

Endoplasmic reticulum stress as target for treatment of hearing loss

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ABSTRACT

The endoplasmic reticulum (ER) plays pivotal roles in coordinating protein biosynthesis and processing. Under ER stress, when excessive misfolded or unfolded proteins are accumulated in the ER, the unfolded protein response (UPR) is activated. The UPR blocks global protein synthesis while activates chaperone expression, eventually leading to the alleviation of ER stress. However, prolonged UPR induces cell death. ER stress has been associated with various types of diseases. Recently, increasing evidences suggest that ER stress and UPR are also involved in hearing loss. In the present review, we will discuss the role of ER stress in hereditary hearing loss as well as acquired hearing loss. Moreover, we will discuss the emerging ER stress-based treatment of hearing loss. Further investigations are warranted to understand the mechanisms in detail how ER stress contributes to hearing loss, which will help us develop better ER stress-related treatments.

Keywords: ER stress · Unfolded protein response (UPR) · Hearing loss · Inner ear · Cochlea

1. Introduction

The endoplasmic reticulum (ER) is a highly dynamic organelle in eukaryotic cells, playing important roles in protein synthesis, processing, folding, and transportation, as well as lipid synthesis and calcium homeostasis. Newly synthesized transmembrane and secretory proteins must be folded and processed in the ER before being targeted to their final destinations. Misfolded proteins will be folded into correct conformations in the ER with the help of chaperon proteins such as binding immunoglobulin protein (BiP)/glucose regulated protein 78 (GRP78) (1). Alternatively, misfolded proteins are subjected to degradation through the proteasome-dependent ER-associated protein degradation (ERAD) pathway (2). Physiological or pathological conditions such as hypoxia, acidosis, or calcium fluxes can disturb ER homeostasis and result in an accumulation of unfolded or misfolded proteins in the ER, commonly referred to as ER stress. To alleviate ER stress, the so-called unfolded protein response (UPR) is activated, which blocks protein synthesis and activates chaperone gene expression (3).

Three main UPR pathways have been identified so

far, which are mediated by ER stress sensors that reside on the ER membranes, namely the inositol-requiring enzyme 1 α (IRE1 α), the PKR-like ER kinase (PERK), and the activating transcription factor 6 α (ATF6 α) (Figure 1). These sensors are all transmembrane proteins that are normally inactivated by BiP binding at the ER lumen side. Under ER stress, accumulated unfolded proteins sequester BiP from the ER stress sensors and activate UPR through three independent pathways: (i) Upon release from BiP, IRE1 α oligomerizes and trans-autophosphorylates itself, which then activates its endoribonuclease activity. Activated IRE1 α catalyzes the splicing of *XBPI* mRNA into *XBPIs* that encodes an active transcription factor. *XBPIs* then enters the nucleus and activates gene expression involved in ER membrane biogenesis and protein folding. (ii) Similar to IRE1 α , PERK obtains its kinase activity through oligomerization and trans-autophosphorylation after being released from BiP. After activation, PERK phosphorylates the α subunit of eukaryotic translational initiation factor 2 α (eIF2 α) on Ser51. Phosphorylated eIF2 α attenuates global protein synthesis to reduce the ER protein-folding load. Meanwhile, it enhances the translation of certain proteins such as activating transcription factor 4 (ATF4). ATF4 enters the nucleus and induces gene expression that are involved in ER function and reactive oxygen species (ROS) production. (iii) Upon release from BiP, ATF6 α translocates from the ER to the Golgi apparatus. At the

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Golgi, site-1 protease (S1P) and site-2 protease (S2P) sequentially cleaves ATF6 α , releasing the transcription-activating form of ATF6 α that enters the nucleus and induces ER chaperon gene expression.

