A Novel Animal Model of Hearing Loss Caused by Acute Endoplasmic Reticulum Stress in the Cochlea

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Abstract. Many stimuli such as ischemia, hypoxia, heat shock, amino acid starvation, and gene mutation, exhibit a cellular response called endoplasmic reticulum (ER) stress. ER stress induces expression of a series of genes, leading to cell survival or apoptosis. Previously, we found that in an animal model of hearing loss caused by acute mitochondrial dysfunction, several ER stress markers including C/EBP homologous protein were induced in the cochlear lateral wall. To elucidate the mechanism of hearing loss caused by ER stress, we established a novel animal model of hearing loss by perilymphatic perfusion of tunicamycin, an ER stress activator that inhibits \( \text{N} \)-acetylglucosamine transferases. Subacute and progressive hearing loss was observed at all sound frequencies studied, and stimulation of ER stress marker genes was noted in the cochlea. The outer hair cells were the most sensitive to ER stress in the cochlea. Electron microscopic analysis demonstrated degeneration of the subcellular organelles of the inner hair cells and nerve endings of the spiral ganglion cells. This newly established animal model of hearing loss from ER stress will provide additional insight into the mechanism of sensorineural hearing loss.

Keywords: endoplasmic reticulum stress, tunicamycin, hearing loss, animal model

Introduction

Hearing loss is a major communicative disorder that influences personal and social activities of afflicted patients. Although developments in medicine and engineering have significantly improved adaptive strategies for patients with hearing loss, many types of sensorineural hearing loss, such as sudden deafness and Meniere’s disease, still require effective therapeutic strategies. One of the difficulties in developing effective therapeutic methods for such patients is the lack of knowledge about the molecular and cellular events causing the hearing disorders. To elucidate the mechanism underlying acute hearing loss due to cochlear energy failure such as what occurs in cochlear ischemia, we previously established an animal model of acute hearing loss using the mitochondrial toxin 3-nitropropionic acid (3-NP) (1, 2). In this model, expression of endoplasmic reticulum (ER) stress marker genes such as C/EBP homologous protein (\( \text{chop} \), also called \( \text{Ddit3} \) or \( \text{Gadd153} \)) and activating transcription factor 4 (\( \text{Atf4} \), also called \( \text{Creb2} \)) was upregulated in the cochlear lateral wall, suggesting that ER stress plays a role during the onset or exacerbation of hearing loss in some types of auditory disorders.

A number of reports have indicated that ER stress in various organs is related to conditions such as Alzheimer’s disease (4), Parkinson’s disease (5), ischemia-reperfusion injury (6), diabetes (7), and cystic fibrosis (8). Tunicamycin, an inhibitor of \( \text{N} \)-acetylglucosamine transferases, has been widely used to examine ER stress and subsequent molecular and cellular events (9). Tunicamycin inhibits the first step of \( \text{N} \)-linked glycosylation of immature proteins (10) and ultimately causes apoptosis in vitro (11). Blockage of \( \text{N} \)-linked glycosylation results in the accumulation of misfolded and/or misassembled proteins in the ER, which typically stimulates the so-called ER stress marker genes, including \( \text{chop} \), \( \text{Atf4} \), glucose-regulated protein 78 (\( \text{grp78} \), also called \( \text{Bip} \) or \( \text{Hspa5} \)), and \( \text{grp94} \) (also called \( \text{Hsp90\beta1} \) or \( \text{Tra1} \)) (12). In vivo injec-
tion of tunicamycin causes death of retinal ganglion cells (13, 14). No studies on the cellular and molecular mechanisms of tunicamycin for ER stress in the inner ear have been performed.

In this study, we established a novel animal model of ER stress–induced hearing loss by perilymphatic perfusion of tunicamycin into the rat inner ear and elucidated the pathological mechanism at the physiological, morphological, and molecular levels to reveal unique features of cochlear responses to ER stress.

Materials and Methods

Animals and drug administration

Male 6- to 8-week-old Sprague-Dawley rats (weighing 170 – 230 g; Charles River Lab, Japan, Yokohama) were used to establish the hearing loss model. They were housed in metallic breeding cages in a room with a 12-h light/dark cycle, 55% humidity, and at 23°C, and the rats were permitted free access to food and water for at least 7 days before use.

