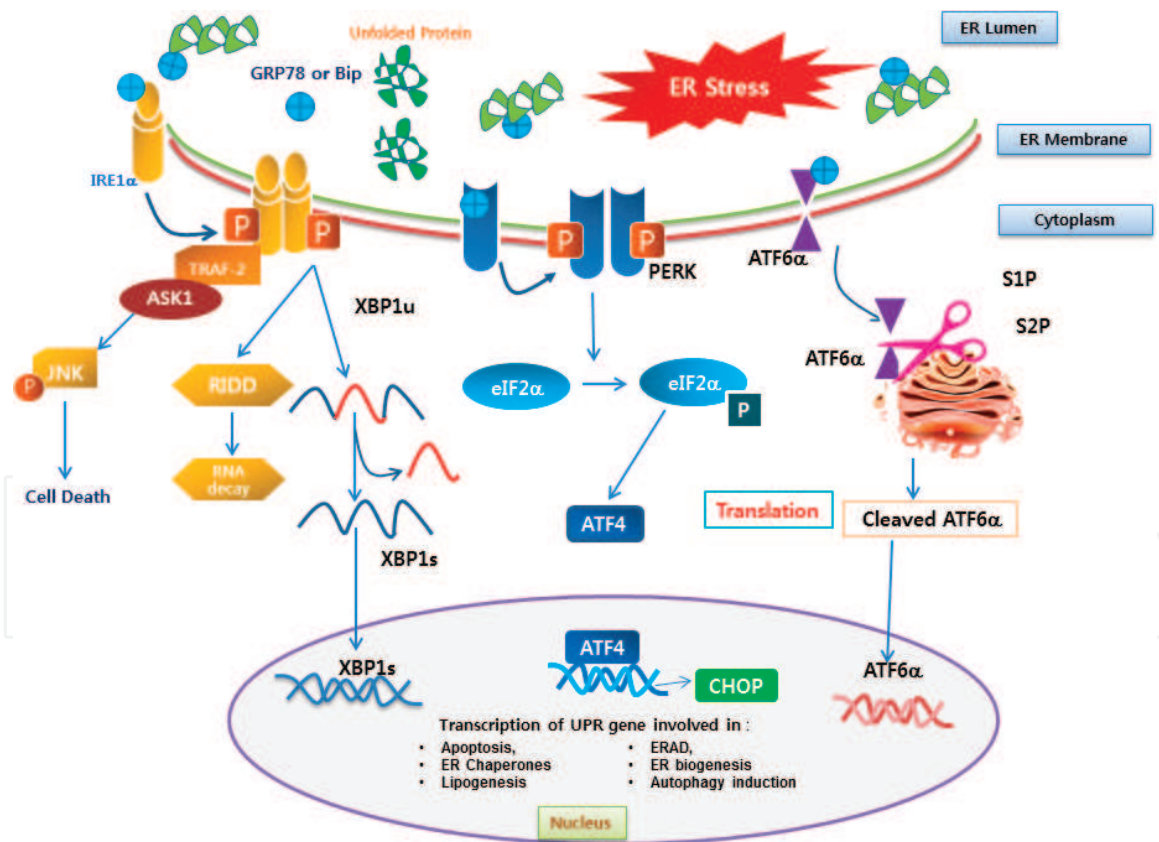
## **4. The UPR signaling pathway**

The UPR and misfolded or unfolded proteins as a prominent characteristic of mammalian cell ER stress were first reported in the 1980s by Kozutsumi et al. [10].

The UPR signaling pathway consists of three main branches involving the proteins IRE1 $\alpha$ , PERK, and ATF6 $\alpha$ . These proteins present in the ER resident transmembrane are major signaling elements. These three signaling sensors are all confederate with the ER chaperones, such as GRP78 (glucose-regulated protein 78 kDa, the main ER chaperone that is also named as BiP), which regulate their activation by fastening or discharge mechanism [15]. Primarily, IRE1 $\alpha$ , PERK, and ATF6 $\alpha$  are activated by the interaction with GRP78. GRP78 is well established to bind to the hydrophobic domains of proteins with its C-terminal binding domain, to prevent misfolding and unfolded protein aggregation (**Figure 1**). GRP78 accelerates the oligomerization and autophosphorylation of IRE1 $\alpha$  and PERK activates them, while ATF6 $\alpha$  is translocated to the Golgi apparatus [16].

#### 4.1 IRE1 $\alpha$

IRE1 $\alpha$  is the most conserved ER stress signaling branch, and its activation mechanism has been studied thoroughly. IRE1 $\alpha$  is a bifunctional type 1 transmembrane protein kinase containing three domains: an N-terminal luminal domain, a cytosolic endoribonuclease (RNase) domain, and a cytosolic serine/threonine kinase domain [17]. In response to the accumulation of unfolded proteins under the ER stress condition, IRE1 $\alpha$  dimerizes and transautophosphorylates leading to activation of



**Figure 1.** Schematic overview of UPR signaling. The three sensors of UPR, namely, IRE1 $\alpha$ , PERK, and ATF6 $\alpha$ , are activated when the misfolded protein aggregates recruit GRP78 or Bip, by dissociating them from the sensors. Activated IRE1 $\alpha$  dimerization and phosphorylation induces XBP1 mRNA splicing to generate active XBP1s, which increase the expression of UPR functional gene. UPR also activates another cellular pathway by interacting with Jun N-terminal kinase (JNK), via recruit TRAF2 and ASK1. PERK phosphorylates the downstream translation initiation factor eIF2 $\alpha$ , leading to the attenuation of overall protein translation and the activation of ATF4, which activates the expression of CHOP. Under ER stress conditions, the ATF6 $\alpha$  is transported to the Golgi apparatus and its cytosolic domain is cleaved by S1P and S2P proteases, which triggers the transcription of the ER chaperones. XBP1, ATF4, and ATF6 $\alpha$  transcription factors are translocated to the nucleus where they actuate the expression of target genes.