The activation of UPR pathways usually leads to the clearance of unfolded proteins and the restoration of ER homeostasis through reducing global protein synthesis and increasing chaperon protein expression. However, cell death occurs under prolonged or excessive ER stress when the ER protein load greatly exceeds its folding capacity (4). One of the UPR target genes encodes C/EBP homologous protein (CHOP)/growth arrest and DNA damage-inducible gene 153 (GADD153) (5). As a transcription factor, CHOP activates pro-apoptotic gene expression encoding growth arrest and DNA damage-inducible 34 (GADD34), death receptor 5 (DR5), endoplasmic reticulum oxidoreductase-1 (Ero1 α), and Bim (6). CHOP also represses anti-apoptotic gene *Bcl-2* expression (7). Other possible cell death pathways induced by ER stress include activation of apoptotic-signaling kinase-1 (ASK1) and p38 MAPK downstream of IRE1 α (6). Currently, one intriguing question that requires further investigation is what determines the pro-survival versus pro-death role of ER stress.

ER stress is involved in various diseases, ranging from cancer, diabetes, metabolic syndromes, to

neurodegenerative diseases (8). Recently, the role of ER stress in hearing loss has attracted increasing research attention. Hearing loss (deafness) is the most prevalent sensory impairment in humans, affecting around 466 million worldwide (9). Both genetic and environmental factors contribute to hearing loss. Hereditary hearing loss is clinically divided into non-syndromic hearing loss and syndromic hearing loss, depending on the presence of other symptoms besides deafness. Mutations in more than 100 genes have been identified to be responsible for non-syndromic hearing loss, and it is estimated that additional hundreds of genes are involved in hereditary hearing loss. Environmental factors, such as exposure to ototoxic chemicals, noise and ageing, lead to drug-induced hearing loss (DIHL), noise-induced hearing loss (NIHL) and age-related hearing loss (ARHL), respectively. ARHL (also referred to as presbycusis) is especially important today, affecting nearly one-third of individuals over 65 years of age (9). In this review we will discuss the role of ER stress and its therapeutic potentials in both hereditary and acquired hearing loss.

