IRE1α Signaling Pathways Involved in Mammalian Cell Fate Determination

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Abstract
A diverse array of cellular stresses can lead to accumulation of misfolded or unfolded proteins in endoplasmic reticulum (ER), which subsequently elicits ER stress. Inositol-requiring enzyme 1α (IRE1α) is the most sensitive of the three unfolded protein response (UPR) branches which are triggered to cope with ER stress in mammalian cells. IRE1α signaling is quite context-specific on account of many adaptor and modulator proteins that directly interact with it, including heat shock proteins (HSPs), RING finger protein 13 (RNF13), poly (ADP-ribose) polymerase 16 (PARP16), Bax/Bak, and Bax inhibitor-1 (BI-1). The activated IRE1α triggers different downstream pathways depending on the UPRosome formed by distinct modulator proteins. At the initial phase of ER stress, IRE1α-XBP1 axis functions as an adaptive response. While ER stress sustains or intensifies, signals shift to apoptotic responses. Furthermore, IRE1α signaling can be exploited to the development of a wide range of prevalent human diseases, with cancer the most characterized. Here we provide an overview of recent insights into the complex IRE1α signaling network which makes IRE1α an intriguing cell fate switch. Besides, the functional relevance is presented since IRE1α activation also participates in some other physiological processes beyond protein-folding status.

Introduction
IRE1α, as an evolutionarily conserved protein, has been long considered to play a crucial role in the decision of mammalian cell fate. The precise mechanisms of IRE1α signaling haven’t been fully illustrated for their complexity and diversity. Data implied that IRE1α activation was critical in promoting the survival of mouse spermatocytes after testicular hyperthermia [1]. However, a recent study revealed that IRE1α expression was...
involved in wogonin-induced apoptosis, while IRE1α knockdown abolished the cytotoxic effects of wogonin on malignant neuroblastoma cells [2]. Accordingly, IRE1α can no longer be considered simply as a positive regulator of cell survival, but a cell fate switch from pro-survival to pro-apoptotic, depending on different mechanisms under accurate regulation control.

Endoplasmic reticulum (ER) stress and the unfolded protein response (UPR)

ER is an essential organelle in eukaryotic cells responsible for the proper folding and assembly of most secretory and membrane proteins. ER is also involved in lipid biosynthesis, calcium homeostasis, cell motility, gene expression, cell cycle progression and apoptosis [3, 4].

However, the ER proteostasis may be fluctuated by a wide range of stresses, such as glucose deprivation, hypoxia, redox changes and acidosis [5-7]. Stresses causing accumulation of unfolded or misfolded proteins drive cell into a condition referred to as ER stress. To maintain the fidelity of ER functions, the cell initiates an adaptive mechanism called UPR [8]. The UPR aims to restore protein folding homeostasis at the initial phase, while this signal transduction pathway triggers apoptosis if ER stress remains unmitigated [9, 10]. In mammalian cells, the UPR is mediated by three classes of signaling components: PKR-like ER protein kinase (PERK), activating transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1). Among the three branches, IRE1 is the most evolutionarily conserved arm from yeast to human [11]. IRE1 has two isoforms: IRE1α and IRE1β; IRE1α is widely expressed in most cells and tissues while IRE1β is restricted to intestinal epithelial cells [12].

The localization, structure, and allosteric change of IRE1α

IRE1α is a 100 kDa type I ER-membrane-resident protein consisting of a luminal domain, a transmembrane domain and a cytoplasmic region. The cytoplasmic region contains a kinase domain and an endoribonuclease (RNase) domain, making IRE1α a bifunctional enzyme.

The luminal domain of IRE1α acts as a sensor of the ER unfolded protein load. Combining with the ER chaperone binding immunoglobulin-protein (Bip, also termed GRP78), the luminal domain stays in an inactive monomeric state [13]. The activation of this luminal fragment in mammalian depends on the dissociation with Bip, rather than direct interaction with unfolded proteins like yeast IRE1. As for the transmembrane domain, very little has been known about its functional and structural roles thus far. But a recent study revealed the possibility that it might manipulate the conformation of cytosolic domain [14]. The kinase domain of IRE1α serves as a substrate for IRE1α trans-autophosphorylation and provides ATP-binding pocket [15, 16].