the cytosolic region RNase domain, resulting in the conformational change that activates the excision of the 26-nucleotide intron from the mRNA encoding the transcription factor XBP1 (**Figure 1**) [18–21]. This splicing event results in a frame shift in the mRNA and leads to the expression of an active and stable form of the transcription factor XBP1. The XBP1 is then translocated to the nucleus where it upregulates target genes that are involved in prosurvival events, such as quality control, maintaining ER homeostasis (via the ER chaperones GRP78, ERDj4, HEDJ, and PDI-P5) and ERAD (ER-associated degradation) [22, 23]. In the ER and Golgi compartment, XBP1 also increases the secretion rate of proteins. In addition, the RNase domain of IRE1 $\alpha$  can rapidly cleave a group of mRNAs and microRNAs, degradation through a process known as IRE1 $\alpha$ -dependent decay (RIDD) [24, 25]. IRE1 $\alpha$  activation is associated with the reduction of levels of a myriad of cytosolic RNAs, ribosomal RNAs, and microRNAs that have significant roles in inflammation, glucose metabolism, and apoptosis. Furthermore, active IRE1 $\alpha$  not only promotes UPR but also mediates other pathways, including the mitogen-activated protein (MAPK) kinase pathway, where the activated IRE1 $\alpha$  interacts with the adaptor protein tumor necrosis factor receptor-associated protein (TRAF-2) to form the complex IRE1 $\alpha$ -TRAF2. This complex interacts with the apoptosis signal-regulated kinase 1 (ASK1) to form the IRE1 $\alpha$ -TRAF2-ASK1 complex, which interacts with the ER stress-triggered c-Jun N terminal kinase (JNK) and results in the production of reactive oxygen species and activation of the autophagy and inflammatory pathways that involve the nuclear factor- $\kappa$ B [26–28].

#### 4.2 PERK

PERK is a type 1 transmembrane kinase that is structurally and functionally related to IRE1 $\alpha$  and is activated by transautophosphorylation and dimerization [29]. Under the ER stress conditions, PERK phosphorylates the downstream substrate eukaryotic translation initiator factor-2 $\alpha$  (eIF2 $\alpha$ ) at serine 51, which leads to the inhibition of protein synthesis within the ER lumen (**Figure 1**) [30–32]. This blockade reduces the continuous accumulation of unfolded proteins in the ER, thus reducing the ER stress. In addition, it allows the selective translation of the mRNA encoding the transcription factor ATF4, which has a ribosome entry site in its 5' nontranslated region, enabling its cap-independent translation [33, 34]. ATF4 is translocated to the nucleus where it upregulates the expression of the ER chaperone proteins (GRP78 and GRP94), the genes involved in macroautophagy, amino acid biosynthesis, protein secretion, antioxidant response, and the proapoptotic transcription factor CHOP [34, 35]. In addition to its role in UPR, eIF2 $\alpha$  phosphorylation assumes the role of a confluent marker of a particular stress pathway known as the integrated stress response," which is led by the unambiguous kinase that triggered during nutrient deficiency, viral infection, inflammation, and heme deficiency [35].

#### 4.3 ATF6 $\alpha$

A third sensor of the ER stress, ATF6 $\alpha$ , is an ER-targeted type 2 transmembrane protein that includes a basic leucine zipper transcription factor domain (**Figure 1**) [36, 37]. Under upregulation of UPR, ATF6 $\alpha$  is translocated to the Golgi apparatus for cleavage by the endopeptidases S1P and S2P, thereby releasing the activated form of ATF6 $\alpha$ . In response to the ER stress condition and GRP78, GRP94 agglomeration, similar to that of IRE1 $\alpha$  and PERK activation, the redox state is involved in the activation of ATF6 $\alpha$  [38].

The activation of IRE1 $\alpha$ , PERK, and ATF6 $\alpha$  has several effects, such as reduced translation, enhanced ER protein-folding capacity, and clearance of misfolded ER

proteins. The UPR stress sensors interact with and activate several transcription factors, which indicate the functional role of the UPR in proteostasis.

## 5. Autophagy

Autophagy, derived from the Greek words “auto,” meaning “self,” and “phagy,” meaning “to eat,” is a lysosomal pathway for cell survival used by eukaryotes, in which the cells digest and recycle their own cytoplasmic contents [39]. In the past three decades, several studies, especially in yeast, have revealed the molecular mechanisms involved in autophagy. Cells attune the number of components or vitiate parts of the organelle to maintain the optimum activity by assisting the minimal basal level of autophagy [40, 41]. In response, the basal autophagy can be activated to play a crucial role in cellular starvation and other cellular stresses, by lysosomal degradation and the exclusion of perennial and misfolded proteins, pernicious cellular substances, and pernicious organelles and infecting pathogens [42, 43]. In addition, autophagy can involve the rearrangement of the cellular membrane to concede parts of the cytoplasm being transported to the compartment, and it also acts as an energy source for the biosynthesis of new macromolecules produced by recycling metabolites of lysosomal proteolysis [44, 45]. Autophagy can maintain the energy homeostasis not only in particular organelle but also in the entire cell, through the increase of metabolic activity [45]. Moreover, autophagy plays critical roles in physiological processes such as cell growth, cell cycle, differentiation, tumor suppression, and programmed cell death including apoptosis and cellular senescence. In these ways, autophagy plays crucial roles throughout the life cycle of the cells [46, 47].

In mammal cells, there are three types of autophagy that have been documented; they are distinguished according to their physiological function and mechanism of cargo sequestration at the known destination lysosomes. These subtypes include macroautophagy, microautophagy, and chaperone-mediated autophagy [43, 48]. Macroautophagy is a major type of autophagy, and it has been the most studied compared to microautophagy and chaperone-mediated autophagy. It uses cytosolic double-membrane sequestering vesicles formed from phagophores, known as the autophagosomes, which transport cytosolic content to the lysosome [43, 49]. In microautophagy, the lysosome itself is a component of the cytoplasm where it engulfs cytoplasmic protein and small components of the lysosomal membrane. Macroautophagy and microautophagy both carry out the nonselective degradation of proteins, lipids, and organelles [50, 51]. In contrast, the chaperone-mediated autophagy does not involve the membrane rearrangement; instead, the protease of the lysosomal matrix acts on the substrate unfolded protein by directly translocating across the lysosomal membrane, which is dependent on LAMP2A (lysosomal-associated membrane protein 2A) and the lysosomal molecular chaperon HSPA8/HSC73/lys-HSC70 (heat shock cognate 70) [52, 53].