2. ER stress and hereditary hearing loss

2.1 Wolfram syndrome 1 (WFS1)

WFS1, also called wolframin, is a transmembrane protein

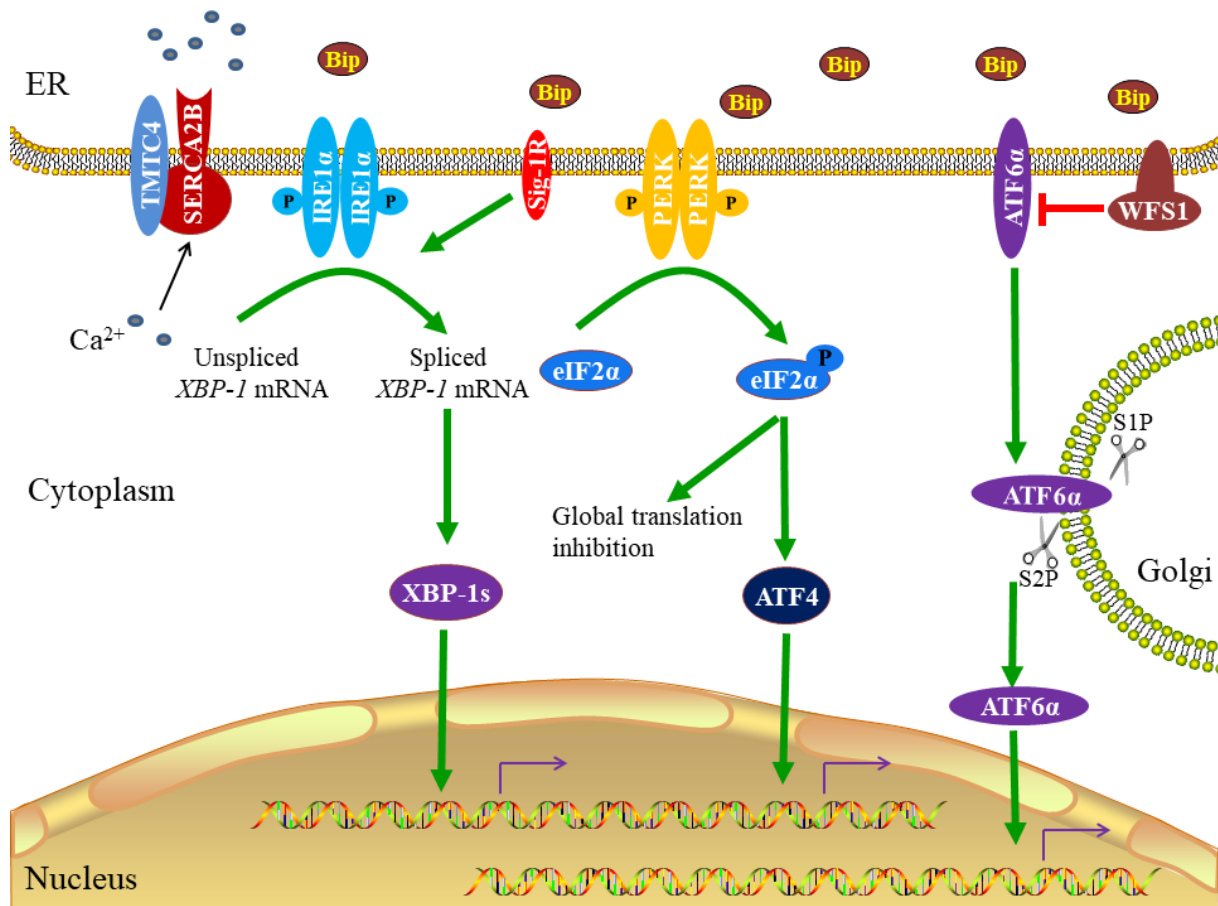


Figure 1. Endoplasmic reticulum (ER) stress and unfolded protein response (UPR) in mammals. See main text for details.

that localizes on the ER membrane. WFS1 interacts with the ER-localized vacuolar-type H⁺-ATPase V1A subunit (ATP6V1A), Na⁺/K⁺ ATPase 1 subunit (ATP1B1), and calmodulin (CaM), suggesting that it might play important roles in ER function and/or homeostasis (10-12). Under ER stress, IRE1 α and PERK pathways induce the expression of WFS1, which in turn recruits ATF6 α to E3 ligase HMG-CoA reductase degradation protein 1 (HRD1) for proteasomal degradation, therefore provides a negative feedback loop of UPR (13, 14).

Mutations in the *WFS1* gene cause syndromic deafness Wolfram syndrome (WS) or non-syndromic deafness DFNA6/14/38 (15-18). WS is characterized by diabetes insipidus, diabetes mellitus, psychiatric illness, optic atrophy, and hearing loss, and is mostly caused by recessive *WFS1* mutations (15, 16). In contrast, DFNA6/14/38 is caused by dominant *WFS1* mutations (17, 18). *In vitro* studies showed that WS-associated *WFS1* dominant mutants induce constitutive ER stress (19, 20). Moreover, in *Wfs1* knockout mice or rats, elevated ER stress could be detected in pancreatic β -cells and retinal cells, consistent with its negative regulatory role in ER stress (21-24).

In the mouse inner ear, WFS1 is widely expressed in hair cells (HCs), spiral ganglion cells (SGCs), supporting cells (SCs), and stria vascularis marginal cells (25). Although *Wfs1* knockout mice or rats develop diabetes and optic atrophy, hearing phenotypes have not been reported in these animals. Recently, WFS1 expression was examined in the cochlea of marmoset (*Callithrix jacchus*), which is a nonhuman primate (26). The results showed that besides those cell types reported in the mouse study, WFS1 immunoreactivity was also detected in the stria vascularis basal cells. The differential expression pattern of WFS1 might help to explain the different hearing phenotypes in *WFS1* deficient rodents and primates. Development of primate models would be helpful for understanding the role of WFS1 in hearing and deafness.