The rats were anesthetized by inhalation of 1.5% – 3.0% isoflurane (2.0 L/min air flow) delivered via a mask using an anesthetizer (model TK-5; Muromachi Kikai, Tokyo), together with local anesthesia by injection of 1% lidocaine. The surfaces of the posterior and lateral semicircular canals of the left inner ear were exposed, and a small hole was made in each canal. A small tube (Eicom, Tokyo) was inserted into the lateral semicircular canal toward the ampulla. Through this tube, the perilymph was perfused with tunicamycin (Wako Pure Chemical, Osaka) for 8 min at a rate of 5 μL/min using a syringe pump. Drainage was allowed through a hole on the posterior semicircular canal. The tube was then removed, the holes on the semicircular canals were sealed with a piece of muscle and fibrin adhesive (Beriplast P Combi-set; CSL Behring, King of Prussia, PA, USA), and the wound on the neck was closed. Tunicamycin was dissolved at 0.5 to 500 ng/μL in saline containing PURE BRIGHT MB-37-50T (NOF Corp., Tokyo), which enhances drug solubility (15). An equal volume of vehicle without tunicamycin was injected into the semicircular canal of another group of animals as a control. The cochlea on the contralateral side (right side) was surgically destroyed to avoid cross hearing during ABR measurement. All experimental procedures were approved by the Institutional Animal Care and Use Committee of National Tokyo Medical Center.

Measurement of auditory brainstem response (ABR)

ABR thresholds were recorded from rats before surgery and at 1, 2, 3, and 7 days after treatment (DAT) with serial doses of tunicamycin (n = 3 in each group) or vehicle (n = 4), and additional 14, 21, and 28 DAT with 5 ng/μL of tunicamycin, vehicle, or untreated (n = 3) using Scope wave form storing and stimulus control software and the PowerLab data acquisition and analysis system (PowerLab2/20; AD Instruments, Castle Hill, Australia). Pure tone bursts of 8, 20, and 40 kHz (0.2 ms rise/fall time, 1 ms flat segment) and the amplitudes were specified by a real-time processor and programmable attenuator (RP2.1 and PA5; Tucker-Davis Technologies, Alachua, FL, USA). Sound levels were calibrated using a sound level meter (NL32; RION, Tokyo). Waveforms of 512 stimuli at each frequency were averaged and the visual detection threshold was determined with increment or decrement of sound pressure level by 5-dB steps. Details of ABR recording have been described previously (1). The thresholds were evaluated as the means ± S.E.M., and statistical significance was determined with two-way ANOVA.

Light microscopy and fluorescent microscopy

For morphological analysis with light microscopy using tissue sections, rats (n ≥ 3 for each period in a series of experiments) were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and perfused intracardially with 0.01 M phosphate–buffered saline (PBS, pH 7.4) containing 8.6% sucrose, followed by 4% paraformaldehyde (PFA) in PBS. After decapitation, temporal bones were removed quickly and placed in the same fixative. Small openings were made at the round window, oval window, and apex of the cochlea. After immersion in the fixative overnight, the temporal bones were decalcified in 5% EDTA and 4% sucrose in PBS at 4°C for 2 weeks, dehydrated, and embedded in paraffin. Transverse cochlear sections (5-μm-thick) were cut and mounted on glass slides. After rehydration, sections were stained with hematoxylin and eosin. To analyze hair cells using surface preparation of cochleae, the cochleae from rats treated with vehicle or 5 ng/μL tunicamycin at 7 DAT were immediately fixed with 4% PFA in PBS overnight at 4°C and then decalcified in 5% EDTA and 4% sucrose in PBS at 4°C for 3 days. Following decalcification, the optic capsules were removed, and the samples were treated with 0.3% Triton-100 in PBS for 5 min. The samples were stained with rhodamine-phalloidin (70 nM in PBS) for 60 min at room temperature (16). Then, the organ of Corti was separated from the lateral wall and modiolus, microdissected into individual turns, counterstained with DAPI (1 μg/mL; DOJINDO, Kumamoto), and mounted on glass sides in PermaFluor Aqueous Mounting Medium (Thermo Fisher Scientific, Waltham, MA, USA).
Quantitative reverse transcription PCR (qRT-PCR)