## 6. Molecular mechanism of autophagy

The mechanism of autophagy is a complex process that can be categorized into multiple steps. It involves the formation of double-membrane vesicles containing cellular and external malformed proteins. Long-lived proteins can be induced autophagy, which are ensued by cargo recognition and packaging, an extension of the phagophore membrane, and closure to form the complete autophagosome. Fusion of the autophagosome with the lysosome occurs, which leads to the derogation of the autophagosomal contents, and the breakdown products are finally

eliminated [54–56]. The initiation of autophagy can be observed by TEM (transmission electron microscopy) during the expansion of phagophore and autophagosome. The induction of autophagy, vesicle nucleation, and formation of autophagosomes are regulated by the proteins named as autophagy-related genes (ATGs) [50]. They are highly conserved genes and were originally discovered in yeasts. Mammalian orthologs of the ATGs have also been discovered [57]. Autophagy induction is controlled at the molecular level by the multiprotein complex of unc-51-like autophagy-activating kinase 1 (ULK1, the mammalian homolog of yeast Atg1), ATG13, ATG101a, and RB1 inducible coiled coil 1 (RB1CC1, also known as FIP200) [58, 59]. This complex is regulated by the mammalian target of rapamycin complex 1 (mTORC1), which remains inhibited by mTORC1 by the phosphorylation of ULK1/2 and ATG13, which suppresses the phosphorylation activity of ULK1/2-ATG13-FIP200 complex [59–61]. Under starvation and other stress conditions, the inhibition of mTORC1 dissociates it from the ULK complex followed by the dephosphorylation of specific residues within the ULK1/2 and ATG13 (phosphorylated by mTORC1) complex, which in turn promotes the induction of the phagophores [61]. Formation of phagophores includes a class III phosphatidylinositol 3-kinase complex (PtdIns3K) consisting of Beclin-1 (ATG6 in yeast), VPS34 (class III PI3K), VPS15 (also known as p150 in mammals), PIK3R4/p150, ATG14, UV radiation resistance-associated gene (UVRAG), and nuclear receptor binding factor 2 (NRBF2) [62–64]. In addition, the nonapoptotic proteins, such as the B-cell lymphoma-2 (BCL2) and BCL2L1/BCL-XL, hold Beclin-1 directly interacting with Beclin-1 (BECN-1s) BH3 domain and negatively regulating autophagy inducing the PtdIns3K. The c-Jun protein kinase (JNK1) and death-associated protein kinase (DAPK) phosphorylate BCL2 and are positive regulators involved in the induction of autophagy [65, 66].

The elongation or obstruction of phagophore depends on two diverse ubiquitin-like protein conjugation reactions [67, 68]. The first pathway involves the covalent conjugation reaction of ATG12 to ATG5, with the assist of the E1-like enzyme ATG7 and the E2-like enzyme ATG10. This conjugate ATG12-ATG5 complex interacts with ATG16L in a non-covalent reaction to form the multiprotein complex ATG12-ATG5-ATG16L, which performs the E3 ligase reaction of the cytosolic MAP1LC3/LC3 (microtubule-associated protein 1 light chain 3), LC3-I to the membrane-bound lipidated form, LC3-II [50, 69–71]. The second pathway includes the ubiquitin-like system, which plays a role in the conjugation to phosphatidylethanolamine (PE) lipid and glycine residue of the yeast ATG8 (LC3 in the mammal), and is processed by the cysteine protease ATG4 and then ATG8 is conjugated to PE by E1-like enzyme ATG7 and E2-like enzyme ATG3. Based on that, the ATG4 can act as delipidation or deconjugation enzyme which is involved in the recycling of membrane bound LC3-II on the external layer to the internal layer of the autophagosome [50, 67, 72]. Accordingly, the lipidated form of LC3-II is a stable marker protein associated with the biochemical and microscopic detection of cellular autophagy [73]. In mammals, six orthologs of ATG8 and four of ATG4 exist, among which the LC3, GATE-16 (Golgi-associated ATPase enhancer of 16 kDa), and GABARAP (G-amino butyric acid type A receptor-associated protein) have been the most studied [74]. The lipidation of ATG8/LC3 expedites the interaction with the autophagosome membrane, which leads to the autophagosome maturation steps, such as the extension and shrinkage of the membranes and cargo induction to autophagosome [75]. Once the autophagosome has surrounded the substrate of autophagy, it may merge with the late lysosome or endosome to create the autolysosome [76]. The cellular and molecular machinery that important for the fusion is activated by the small GTPase, RAB7A/RAB7 member of RAS oncogene family, which is necessary for autophagosome maturation [77]; and the RAB7 effector pleckstrin homology and RUN domain containing M1 (PLEKHM1) [78]; other soluble

N-ethylmaleimide-sensitive factor proteins trigger set of SNARE protein including syntaxin-17 (STX17), SNAP29, and VAMP8 [79, 80]; the PI3P-binding protein tectonin beta-propeller repeat containing 1 (TECPR1) [81]; inositol polyphosphate-5-phosphatase-E (INPP5E) [82]; ectopic P-granules autophagy protein 5 homolog (EPG5) [83]; as well as the homotypic fusion and vacuole protein sorting (HOPS) complexes ATG14 [78]; LAMP2B (but not LAMP2A) as well as the phosphorylated and lipidated LC3 which are also involved in the formation of autolysosomes [84]; finally, the autophagosomal-sequestered cargo undergoes degradation upon the acidification of the lysosomal lumen (by the activity of an ATP-dependent proton pump known as the V-type ATPase) [85].

## 7. ER stress and autophagy

Several studies have demonstrated that the ER stress and autophagy are mechanistically interconnected, in which the UPR, the key ER stress pathway, stimulates the autophagy. The three canonical divisions of the UPR intervened by the three ER membrane-associated proteins, IRE1 $\alpha$  (inositol-requiring enzyme 1), PERK (PKR-like eIF2 $\alpha$  also known as EIF2AK3), and ATF6 $\alpha$  (activating transcription factor-6), regulate the autophagy in distinctive manners during the ER stress. The relationship between autophagosome and the ER stress was first described in 2006 [86, 87].