A smaller portion of WS is caused by recessive mutations in the gene that encodes CDGSH iron-sulfur domain-containing protein 2 (CISD2) (27). CISD2, also named as ERIS or Miner1, is an integral membrane protein and localizes on the mitochondria-associated ER membranes (MAMs). Unlike WFS1, the function of CISD2 still remains poorly defined. Nevertheless, CISD2 has been suggested to regulate Ca²⁺ homeostasis and ER stress. *Cisd2*^{-/-} mouse embryonic fibroblasts (MEFs) show dysregulated Ca²⁺ homeostasis and elevated ER stress (28). Similarly, fibroblasts from a WS patient with homozygous *CISD2* mutation (Asn72Ser) show disturbed cellular Ca²⁺ homeostasis and expanded ER compartment, although no overt ER stress phenotype (29). Further investigations are warranted to understand the role of CISD2 in ER stress as well as hearing loss in more detail.

2.2 Transmembrane and tetratricopeptide repeat containing 4 (TMTC4)

TMTC4 is an ER transmembrane protein and is suggested to play important roles in regulating Ca²⁺

dynamics (30). TMTC4 interacts with the Ca²⁺ pump SERCA2B and is involved in maintaining the Ca²⁺ gradient between the cytoplasm and the ER. Inactivation of *Tmtc4* in mice causes increased ER stress and UPR, possibly through dysregulation of ER Ca²⁺ level (30).

TMTC4 is expressed in the HCs and various SCs in the mouse cochlea (30). Mutation in the *TMTC4* gene has not been associated with any diseases including hearing loss in human. However, *Tmtc4* knockout mice show progressive HC loss that leads to early onset hearing loss (30). Consistent with the proposed role of TMTC4 in Ca²⁺ homeostasis and ER stress, cochlear cells of *Tmtc4* knockout mice show enhanced sensitivity to ER-induced apoptosis. In line with this, disruption of *Chop* gene partially improves the auditory function of *Tmtc4* knockout mice (30).

2.3 Cadherin 23 (CDH23)

CDH23 is a large atypical cadherin, consisting of 27 extracellular cadherin repeats, a single transmembrane domain, and a short cytoplasmic part. *CDH23* gene mutations cause syndromic hearing loss Usher syndrome (USH) 1D or non-syndromic hearing loss DFNB12 (31-33). USH is the most common inherited deaf-blindness, and so far ten genes have been associated with USH (34). These genes encode the so-called USH proteins, which bind to each other and play pivotal roles in the stereocilia and ribbon synapses of the HCs.

Evidences suggest that before being transported to the plasma membrane, CDH23 is preassembled into a complex with other USH proteins harmonin and MYO7A at the ER in zebrafish HCs (35). Disruption of the complex induces ER stress characterized by expanded ER membrane and elevated BiP expression, which eventually leads to HC apoptosis (35). These results led to the hypothesis that USH proteins are transported from the ER to their destinations as a protein complex. When one USH protein is defective or missing, other complex components are exposed abnormally and recognized as misfolded proteins, hence triggering ER stress (35).

The involvement of deficient CDH23 in ER stress is further supported by a mutant *Cdh23* mouse line *erlong* (*erl*) with a point mutation T208C. The *erl* mice suffer HC loss that eventually leads to early-onset progressive deafness (36). Further investigation showed that the mutant CDH23 failed to reach the stereocilia. Instead, it colocalized with BiP in the subapical regions of HCs (37). This may activate the PERK-eIF2 α -ATF4-CHOP pathway, which eventually leads to HC apoptosis (37). Inactivation of the *Chop* gene, treatment with ER stress modulator salubrinal, chemical chaperone 4-phenylbutyrate (PBA) or tauroursodeoxycholic acid (TUDCA) preserves HCs and delays the progression of hearing loss in the *erl* mice (37-39).

2.4 Connexins

Connexins are a family of membrane-spanning proteins, acting as the building blocks of gap junctions. By connecting the cytoplasm of adjacent animal cells,

gap junctions provide direct intercellular communication for exchange of ions, metabolites, and second messengers. More than 20 mammalian connexin genes have been identified, whose mutations are responsible for various diseases such as peripheral neuropathy, skin disease, cataracts, and hearing loss (40).