Autophosphorylation of the kinase domain and binding of ADP (or ATP in vivo) allosterically regulate dimerization/oligomerization and lead to IRE1α RNase activation [15, 17]. Previously, IRE1α’s kinase phosphotransfer function was considered to be essential for RNase activation. However, recent evidence has been shown that it is the conformational change in the kinase domain triggered by ligand binding to the ATP binding pocket that leads to the activation of IRE1α, rather than phosphotransfer per se. In the presence of an ATP mimetic (1NM-PP1), drug-sensitized IRE1α bypasses mutation that lacks its kinase activity and induces a full UPR [18]. Other ligands that interact with the ATP-binding site of wild-type IRE1α also activate the RNase directly. For example, a potent small molecule, IPA, binds to IRE1α’s ATP-binding pocket and predisposes the kinase domain to oligomerization, activating its RNase [19]. Notably, the kinase domain being in its active conformational state (DFG/αC-in conformation) is required for IRE1α’s activation [20].
Proteins interacting with IRE1α

The IRE1α protein complex is a dynamic scaffold where many regulatory components assemble [21]. To demonstrate the platform modulating the activation status of IRE1α, the concept “UPRosome” emerged [22]. This term refers to a macromolecular complex comprising IRE1α and modulator proteins directly interacting with it. A lot of modulator proteins have been discovered, and more interactors are being identified. Different UPRosome elicits different downstream signal and leads to different cell fate.

Heat shock protein (HSP)

HSPs are characterized as stress-induced molecular chaperones performing cell survival functions, classified according to their approximate molecular weight [23]. To date, HSP70 (also named HSP72) and HSP90 have been found to interact directly with IRE1α and initiate downstream signal.

HSP70 has strong cytoprotective effects related to its ability to inhibit apoptosis. Binding of HSP70 to IRE1α enhances IRE1α RNase activity, promoting adaptation to ER stress and facilitating cell survival [24]. Specific silence of HSP70 abrogated ER stress-induced upregulation of IRE1α and promoted cell death [25]. This finding provides direct evidence that HSP70 is indispensable for cell adaptation and survival under ER stress.

HSP90 is a protein abundant in cytoplasm that functions with its cochaperone Cdc37. The modulation of HSP90 on IRE1α protein stability was demonstrated early in 2002 [26]. Later research showed that Cdc37 knockdown results in an increased phosphorylation and oligomerization of IRE1α, whereas no effect on IRE1α protein stability is found [27]. This evidence indicates that HSP90 regulates IRE1α oligomerization and auto-phosphorylation in a Cdc37-dependent manner, while the regulation of IRE1α protein stability is independent of Cdc37.

RING finger protein 13 (RNF13)

RNF13 is a recently identified ubiquitin ligase which belongs to Goliath family and is highly enriched in ER membrane [28]. As an evolutionarily conserved protein, RNF13 contains an N-terminal protease-associated domain and a C-terminal RING finger domain [29, 30].

The interaction between RNF13 and IRE1α enhances the stability of IRE1α. The intact RING domain is indispensable in mediating this interaction. Acting as a regulator of IRE1α, RNF13 plays an important role in ER stress-mediated apoptosis. Knockdown approach proved that RNF13 facilitates the activation of IRE1α-TRAF2 signaling, which further promotes JNK activation and apoptosis [31].

Poly (ADP-ribose) polymerase 16 (PARP16)

PARPs were found to regulate DNA damage repair and the cytoplasmic stress response [32]. Human PARP16 (also known as ARTD15) is a tail-anchored ER transmembrane protein with a cytosolic catalytic domain. The luminal C-tail of PARP16 is required for its function, indicating that stress signals may be transduced from ER domain to cytosolic domain [33].