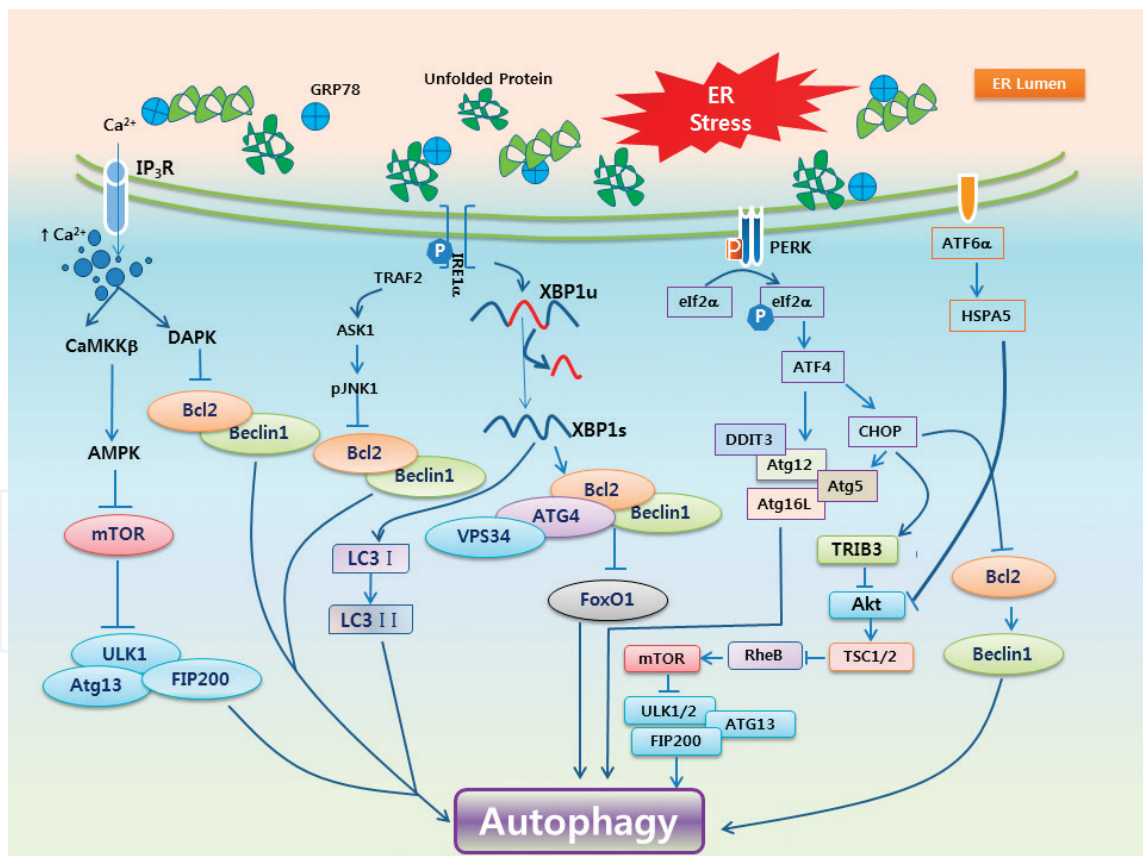
IRE1 $\alpha$ -mediated MAPK8 (mitogen-activated protein kinases 8) phosphorylation is the major regulatory step in this pathway [88]. MAPK8 is considered stress-associated protein kinase, which is involved in numerous manners in stress-induced autophagy and apoptosis, which depend on MAPK8 activation [89]. In particular, the activation of IRE1 $\alpha$  leads to MAPK8 phosphorylation, which induces autophagy. JNK (c-Jun N-terminal kinase) interacts with the MAPK8 family, which triggers the downstream mediators of autophagy, both directly and indirectly [90]. Directly, JNK can stimulate cell apoptosis in cancer cells by inducing Atg5 and p53. Indirectly, JNK inhibits the association of Bcl-2 with Beclin-1 and upregulates Beclin-1 expression by c-Jun phosphorylation. Beclin-1 is the autophagy-related gene and is the downstream regulator of MAPK8 and is activated by the direct phosphorylation of Bcl-2, which then obstructs the interaction between Beclin-1 and Bcl-2 and activation of the phosphoinositide-3-kinase (PI3K) complex and induces autophagy in the cancer cell (**Figure 2**) [90, 91]. Additionally, SP600125, a pharmacological inhibitor of JNK, also blocks the Beclin-1 expression and autophagy [92]. Wei Y et al [91] elucidated the starvation-induced autophagy by JNK1, via phosphorylation of ER-specific Bcl-2, at multiresidues T69, S70, and S87A, followed by Beclin-1 disruption from ER-localized Bcl-2 and the induction of autophagy [91]. Similarly, Beclin-1 expression is regulated by the JNK1 pathway, which plays a crucial role at the transcription level, following the ceramide-induced autophagy in mammalian CNE2 and Hep3B cancer cell lines [92]. SP600125 inhibited the autophagosome formation and ceramide-induced upregulation of Beclin-1, and similar phenomenon was observed using the small interfering RNA targeting JNK mRNA. Moreover, immunoprecipitation of chromatin and luciferase reporter analysis demonstrated that c-Jun, a target of JNK1, was activated and directly interacted with the Beclin-1 promoter in ceramide-treated cancer cells. In this respect, the IRE1 $\alpha$ /JNK1/c-Jun pathway is the key mechanism for the induction of autophagy. The IRE1 $\alpha$ /JNK1-induced autophagy pathways interact with the ATG protein and Beclin-1, which play a key role in vesicle nucleation [93, 94].

In addition, the IRE1 $\alpha$ -XBP1s axis has been involved in the induction of autophagy [95]. Initially, the spliced XBP1 indirectly regulates the Bcl-2 expression to induce autophagy (**Figure 2**) [66, 96]. Along with this, the autophagy induction



is also observed in endothelial cells that overexpress XBP1s, which enhances the transformation of LC3-I to LC3-II and increases the Beclin-1 expression [95]. Furthermore, XBP1s binds directly to the -537 and -755 region of the Beclin-1 gene promoter in the nucleus and enhances an autophagy induction via the transcriptional upregulated expression of Beclin-1 gene [97]. The deficiency in XBP1s leads to increased expression of Forkhead box O1, a transcriptional factor that elevates the induction of autophagy in neurons [98].