In the cochlea, gap junctions are broadly present in the non-sensory cells including the SCs, the stria vascularis, the spiral limbus, and the spiral ligament (41). Mutations in the *GJB2* gene that encodes connexin 26 (Cx26) are responsible for ~50% of non-syndromic hearing loss (42, 43). Moreover, mutations in *GJB6* (Cx30) and *GJB3* (Cx31) are also associated with non-syndromic hearing loss (44-46). Other deafness-related connexins include Cx29 (GJC3) and Cx43 (GJA1). Although mutations in *GJC3* and *GJA1* have not been clearly associated with hearing loss in humans, auditory function is affected in *Cx29* or *Cx43* mutant mice (47-49).

A possible link has been proposed connecting connexin expression and/or function with ER stress. Treating cultured mesangial cells with ER stress inducers leads to decreased Cx43 expression and reduced gap junctional intercellular communication (50). Therefore, gap junctions might protect cells under ER stress by preventing 'stress' signals from transmitting to adjacent cells (50). On the other hand, connexin gene mutations could induce ER stress. When overexpressed in cultured cells, several Cx26, Cx30 and Cx31 deafness-associated mutants are trapped in the ER instead of being localized on the plasma membrane (51-53). Moreover, Cx31R180X- and Cx31E183K-overexpressing cells show elevated BiP/GRP78 expression, indicating elevated ER stress (51). However, ER stress is not elevated in cultured cells overexpressing deafness-associated Cx31 (66delD), suggesting that ER stress is not the sole underlying mechanism of mutant Cx31-associated cell death (54).

2.5 Elongator acetyltransferase complex subunit 3 (ELP3)

Elongator complex plays a pivotal role in regulating protein translation efficiency through modifying the wobble uridine (U34) in the anticodon of various tRNAs (55). This protein complex consists of two sets of six subunits ELP1-ELP6, among which ELP3 acts as the enzymatic core (56). Elongator-mediated tRNA modifications ensure fidelity and efficiency of protein translation, which are essential to normal proteostasis (57, 58). Elongator complex also plays important roles in α -tubulin acetylation, transcriptional elongation, actin organization, kinase signaling, etc., and dysfunction of this complex is associated with various neurological diseases (59).

Conditional knockout of *Elp3* gene in the cortical neurons decreases translation rates and activates the PERK-eIF2 α -ATF4 pathway, eventually leading to microcephaly (60). In the cochlea, ELP3 is abundantly expressed in the SGNs and nascent HCs (61). Conditional knockout of *Elp3* gene in the cochlea causes protein

misfolding and aggregation, resulting in apoptosis of SGNs and defects in cochlear planar cell polarity (PCP), and eventually leading to severe hearing loss (61). Activation of ER stress was not examined in the cochlea of *Elp3* knockout mice. Nevertheless, chemical chaperone PBA treatment alleviates protein aggregation and PCP deficits in *Elp3* knockout mice, implying that ER stress is likely involved in ELP3-associated hearing loss (61).

3. ER stress and acquired hearing loss

3.1 ER stress and DIHL

Ototoxic chemicals could lead to 'drug-induced' hearing loss (62). Studies in animals and cultured cells suggest that ER stress could be induced by ototoxic chemicals such as aminoglycosides, cisplatin, N-acetyl-para-aminophenol (APAP), 3-nitropropionic acid (3-NP), and N-acetyl-p-benzoquinoneimine (NAPQI) (63-67). Additionally, ER stress activator tunicamycin treatment causes profound hearing loss in rats (68). Further investigations suggest that these chemicals affect different cell types in the cochlea. For example, aminoglycoside gentamicin induces ER stress in SGCs but not HCs, whereas tunicamycin induces ER stress in both HCs and SGCs (65, 68).

Calreticulin (CRT) is an ER-residing chaperone induced under ER stress (69). In the inner ear, CRT is expressed in the HCs and the stria marginal cells, and could bind aminoglycosides such as gentamicin (70), which inhibits the chaperon activity of CRT (71). *Crt* knockout or knockdown MEFs are more susceptible to gentamicin treatment, consistent with a protective role of CRT against gentamicin-induced cytotoxicity (70). CRT has been identified as one of the cisplatin-binding proteins in a screen, which also identified GRP78/BiP albeit at relatively low abundance (72). The significance of CRT and GRP78/BiP binding to gentamicin and cisplatin in hearing and deafness requires further investigation.