During ER stress, PARP16 enzymatic activity is upregulated. Co-immunoprecipitation assay demonstrates a robust association between PARP16 and IRE1α both in the presence and absence of ER stress. The ADP-ribosylation caused by PARP16 increases the kinase and RNase activities of IRE1α. Under ER stress, PARP16 could facilitate Bip dissociation from IRE1α. This effect was impaired in PARP16 knockdown, accompanied by an increase of cell death [34], suggesting PARP16 is involved in cell survival.

Bax/Bak and Bax inhibitor-1 (BI-1)

Bax and Bak are pro-apoptotic Bcl-2 family members that initiate mitochondrial dysfunction, but also localize to ER and modulate steady-state calcium homeostasis [35]. Bax and Bak form a protein complex with the cytosolic domain of IRE1α that is essential
for IRE1α activation. This function is independent of their proapoptotic function at the mitochondria. ER stress contributes to an increased association between Bak and IRE1α. Since BH3 and BH1 domains of Bak are necessary for the interaction, the IRE1α signal may be abolished once the interaction is disrupted [36].

Intriguingly, BI-1, an ER-resident protein suppressing cell death, is also found to form a UPRosome. BI-1-deficient cells showed hyperactivation of IRE1α, especially in RNase activity, suggesting the inhibition effect of BI-1 on IRE1α signaling [37]. This effect may be related to the competence for a similar binding site from Bax and Bak.

Besides the proteins described above, there are some other possible IRE1α interactors uncovered very early, including Ptp-1b [38], Nck1 [39], Aip-1 [40], Rack1 [41], and Bim/Puma [42].

**The action modes of IRE1α**

IRE1α is a key component of cell fate switch. XBP1 splicing promotes cell survival, whereas selective activation of regulated IRE1α-dependent decay (RIDD) initiates apoptosis [43, 44]. Besides, IRE1α is also able to cleave several microRNAs, which may lead to an enhancement in cell death. Furthermore, IRE1α-TRAF2 axis elicits apoptosis as well. The switch between anti-apoptotic and pro-apoptotic signaling may be dependent upon the conformational state of IRE1α.

**IRE1α-XBP1 pathway**

X-box binding protein 1 (XBP1) is a positively acting transcription factor within the cAMP Response Element Binding Protein/Activating Transcription Factor (CREB/ATF) family [45]. It comprises a transcriptional activation domain and a sequence-specific DNA-binding domain [46].

The activated IRE1α cleaves a 26-nucleotide intron from the unspliced form of XBP1 (XBP1u) mRNA, and then the cleaved exons are ligated by RtcB to produce an active transcription factor—XBP1s [47]. XBP1s translocates into the nucleus and binds to the promoters of its target genes such as those encoding molecular chaperones and proteins contributing to ER-associated degradation (ERAD). By enhancing their expression, XBP1s functions as a master coordinator of the adaptive UPR [48]. When ER stress subsides, the remaining XBP1s in the nucleus leads to further production of XBP1u. XBP1u shuttles between the nucleus and the cytoplasm, forming a complex with XBP1s. The complex is rapidly degraded by proteasomes owing to the degradation motif in XBP1u [49, 50]. In other words, XBP1u acts as a negative feedback regulator of XBP1s. A recent study also proposed that in the IRE1α-XBP1 pathway, XBP1s works as an anti-apoptotic protein while XBP1u functions as a pro-apoptotic one [51].

**Regulated IRE1α-dependent decay (RIDD)**

When the attempt to rebalance ER homeostasis fails, IRE1α reinforces the process of cleaving a subset of mRNAs encoding ER-targeted proteins [52, 53]. This cleavage leading to the degradation of mRNAs is termed of RIDD [54]. RIDD is a default pathway for ER-localized mRNA decay and that a cleavage site with a consensus sequence (CUGCAG) and the presence of an XBP1-like stem-loop secondary structure in target mRNAs are required [55]. Nevertheless, many mRNAs that possess XBP1-like cleavage sites are not the target for RIDD [56].

XBP1 cleavage and RIDD are uncoupled. Since the RNA binding and/or RNase catalytic residues in IRE1α are different, these two outputs use the same catalytic residues but different substrate binding sites. As a result, they would not compete with each other. Previous model proposed that higher ordered structures were assigned to the RIDD active form of IRE1α [57]. However, a recent study suggested that IRE1α oligomer generates a catalytically active
IRE1α unit for XBP1 mRNA splicing, while the RIDD substrates cleavage is within IRE1α monomer or dimer [58].