The major events in autophagy, such as the induction of phagophore and maturation, are coordinated by the LC3-II and the ATG12-ATG5 conjugate [99]. To maintain the autophagy flux, the upregulation of the transcription of the congruent autophagy genes is important [100]. Under the ER stress conditions, the PERK branch of UPR aids in the regulation of the autophagy-related genes. The association of PERK in ER stress-mediated induction of autophagy was first reported by Kouroku et al. [101]. In particular, they demonstrated that the aggregated polyglutamine (72Q) protein in the cytosol decreases the activity of proteasomes and leads to autophagy induction through the activation of the PERK branch of the UPR [102]. Under the hypoxic response, PERK mediates the transcriptional activation of LC3 and Atg5 proteins, through the action of the transcription factors ATF4, CHOP, and DDIT3 induction (**Figure 2**) [101, 103]. PERK may also reduce I $\kappa$ B $\alpha$  translation, as well as NF- $\kappa$ B activation, which promotes the induction of



**Figure 2.**

Overview of the mechanism of UPR-mediated autophagy. The ER stress can activate autophagy through  $\text{Ca}^{2+}$ , IRE1 $\alpha$ , PERK, and the ATF6 $\alpha$  signaling pathway.  $\text{Ca}^{2+}$  from ER lumen can be released through the IP<sub>3</sub>R channel, which phosphorylates CaMKK $\beta$  and activates AMPK, which in turn inactivates ULK1 complex through the inhibition of mTOR;  $\text{Ca}^{2+}$  activates DAPK which phosphorylates Beclin1 and Bcl2 lead to autophagy induction. The IRE1 $\alpha$  arm of UPR activation of JNK1 mediates phosphorylation of Bcl2, which causes Beclin-1 dissociation and induction of autophagy. In addition, spliced XBP1 also enhances the formation of LC3-I and LC3-II, which triggers the Beclin-1 via decrease of FoxO1 activity. Another arm of UPR activated PERK induce autophagy via expression of ATG12, DDIT3, ATG12, ATG16L by ATF4 transcription factor similarly CHOP activate TRIB3 which suppress the activity of Akt/mTOR pathway induced autophagy. ATF6 $\alpha$  arm of UPR can also induce autophagy by inhibiting phosphorylation at Akt and mTOR pathway.

autophagy [104]. PERK phosphorylates the downstream regulator eukaryotic initiation factor 2a (eIF2 $\alpha$ ), at the residue serine 51, and also increases the ATG12 mRNA and protein levels [105]. PERK-mediated ATF4 activation is required for expression of the autophagy genes, including MAP1LC3B, BECN1, ATG3, ATG12, and ATG16L1, while interaction of ATF4 and DDIT3 causes the upregulation of the transcription of SQSTM1/p62, BR1, and ATG7 [100]. In addition, ATF4 directly binds to cyclic AMP response component binding site of the promoter of microtubule-associated protein 1 light chain 3 $\beta$  (LC3 $\beta$ ), a vital component of autophagosomal membranes, which alleviates the induction of autophagy. In addition, DDIT3 can activate the formation of autophagosome through downregulation of Bcl-2 expression [106].

CHOP is another potent transcription factor, which is involved in the induction of autophagy [107, 108]. It has been elucidated that the expression levels of ATG5 and BH3 domain proteins are elevated by upregulation of the CHOP expression. Besides, the Bcl-2 expression level is downregulated, which assists in the release of Beclin-1 from Bcl-2. Moreover, the PERK-CHOP pathway instigates tribbles-related protein 3 (TRIB3), which inhibits the activation of the protein kinase B (Akt) [103, 109]. TRIB3-mediated inhibition of Akt regulates the phosphorylation of TSC2 (tuberous sclerosis complex 2) by the serine/threonine kinase, Ras homolog enriched in brain (Rheb), and the inhibition of mTORC1, which dephosphorylates ATG13 and the ULK1/2 complex and results in the induction of autophagosome formation [110].

The ATF6 $\alpha$  branch of the UPR is the least understood branch in relation to ER stress and autophagy. Nonetheless, the ATF6 $\alpha$  transcription regulator is involved in the initiation of autophagy by the elevated expression of HSPA5 (heat shock70kDa protein 5) (**Figure 2**) and followed by the inhibition of expression and activation of protein kinase B of AKT1/AKT [111]. In addition, the ATF6 $\alpha$  interacts with CEBPB (CCAAT/enhancer binding protein) to form a transcriptional heterodimer complex and binds to the CRE/ATF components of DAPK1 (death-associated protein kinase 1) to induce DAPK1 expression. ATF6 $\alpha$  knockdown with specific shRNA and ATF6 $\alpha^{-/-}$  cells leads to reduced expression of DAPK1, followed by the inhibition of formation of autophagosomes [112]. Beclin-1 phosphorylation leads to decreased Bcl-2 expression and initiates the formation of a complex between the autophagosome initiator Beclin-1 and PIK3C3. Simultaneously, the ATF6 $\alpha$ -mediated upregulation of CHOP, XBP1, and GRP78 expression is also initiated, resulting in the induction of autophagy [113].

## **8. ER stress induces autophagy via the PI3K/AKT/mTORC pathway**

The serine/threonine kinase of mTORC is the main regulator of ER stress [114]. It forms two complexes, the mTORC1 and mTORC2, both of which are triggered by extracellular and intracellular stimuli, under favorable conditions for growth [114, 115]. Accordingly, mTORC1 is a critical regulator of the UPR-mediated autophagy and nutrient signaling [116]. mTORC1 is involved in the regulation of the major signaling pathway. Interaction of growth factors with insulin triggers the PI3K complex, which accelerates the plasma membrane adaptation of the lipid phosphatidylinositol-3-phosphate (PtdIns(3)P) to generate PtdIns(3,4,5)P<sub>2</sub> and PtdIns(3,4,5)P<sub>3</sub>. These increase the membrane recruitment of pleckstrin homology domain proteins such as the serine/threonine kinase PDK1 (phosphoinositide-dependent kinase 1) and its substrate Akt protein kinase B to activate Akt in the plasma membrane [117]. The PI3K is elicited as a vesicular protein trafficking mediator, which binds to PtdIns(3)P, resulting in its translocation to intracellular membranes such as endosomal and lysosomal membranes. PI3K is a member of Vps34 family, which plays an important role in the formation of autophagosomes,

by directly interacting with Beclin-1 [118]. Similarly, PtdIns(3)P and PtdIns(3,4,5)P<sub>3</sub> initiate autophagy by phosphorylation of the phosphatidylinositol to activate PtdIns(3,4,5)P<sub>3</sub> and contributes to the autophagic vacuole sequestration [119].

Akt is a serine/threonine kinase, which is an upstream regulator of mTORC. Several hormone growth factors and the phosphorylation of the oncogene PI3K-Akt-mTORC can stimulate mTORC and the ribosomal protein S6 kinase (RPS6KB1) and inhibit the expression and phosphorylation of TSC1 (tuberous sclerosis 1) and TSC2, which under ER stress conditions inhibits mTORC [90]. Similarly, the inhibition of TSC triggers mTORC activity, which suppresses the initiation of ER stress-mediated autophagy. Furthermore, the knockdown of TSC1/2 can regulate the activation of mTORC, which is elevated under ER stress conditions. This indicates that TSC is essential for the canonical ER stress feedback [120, 121]. Thus, TSC1/2 is a crucial coordinator of several signals, including mTORC and the well-known PI3K-Akt pathway, for the induction of autophagy.