Consistent with the potential role of ER stress in DIHL, *Xbp1*^{+/-} mice are more susceptible to aminoglycoside-induced hearing loss compared to wild-type mice (65). Furthermore, chemical chaperone TUDCA attenuates aminoglycoside-induced hearing loss in *Xbp1*^{+/-} mice (65). In addition, it was recently shown that TUDCA treatment also exerted a protective effect on cisplatin-induced hearing loss (66).

3.2 ER stress and NIHL

High-intensity noise could cause HCs and SGNs death, which eventually leads to the so-called NIHL (73). Expression levels of BiP/GRP78, XBP1s, CHOP/GADD153, and caspase-3 are elevated in the cochlea of guinea pigs or mice during NIHL, suggesting that ER stress might play a role in this process (30, 74). Integrated stress response inhibitor (ISRIB) inhibits the PERK-eIF2 α -ATF4 pathway through activating the guanine nucleotide exchange factor (GEF) eIF2B (75, 76). Treatment of mice with ISRIB prior to noise exposure preserves HCs and improves hearing (30).

Sigma-1 receptor (Sig-1R) interacts with BiP at the MAMs,

and regulates Ca^{2+} signaling and cell survival (77). Under ER stress, Sig-1R expression is increased via the PERK-eIF2 α -ATF4 pathway and inhibits cell apoptosis (78). Further investigations have shown that Sig-1R executes protective function through activating the IRE1 α -XBP1 pathway and inhibiting CHOP expression (79, 80). Consistent with the protective role of Sig-1R in hearing loss, noise-induced cell death and hearing loss are significantly reduced in mice by treatment with Sig-1R agonist cutamesine (SA4503) (81).

Glucocorticoid-induced leucine zipper (GILZ) is a transcription factor that has been shown to protect cells from apoptosis (82-84). Under ER stress, overexpression of GILZ up-regulates BiP and down-regulates CHOP, ATF4, and XBP1s, and protects cells from apoptosis through a pathway involving mitochondrial function and oxidative phosphorylation (OXPHOS) (85). Recently, it was suggested that GILZ had similar protective effect in NIHL. Overexpression of GILZ protects rats from NIHL through increasing BiP and Bcl-xL, and decreasing CHOP, Bax, and cleaved caspase-3, whereas GILZ silencing has the opposite effect (86).

3.3 ER stress and ARHL

ARHL, also called presbycusis, is more and more common nowadays, affecting nearly one-third of individuals over 65 years of age (9). There are evidences suggesting that ER stress is also involved in ARHL. For example, BiP/GRP78 expression is decreased, whereas CHOP expression is increased in the cochlea of aged mice (87). Consistently, cleaved caspase-3 and caspase-9, but not caspase-12, are elevated in the cochlea of aged mice, indicating the activation of apoptosis (87).

Geranylgeranylacetone (GGA) is a nontoxic acyclic isoprenoid compound with protective function through increasing the expression of HSP70 (88). It has been shown that GGA treatment attenuates ARHL in mice (89). Moreover, GGA could ameliorate 3-NP-induced deafness as well as NIHL (90, 91). The protection of auditory function by GGA has been attributed to its activity as a HSP70 inducer. However, GGA could also induce BiP expression and enhance ER stress (92-94). The potential role of ER stress in GGA-mediated auditory protection awaits further examination.

4. ER stress-based treatment of hearing loss

Many small molecules are able to interfere with ER stress and provide protection for cells, while only a few of them have been tested in treatment of hearing loss. We will discuss these potential ER stress-based treatment of hearing loss in three categories: (1) restoring ER homeostasis; (2) modulating the PERK-eIF2 α -ATF4 pathway; and (3) modulating the IRE1 α -XBP1 pathway. As mentioned above, HSP70 inducer GGA could also induce BiP expression and enhance ER stress, and has been tested in hearing loss treatment in several animal experiments. At present, the specific target of GGA in ER stress remains elusive.