Until now, 37 putative RIDD substrates have been reported, including IRE1α itself [59, 60]. The RIDD-target mRNAs encode 64% ER-localized/secretory proteins and 36% cytosolic proteins [59].

A basal RIDD activity of IRE1α is necessary for ER homeostasis maintenance. Nonetheless, it turns different when homeostasis is disrupted. The RIDD activity is upregulated proportionally with stress intensity or duration, finally leading to apoptosis by degrading pro-survival protein-encoding mRNAs under irremediable ER stress [61, 62]. Moreover, RIDD activation without XBP1 splicing induces more predominant cell death.

**MicroRNA (miRNA)**

MicroRNAs are generally described as negative regulators of gene expression. It is important to note that IRE1α is able to degrade several pre-miRNAs (miRs -17, -34a, -96, and -125b), though the exact downstream mechanisms of these miRNAs are not fully understood. Upton and colleagues reported that sustained IRE1α RNase activation promotes the cleavage of select miRNAs that normally repress translation of caspase 2 mRNA, resulting in induction of apoptosis [63]. Because the outer nuclear membrane is linked with ER, pre-miRNAs could be degraded as they transit through the nuclear pore when encountering IRE1α. IRE1α-dependent reduction of miR-17 causes cell death by increasing thioredoxin-interacting protein (TXNIP) mRNA stability, which in turn activates the NLRP3 inflammasome, causing procaspase 1 cleavage and interleukin 1β (IL-1β) secretion [64].

**IRE1α-TRAF2 axis**

During prolonged ER stress, however, independent of the above-mentioned signaling pathways, IRE1α recruits tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2) and apoptosis signal-regulating kinase 1 (ASK1). IRE1α-TRAF2-ASK1 complex triggers the activation of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase signaling pathways [65]. Activated JNK translocates to the mitochondrial membrane and promotes the activation of Bim and the inhibition of Bcl-2 [66], resulting in mitochondrial signaling apoptosis, while p38 activates CHOP via phosphorylation of its transactivation domain. CHOP can induce transcriptional activation of genes that ultimately lead to cell death [67, 68].

In addition, the association of IRE1α and TRAF2 possibly promotes clustering and activation of procaspase 12, which subsequently activates caspase 12. Caspase 12 then activates caspase 9, which in turn activates caspase 3 and induces apoptosis [69].

**Involvement of IRE1α signaling in human diseases**

Accumulating evidence supports the idea that altered IRE1α function is implicated in various human diseases such as cancer, diabetes, and neurodegenerative disorders. One of the major areas of active research in IRE1α-associated human diseases is cancer, which is characterized by rapidly proliferating cancer cells in need of increased protein synthesis.

Evidence is emerging that IRE1α plays a key regulatory role in cancer initiation, growth, metastasis, and angiogenesis. IRE1α gene is one of the kinases most frequently mutated in various cancers [70, 71]. Using a dominant-negative IRE1α mutant, glioblastoma cells display a lower proliferation rate and exhibit a reduction in angiogenesis [72]. Importantly, many mutations in IRE1α downstream targets have also been discovered, which concomitantly contribute to cancer development. IRE1α-XBP1 branch is essential for the survival and growth of tumor cells into solid tumors, providing an adaptive capacity to adverse micro environmental conditions [73]. An overexpression of XBP1 has been confirmed in breast, lung and pancreas cancers [74]. Conversely, cells deficient in XBP1 show a large reduction in their ability to form solid tumors in nude mice [75]. The RIDD pathway may also be involved...
The important role of IRE1α signaling in cancer progression presents a potential therapeutic target in anti-cancer therapy. Inhibitors specifically inhibiting IRE1α endonuclease activity and XBP1 splicing show significant anti-cancer effects and provide a potential therapeutic option in multiple myeloma [76, 77]. And the anti-cancer effect of CXC195 on urothelial carcinoma lies on the activation of IRE1α and IRE1α-TRAF2-ASK1 complex induced apoptosis [78].