The opposite branch of this pathway is downregulated by mTORC release, and ULK1 initiates the autophagosome formation [122]. Accordingly, ER stress can inhibit the expression of Akt and suppress the mTORC regulation, which can induce autophagy. ATF6 $\alpha$  increases the expression of ER chaperone HSPA5 (heat shock 70 kDa protein 5), which can block the phosphorylation of Akt activity, in turn activating the induction of autophagy in placental choriocarcinoma cell [90].

TRIB3 (tribbles homolog 3) is an ER stress-associated protein, which can interact with Akt and downregulate the expression of Akt-mTORC [123, 124]. The defective ATF4-DDIT3 complex in malignant gliomas can activate TRIB3 under ER stress condition, which indicates that TRIB3 activation is ATF4-DDIT3 dependent.  $\Delta$ 9-Tetrahydrocannabinol (THC), the main active compound of marijuana, triggers the TRIB3-dependent autophagy pathway of ER stress, by the suppression of the Akt/mTORC1 pathway. The overactivation of TRIB3 can reduce the transcriptional activity of ATF4 and DDIT3. This indicates that the ER stress-mediated induction of autophagy via the PI3K/AKT/mTOR pathway plays a key role in cell survival [123].

## **9. ER stress induces autophagy via the AMPK/TSC/mTORC1 pathway**

The AMP-activated kinase (AMPK) is a key cellular energy sensor that regulates the transcription of the autophagy genes through the regulation of many downstream kinases [125]. AMPK is a cellular energy sensor that detects increased level of intracellular ATP/AMP concentration ratio [126]. Under several metabolic stress conditions, AMPK is phosphorylated by a serine/threonine kinase and activates genes including liver kinase B1 (LKB1, which is activated upon energy depletion), calcium/calmodulin kinase (CaMKK $\beta$ , which is activated by cytosolic Ca<sup>2+</sup>), and TGF $\beta$ -activated kinase-1 (TAK-1, which is involved in IKK activation) [126]. AMPK induces autophagy through the inactivation of mTORC1 via the phosphorylation of the tuberous sclerosis complex 2 (TSC2) and the regulation of the associated protein Raptor, after the dissociation and activation of ULK1 [127]. In addition, AMPK-induced autophagy not only inhibits mTORC1 but also directly phosphorylates ULK1 and Beclin-1. AMPK has a major role in preventing the ER stress-induced autophagy-mediated cytotoxicity. In addition, albumin-treated cellular toxicity leads to the activation of AMPK. Similarly, silenced RPS6KA3 (ribosomal S6 kinase 90 kDa polypeptide 3) decreased expression of AMPK induce autophagy which aggregates ER stress mammalian breast cancer model [128, 129]. Involvement of PERK-AMPK mediated and inactivation mTORC initiate autophagy has also demonstrated detachment of extracellular matrix in human epithelial cell. Moreover, AMPK inhibits synthesis protein by inactivation of mTORC and

phosphorylating EIF4EBP1/4E-BP1 and RPS6KB/p70S6K [130]. Moreover, the phosphorylation of eIF2 $\alpha$  [101] and the activation of IKK [131] are indispensable for induction of autophagy by starvation.

## 10. Ca<sup>2+</sup> in ER stress regulates autophagy

The ER plays a major role in maintaining the intracellular Ca<sup>2+</sup> store that can compile Ca<sup>2+</sup> concentrations of 10–100 mM, while in the cytoplasm and remaining cell concentration, the range is 100–300 nM [132]. The multifunctional organelle ER maintains Ca<sup>2+</sup> homeostasis, which is necessary for proper functioning including protein folding, lipid and protein biosynthesis, and posttranslational modification and regulation of gene expression [133]. The majority of ER-associated proteins participate in maintaining ER Ca<sup>2+</sup> homeostasis. For maintaining ER Ca<sup>2+</sup> homeostasis, most of the ER-associated proteins, such as calreticulin, GRP94 or GRP78, histidine-rich Ca<sup>2+</sup>-binding protein, and protein disulfide isomerase (PDI), uphold to Ca<sup>2+</sup> buffer in the lumen of ER [134]. Ca<sup>2+</sup>-binding protein mainly GRP78 is involved in sensing unfolded protein accumulation in the ER and interacts with three other UPRs of ER transmembrane proteins, ATF6 $\alpha$ , IRE1 $\alpha$ , and PERK [135]. As noted, loss of Ca<sup>2+</sup> homeostasis in the ER followed to initiate ER stress [136]. In addition, ER luminal Ca<sup>2+</sup> can reduce because of ER stress. Upon incitement of plasma membrane ER influx and discharge formation of Ca<sup>2+</sup> signal, whereas ER reservoir influx and release depend on replenishment of Ca<sup>2+</sup>. Activity of Ca<sup>2+</sup> across the membrane of ER is expedited by three kinds of protein receptor: Ca<sup>2+</sup> release channels—RYR (ryanodine receptor) and ITPR/IP3R (inositol 1, 4, 5-trisphosphate receptor); in the ER, cytosolic Ca<sup>2+</sup> enters through a Ca<sup>2+</sup> pump called ATP2A/SERCA (sarco/endoplasmic reticulum Ca<sup>2+</sup>) [137].