4.1 Restoring ER homeostasis with chemical chaperones

Chemical chaperones are small chemical compounds that could improve ER folding capacity and restore ER homeostasis, hence are extensively used to reduce ER stress. Unfolded or misfolded proteins are kept from aggregation by chemical chaperones through the interaction between the hydrophobic regions of each other (95). TUDCA and PBA are two most commonly used chemical chaperones. They could reduce aggregate accumulation and revert ER stress, and have been approved by the Food and Drug Administration (FDA) for clinical uses.

TUDCA is a taurine-conjugated derivative of ursodeoxycholic acid (UDCA), which used to be isolated from black bear gallbladders but can now be synthesized chemically. TUDCA has been widely used in experimental and clinical treatments of diabetes, liver disease, and neurodegenerative diseases (95). Recently, it was also tested in treatment of hearing loss. TUDCA treatment preserves HCs and delays the progression of hearing loss in *Cdh23* mutant mice *erl* (38). Moreover, TUDCA treatment also shows protective effects against cisplatin- or aminoglycoside-induced hearing loss in rodents (65, 66, 96).

First synthesized a century ago, PBA has been approved by the FDA in the treatment of urea cycle disease (97). Moreover, it has potential benefits for cancer, diabetes, thalassemia, cystic fibrosis, spinal muscular atrophy, and neurodegenerative diseases (95). PBA can also inhibit histone deacetylase (HDAC) and stimulate gene transcription. The protective effect of PBA on ER stress mainly involves its chaperone activity, given that removal of HDAC inhibitory activity does not affect its protective effect (98). Recently it was shown that PBA treatment preserved HCs and delayed hearing loss progression in *Cdh23* mutant mice *erl* (39). The protective effect of PBA in hearing is further supported by a report showing that PBA could alleviate protein aggregation and hair cell deficits in *Elp3* knockout mice (61).

4.2 Modulating the PERK-eIF2 α -ATF4 pathway

ISRIB was identified as an inhibitor of PERK signaling in a cell-based screen (75). It was then shown that ISRIB inhibited the PERK-eIF2 α -ATF4 pathway through activating the GEF eIF2B (76). ISRIB treatment shows protective effects in neurodegenerative diseases (99, 100). Consistently, treatment with ISRIB preserves HCs and protects mice from NIHL (30). However, ISRIB treatment needs to be applied with caution. Besides inhibiting the PERK signaling, ISRIB could also inhibit stress granule (SG) formation induced by eIF2 α phosphorylation (101). It was recently reported that inhibition of SG formation by ISRIB increases HC death in cochlear explants during ototoxicity (102). The controversial effects of ISRIB on HC survival in these two studies require further investigation.

Salubrinal is a selective inhibitor of eIF2 α phosphatase complexes GADD34-PP1C, and could prevent ER stress-induced cell death (103, 104). However, its protective effect seems to be cell- and context-dependent. For

example, salubrinal has been shown to enhance fatty acid-induced ER stress and increase rat pancreatic β -cell apoptosis (105). Salubrinal treatment preserves HCs and delays hearing loss progression in the *erl* mice, indicating a protective effect of salubrinal in hearing (37). Taken together, both PERK inhibitor ISRIB and PERK enhancer salubrinal show protective roles in treatment of hearing loss, suggesting that PERK signaling has a dual role in hearing that might be context-dependent.

4.3 Modulating the IRE1 α -XBP1 pathway

As mentioned above, Sig-1R is an important ER membrane protein that interacts with BiP at the MAMs and regulates Ca²⁺ signaling (77). Sig-1R stabilizes IRE1 α at the MAMs and prolongs IRE1 α 's activity under ER stress (79, 80). Sig-1R agonists have protective effects in various neurodegenerative diseases (106). Consistently, treatment of Sig-1R agonist SA4503 in mice significantly reduces noise-induced cell death and hearing loss, whereas shows no effect on ARHL (81).

5. Perspectives

ER stress has attracted more and more attentions in recent years. As discussed above, ER stress plays important roles in hearing loss, and could act as an effective target for deafness treatment (Table 1). Animal experiments showed that chemicals such as TUDCA, PBA, ISRIB, salubrinal, SA4503, and GGA have protective effects on both hereditary and acquired hearing loss through modulating ER stress. Meanwhile, deletion or overexpression of ER stress-related genes such as *Chop* and *Gilz* also show protective effect on hearing loss. These results suggest that ER stress could act as an effective and promising target for treatment of hearing loss.