Other physiological functions of IRE1α signaling

Increasing evidence reveals the unexpected functions of IRE1α in many physiological processes far outside the realm of protein misfolding, including lipid metabolism, cell differentiation, inflammation and energetic regulations. In this section, we delineate the former two aspects.

Lipid regulation

IRE1α is also implicated in the regulation of lipid metabolism via two distinct mechanisms. First, the IRE1α downstream target XBP1 can regulate expression and activities of key enzymes in phospholipid biosynthesis and contribute to ER membrane expansion [79]. Selective deletion of XBP1 in liver results in a decreased production of lipids [80]. 3T3-L1 cells with knockdown of IRE1α or XBP1 exhibit dramatic defects in adipogenesis [81]. Second, some mRNAs encoding proteins involved in the de novo lipogenesis, hydrolysis of cholesterol ester and triglyceride, and lipoprotein catabolism are regulated by RIDD pathway. XBP1 deficiency triggers feedback activation of IRE1α and enhances the RIDD. Suppression of RIDD by IRE1α ablation reverses hypolipidemia in XBP1-deficient mice [44]. Together, these observations reveal that direct targeting of either IRE1α or XBP1 might be a promising strategy to treat dyslipidemias.

Notably, IRE1α is both a sensor of primary lipid perturbations and a regulator of lipid homeostasis. The direct measure of Kar2p (the yeast homologue of Bip) mobility suggested that lipid imbalance can lead to the activation of yeast IRE1, independently of luminal unfolded proteins [82]. In mammalian cells, mutant IRE1α lacking luminal unfolded protein stress-sensing domain, nonetheless retains responsiveness to increased lipid saturation, occurring dependently of the transmembrane domain [83].

Cell differentiation

Intact IRE1α-XBP1 relay is required for the development of plasma cells, and the RIDD participates in this process as well. Observation demonstrated that XBP1 is the transcription factor to be selectively and specifically required for the transition of mature activated B cells to antibody secreting plasma cells [84]. Indeed, XBP1 contributes to the expression of many proteins that lead to the phenotypic changes characterizing secretory cells, such as expansion of ER and induction of enzymes and chaperones [85]. The mRNA of secretory μ chain is one of the IRE1α substrates. In the absence of XBP1, the IRE1α’s RIDD potential is unleashed and the synthesis of secretory Ig-μ chain (μs) proteins is compromised [86]. Thus, the antibody level is even further restored when both XBP1 and IRE1α are deficient. In addition, IRE1α signaling also mediates adipocyte differentiation [81].

Concluding remarks

Increasing attention has been given to ER stress for the roles it plays in many diseases, including diabetes [87], cardiovascular diseases [88], cancer [89], inflammation [90] and neurodegeneration [91]. IRE1α is the most sensitive UPR branch in mammalian cells upon in tumor development since 68% identified substrates are associated with cancer [59].
ER stress. Once an ER stress is resolved, the IRE1α activity is the first to be terminated, followed by ATF6 and PERK [92].

IRE1α signaling involved in the determination of mammalian cell fate is highly regulated in a context-specific manner (Fig. 1). A complex array of modulator proteins interact directly with IRE1α and form different "UPRosome" which subsequently activate IRE1α. The IRE1α-XBP1 pathway promotes cell survival, while regulated IRE1α-dependent decay (RIDD), degradation of certain miRNAs, and IRE1α-TRAF2 axis promote apoptosis.

Fig. 1. Overview of IRE1α signaling pathways involved in mammalian cell survival and apoptosis. In response to ER stress, IRE1α disassociate with chaperone protein Bip and then dimerize or oligomerize. Many adaptor proteins directly interact with IRE1α and form different "UPRosome" which subsequently activate IRE1α. The IRE1α-XBP1 pathway promotes cell survival, while regulated IRE1α-dependent decay (RIDD), degradation of certain miRNAs, and IRE1α-TRAF2 axis promote apoptosis.

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**Disclosure Statement**

We confirm that there is no potential conflict of interest or financial dependence regarding this paper.

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857

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