There is multitudinous Ca<sup>2+</sup> movement through the membrane of ER that assures appropriate functioning of numerous kinases and proteases. It is already well established that cytosolic Ca<sup>2+</sup> signal regulates protein intricate in several stages of autophagosome formation [138]. In addition, a number of Ca<sup>2+</sup> dependent pathways involved in autophagy induction have been studied. Indeed, cytosolic Ca<sup>2+</sup> initiation of autophagy it is ambiguous in many conditions. The numerous Ca<sup>2+</sup> origin has already involved merely various downstream effectors containing protein kinase C, Ca<sup>2+</sup>/calmodulin-dependent kinase  $\beta$  (CaMKK $\beta$  or CaMKK2), ERK, and Vps34 (a calmodulin protein) [139, 140]. It is already proven that CaMKK $\beta$  or CaMKK2 has perceived the majority experimental support, whereas Ca<sup>2+</sup> refinement of Vps34 and ERK is unsupportable. Activation of Vps34 by Ca<sup>2+</sup> or calmodulin is insinuated although the activity of Vps34 in cellulo was not affected by cytosolic Ca<sup>2+</sup> or calmodulin antagonist [139]. CaMKK $\beta$  is an increase the activity of AMPK, thereby inhibition of mTORC1 leads to activate autophagy [141]. Høyer-Hansen et al. demonstrated that in MCF-7 breast cancer cells the mobilize of cytosolic Ca<sup>2+</sup> from ER by stimulate IP3R generating agonist, such as thapsigargin, ionomycin and vitamin D analogue activate CaMKK $\beta$  which is initiate autophagy by downregulating of mTORC1 and activation AMPK dependent pathway [142]. In addition, deficient autophagy in T lymphocyte has an extension of ER compartment due to more Ca<sup>2+</sup> in the ER. Depletion of Ca<sup>2+</sup> in the ER leads to extension of Ca<sup>2+</sup> reservoir, which could be the purpose behind unfit to store diminished. This invasion of Ca<sup>2+</sup> can be recovered by SERCA/ATPase pump blocking with thapsigargin, which means autophagy can maintain Ca<sup>2+</sup> mobilization across the ER [143]. In total, the connection between autophagy and Ca<sup>2+</sup> mobilization intimates that they can have impact on each other. Moreover, the elevation of cytosolic Ca<sup>2+</sup> endogenously induction of autophagy by precipitation of Ca<sup>2+</sup> phosphate without modifying the condition of ER. In consequence, ER Ca<sup>2+</sup> plays a key role for induced

autophagy by the UPR, while other sources of  $\text{Ca}^{2+}$  can induce autophagy but not interaction with the UPR [144, 145].

IP3R receptor is another important cellular pathways which is involved in regulating  $\text{Ca}^{2+}$  and induced autophagy. This pathway is mTORC-dependent autophagy and ER stress through upon activation of UPR [146]. IP3R is a second messenger which is known for regulating cell survival signaling although its negative role initiating autophagy is also emerging from several experimental studies that suggest the pharmacological and genetic inhibition of IP3R induction of autophagy-independent  $\text{Ca}^{2+}$  flux [147]. The role of ER  $\text{Ca}^{2+}$  depletion (SERCA/ATPase antagonist thapsigargin) and luminal ER  $\text{Ca}^{2+}$ -stimulating compound IP3R antagonist xetospongins B, both of contradictory role, can activate autophagy. Inversely, inhibition of IP3Rs can activate autophagy signal that might be mechanically different from ER stress-attenuated autophagy. Apart from IP3Rs, RYRs have also induced autophagy. In hippocampal neuronal stem cells treated of insulin lead to increase expression of RYR3 isoform which instigate cell death through elevate induction of autophagy [148]. Accordingly, endogenous expression of RYRs in skeletal muscle cells and HEK cells segregates rat hippocampal neurons inhibit the autophagy flux particularly at the autophagosome-lysosome fusion. Inhibition of RYRs increased autophagy flux by mTORC independent pathway [149].

Under ER stress condition,  $\text{Ca}^{2+}$ -mediated autophagy is induced by known tumor inhibitor DAPK1. Activated DAPK1 mediated direct phosphorylation on BH3 domain of Beclin-1 elevated from Bcl2L1, which promotes autophagy [113]. Accordingly, under hypoxic condition, decrease synthesis of protein through PERK-eIF2 $\alpha$ -ATF4 and AMPK-mTORC1 pathway. Similarly, autophagy can be induced upon hypoxic condition, whereas  $\text{Ca}^{2+}$  influx by initiation of hypoxia and triggered CaMKK $\beta$  or CaMKK2 promotes WIPI1 and autophagosome formation [150, 151].

Many evidences suggest that cytosolic  $\text{Ca}^{2+}$  can initiate autophagy although many reports demonstrate that chelating  $\text{Ca}^{2+}$  suppresses autophagy. BAPTA-AM (1,2-bis (O-aminophenoxy) ethane-N, N, N', N'-tetraacetic acid tetra (acetoxymethyl) ester), a cell permeable  $\text{Ca}^{2+}$  buffering agent, can also suppress autophagy initiation following ER stress induced by inhibition of proteasome [152]. In many studies, stimulation of exogenous cytosolic  $\text{Ca}^{2+}$  signal and the BAPTA-AM effect on autophagy can be rational inhibition activate the influx of  $\text{Ca}^{2+}$ . In addition, BAPTA-AM effect on cell did not alter the production of IP3Rs by Vps34 but mutated the aggregation of the IP3Rs protein receptor WIPI-1 to the formation of phagophore. Likewise, BAPTA-AM was observed to suppress lysosome fusion [153]. Furthermore, BAPTA-AM inhibits initiating autophagy by experimentally increasing influx  $\text{Ca}^{2+}$  signal but blocks formation of autophagosome. In the meantime, autophagy inhibition by BAPTA-AM continuously remarks that there are some consequences using  $\text{Ca}^{2+}$  chelating agents which also defect lysosomal function followed by inhibiting degradation of autophagosome [154]. In addition, hydrolysis of the acetoxymethyl ester modification of  $\text{Ca}^{2+}$ -dependent intracellular signaling process directly involved autophagy [154]. Nevertheless, BAPTA is  $\text{Ca}^{2+}$  chelator and limitation is when  $\text{Ca}^{2+}$  enters the cell and it can be replete by the influx of  $\text{Ca}^{2+}$ . In a similar way, mobilization of intracellular  $\text{Ca}^{2+}$  led to defects in plasma membrane, resulting in the expanded interplay between lysosome and SNAREs, which are more important for membrane fusion, and thereby increase of  $\text{Ca}^{2+}$  could alleviate autophagosome-lysosome fusion, which induces autophagy [155].

Alternatively, many compounds that inhibit  $\text{Ca}^{2+}$  signaling led to an ascent of cytosolic  $\text{Ca}^{2+}$  that blocks initiation of autophagy. Particularly, voltage-operated  $\text{Ca}^{2+}$  channel antagonist and the IP3R signal can induce autophagy by suppressing activity of  $\text{Ca}^{2+}$ -sensitive protease called as calpain [156]. Calpain is activated by elevation of cytosolic  $\text{Ca}^{2+}$ . Inhibition of calpain by pharmacological calpestatin

and calpeptin or knockdown of calpain enhances autophagy flux without turbulence mTORC1 [156]. In addition, in neuronal disease cells, abnormal  $\text{Ca}^{2+}$  signal obstructs the clearance accumulation of nascent protein through inhibition of autophagy induction. Nonetheless, these studies demonstrate that calpain can suppress autophagy induction although other experimental studies suggest that the activation of calpain is essential for autophagy induction [156]. Cytosolic  $\text{Ca}^{2+}$  can activate mTORC1, which led to inhibition of autophagy induction. For instance, knockdown of TRPML1 (transient receptor potential cation channel, mucolipin subfamily, member 1) lysosomal  $\text{Ca}^{2+}$  channel inhibits mTORC1 activity. However, knockdown of TRPML1 channel reversed by thapsigargin, lead to downstream cytosolic  $\text{Ca}^{2+}$  signal activated by mTORC1 [157].