However, ER stress-based treatment of hearing loss is still very limited at present. Among the small molecules that have been successfully used clinically or preclinically in treatments of diseases (such as

neurodegenerative diseases), only a few have been tested in animal experiments for treatment of hearing loss. Many promising ER stress-related small molecules await further testing in deafness treatment in the future. Meanwhile, cautions must be taken because we now know that ER stress might play different roles in different types of hearing loss, and that the ER stress-targeted drugs might not be that specific. Moreover, ER stress play important roles in various cell types and organs, hence long-term administration of ER stress-targeted drugs might lead to serious adverse effects. Local drug delivery into the inner ear might help to solve the last problem.

Besides reducing side effects, local drug delivery can also help to bypass the blood labyrinth barrier (BLB) that prevents effective drug delivery into the inner ear by systemic administration. The above-mentioned studies delivered drugs systematically via intraperitoneal injection, subcutaneous injection, or oral administration, which are less effective compared with local drug delivery. At present, two main local drug delivery routes are employed in the inner ear, which are intratympanic administration and intracochlear administration (107). In the less invasive intratympanic administration, a drug is delivered to the middle ear through the tympanic membrane, followed by diffusion into the inner ear through the round window. In the rather invasive intracochlear administration, a drug is applied directly to the cochlea, which is more efficient but has a significant risk of damaging the delicate cochlea. Further investigations are warranted to develop non-invasive or minimally invasive local delivery methods for deafness treatment (108). At present, local drug delivery is more frequently used in gene therapy, which is recently emerging as a promising alternative to small molecules in disease treatment.

Local delivery of viruses that encode XBP1s or BiP were shown to improve neuron survival in animal models of neurological diseases (109). Lentivirus-mediated

Table 1. Potential ER stress-base treatment of deafness.

Deafness	Models	ER stress-related treatment	References
DIHL	mice, rats, guinea pigs	GGA, TUDCA	(65, 66, 90, 96)
NIHL	mice, guinea pigs	GGA, ISRIB, SA4503	(30, 81, 91)
	rats	GILZ overexpression	(86)
ARHL	mice	GGA	(89)
HHL	<i>Cdh23</i> mutant mice (<i>erl</i>)	TUDCA, PBA, salubrinal	(37-39)
	<i>Cdh23</i> mutant mice (<i>erl</i>)	<i>Chop</i> gene deletion	(37)
	<i>Tmtc4</i> ko mice	<i>Chop</i> gene deletion	(30)
	<i>Elp3</i> cko mice	PBA	(61)

overexpression of GILZ has been shown to be protective in NIHL (86). However, the specific target cells of lentivirus are unclear in this study. Moreover, lentivirus has long-term safety concerns and is not commonly used in clinical trials (110). Recently, adeno-associated viruses (AAVs) become increasingly used in gene therapy because of its excellent safety and high efficiency (111). Several AAVs have been developed to efficiently deliver genes into HCs or SCs (112, 113). Hence it will be interesting to examine the therapeutic effect of AAV-mediated delivery of XBP1s, BiP, or GILZ into the cochlea.

As mentioned above, *Chop* gene deletion shows a protective effect in *Cdh23* mutant mice and *Tmtc4* knockout mice (30, 37). AAV-mediated RNA interference (RNAi) against dominant deafness-associated *Tmc1* mutation has been shown to improve HC survival and prevent hearing loss (114). It will be interesting to test whether AAV-mediated RNAi against CHOP also has a protective effect in hearing. As an attractive alternative to RNAi, CRISPR/Cas9-mediated genome editing has been employed to disrupt the dominant deafness-associated allele in the *Tmc1* mutant mice (115). In that study, Cas9-sgRNA complex was delivered via cationic lipid, which pointed out a new direction of developing a DNA- and virus-free treatment of hearing loss (115, 116).

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Conflict of interest

The authors declare that they have no conflict of interest.

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