## 11. ER stress mediates autophagy in pathological condition

The UPR pathway is not always a reason for autophagy induction. When ER stress is divergent in some contagious situation, defective regulation of autophagy occurs. Notably, in some pathological conditions such as neurodegenerative, cardiovascular, and liver diseases, ER stress negatively regulates autophagy. Alzheimer disease (AD) is one of the most common neurodegenerative diseases, which is mainly caused by the accumulation of extracellular amyloid- $\beta$  ( $\text{A}\beta$ ), senile plaques, and neurofibrillary tangles protein.  $\text{A}\beta$  is originating from the cleavage of the amyloid precursor protein (APP) by two aspartic enzymes  $\beta$ -secretase (BACE1) and  $\gamma$ -secretase. This  $\gamma$ -secretase is a membrane-associated complex consisting of a presenilin-1/2 (PS1/PS2) in the ER [158]. UPR and autophagy play a key role in maintaining normal neuron against aggregation of  $\text{A}\beta$  and PS1 mutation that affect the form of AD. Many reports suggest that mutation in PS1 and accumulation of intracellular  $\text{A}\beta$  activate ER stress in neurons [159]. However, mutation of  $\text{A}\beta$  leads to upregulation of the HSPA5 (heat shock 70 kDa protein 5) expression in the neuron, which is the main neuroprotective role despite the ER stress-associated cell death and sustaining  $\text{Ca}^{2+}$  stability [160]. Interestingly, mutation of ps1 and  $\text{A}\beta$  suppresses the main arms of UPR, including IRE1 $\alpha$ , PERK, and ATF6 $\alpha$  [161]. Activation of ER stress is an early sequence of the AD, which initiates autophagy by phosphorylation of PERK-positive neuron via accumulation of MAP1LC3B induced autophagy in cardinal direction for abasement of  $\text{A}\beta$  and APP [162]. Defective regulation of autophagic function leads to AD progression; Pickford et al. report that downregulation of Beclin-1 was observed in the middle frontal lobe in the brain cortex of AD patients similar to the observation in the mouse model of AD [163]. Similarly, in Parkinson disease model, synaptic protein  $\alpha$ -synuclein ( $\alpha$ -syn) decreases accumulation of the expression of Beclin-1 gene that suppresses the induction of autophagy [164]. In addition, Huntington's diseases (HD) is also neuropathological disease condition, whereas ER stress impaired the regulation of autophagy. Knockdown of IRE1 $\alpha$ -XBP1 increases autophagy in HD model which initiates pathological condition [165, 166]. Similarly, in HD-upregulated expression, USP14 is the deubiquitinating enzyme with His and Cys domains that increase autophagic discharge of mutant HTT protein (huntingtin protein) through non-phosphorylated IRE1 $\alpha$ . Phosphorylated IRE1 $\alpha$  has not much affinity to interact with USP14, thus increasing accumulation of mutant HTT by suppressing autophagy regulation [167]. Therefore, activation of UPR will not be regulated properly as a result of negative induction of autophagy, which fails to eradicate the accumulation of contagious protein and then consequently leads to neurodegenerative diseases.

UPR and autophagy are also interconnected for inflammation of bowel in the epithelial cell. In cultured intestinal epithelial cell initiate PERK-eIF2 $\alpha$  dependent

pathway autophagy because of loss IRE1 $\alpha$  activity which intimate that UPR signal maintaining normal mechanism also conserve balance need to possible rebuttal mechanism [168]. In addition, XBP1 conditional knock in intestinal epithelial cell lead to induced autophagy in small intestinal paneth cell, essential for the formation of antimicrobial agents followed by inflammation in small intestine, which is more exacerbated when codeletion of ATG gene like ATG7 or ATG16L1. Double knockout mice XBP1<sup>-/-</sup>, ATG7<sup>-/-</sup> and XBP1<sup>-/-</sup>, ATG16L<sup>-/-</sup> demonstrate that Crohn diseases stimulate nuclear factor kappa B (NF- $\kappa$ B) in IRE1 $\alpha$ -dependent manner. Moreover, In ATG16L conditional knockout mice enhance GRP78 expression along with phosphorylation of eIF2a and activation of JNK, terminating the expression of IRE1a and increased the XBP1 spicing in intestinal glands, these circumstances increase the inflammation state, which changes the interaction between ER stress and autophagy that increases cell death, which is negative retroaction of ER stress-induced autophagy [168]. Notably, inactivation of XBP1 can induce autophagy but this UPR also can downregulate the induction of autophagy. Nevertheless, defective regulation of XBP1 integrates FoxO1 (Forkhead box O1), a transcription factor that sequentially provokes expression of many genes that positively induce autophagy [98]. The unspliced XBP1 (uXBP1) under glutamine starvation condition regulated FoxO1 depravation by interacting FoxO1 for the 20s proteasome. Similarly, this interaction between uXBP1 and FoxO1 based on phosphorylation of uXBP1 by the extracellular signal-regulated kinase 1/2 (ERK1/2), as well as spliced XBP1 (XBP1s) in overexpression which also interacted, evolved degradation of FoxO1 [169]. Accordingly, recently, the FoxO1 and XBP1 interaction in auditory cells regulates autophagy [170]. Prominently, the consistent mechanism has been proved under severe ER stress in which the UPR loses its activity, whereas it can be considered that another regulatory mechanism FoxO1 maintains the autophagy induction.

## 12. Conclusion

During the last decade, research has been conducted to determine the mechanism by which ER stress and autophagy maintain intracellular homeostasis. Here, we described the UPR and autophagy in detail with respect to their molecular mechanism and interaction between ER stress and autophagy. However, the detailed mechanism of ER stress and autophagy is yet to be fully understood. In the last few years, research has shown that the ER stress response can not only initiate autophagy but can also negatively regulate autophagy to maintain cell survival. Elucidation of the interactions between the UPR and autophagy will help in the development of novel treatments for several diseases.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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