After the rats were anesthetized, temporal bones were quickly removed, and immersed in RNAlater (TaKaRa Bio, Shiga) on ice, followed by dissection of whole cochleae. Total RNA was isolated with TRIzol Reagent (Life Technologies, Carlsbad, CA, USA) and dissolved in DEPC-treated water, and the purity of total RNA was determined by the ratio of OD260/OD280 as measured on a UV/visible spectrophotometer (Ultrospec 2100 pro; GE Healthcare, Uppsala, Sweden). First-strand cDNA synthesis was performed using 100 ng of total RNA and Oligo (dT)12-18 primers (Life Technologies) in a total volume of 20 μL according to the SuperScript III RNase H− Reverse Transcriptase protocol (Life Technologies). qRT-PCR was performed according to the manufacturer’s protocols for the ABI PRISM 7000 Sequence Detection System (Life Technologies). We designed PCR primers specific for chop, Atf4, grp78, grp94, and glyceraldehyde-3-phosphate dehydrogenase (gapdh). The specificity of these primer sets has been previously confirmed (3). qRT-PCR was performed in a 25-μL reaction containing 1 × SYBR Premix Ex Taq (TaKaRa Bio), 1 × ROX Reference Dye, 0.2 μM of each primer, and cDNA. The PCR conditions were 5 s at 95°C and 31 s at 60°C for 40 cycles. Gene expression levels were normalized using gapdh as an internal control. Statistical significance was evaluated using Student’s t-tests adjusted with Holm’s procedure.

Electron microscopy

Rats were deeply anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg) and perfused intracardially with Lactec buffer (Otsuka Pharmaceutical, Tokyo), followed by perfusion-fixation with 4% PFA and 4% glutaraldehyde in 30 mM HEPES buffer (pH 7.4). After decapitation, temporal bones were removed quickly and placed in the same fixative. Small openings were made at the round window, oval window, and apex of the cochlea. After immersion in the fixative overnight, the temporal bones were decalcified in 4% EDTA and 5% sucrose in PBS at 4°C for 5 days. After washing in 60 mM HEPES buffer, the tissue was postfixed in 1% OsO4 in 30 mM HEPES buffer for 4 h at 4°C, dehydrated in a graded ethanol series and QY-1 (Nisshin EM, Tokyo), and embedded in Epon resin. Ultra-thin sections in the horizontal plane parallel to the cochlear modiolus were cut with a MT2-B ultra micro-tome (Sorvall, Newtown, CT, USA) and mounted on 100-mesh grids. The sections were stained with uranyl acetate and lead citrate and were examined with a H600 electron microscope (Hitachi, Tokyo).

Results

ABR threshold shifts after ER stress and distinct vulnerability to ER stress among different cell types in the cochlea

We first examined changes in the hearing level after exposure to various degrees of acute ER stress by measuring ABR in rats up to 7 DAT with tunicamycin (Fig. 1: A – D). Then, we examined the differences in vulnerability to ER stress among different cell types in the cochlea by morphological analysis.

Rats treated with 0.5 ng/μL tunicamycin demonstrated a moderate increase in the ABR threshold from below 25 dB before treatment to 51.0 ± 14.1 dB at 40 kHz at 1 DAT.

![Fig. 1](https://example.com/fig1.png)

Fig. 1. Hearing loss following treatment with tunicamycin. A – D) ABR thresholds after treatment with different dosages of tunicamycin were measured at 8 kHz (open triangles), 20 kHz (closed circles), and 40 kHz (closed diamonds) for 7 DAT. 0 DAT: pretreatment. Data are shown as averages ± S.E.M.
DAT (Fig. 1A). The hearing level remained stable up to 7 DAT. The hearing levels at 8 and 20 kHz were not affected by this tunicamycin concentration. Treatment with 5 ng/μL tunicamycin induced ABR threshold shifts at all frequencies as early as 1 DAT. The thresholds developed to 66.6 ± 7.4 dB at 8 kHz, to 77.7 ± 5.5 dB at 20 kHz, and 82.7 ± 2.9 dB at 40 kHz 7 DAT (Fig. 1B). Increasing the tunicamycin dose to 50 ng/μL resulted in no ABR response to the maximum output of the system at all three frequencies measured 7 DAT, indicating that the rats lost hearing completely (Fig. 1C). With 500 ng/μL tunicamycin, the rats lost hearing completely at all three frequencies at 3 DAT (Fig. 1D). A mild temporary threshold shift at 40 kHz was observed in vehicle-treated animals at 3 DAT, but not in untreated animals, indicating an effect of surgical manipulation (data not shown).

Because the differences in ABR threshold shifts among the various tunicamycin concentrations were most prominent at 8 kHz, morphological changes in the middle turn of the cochlea, which is known to respond to approximately 5 to 15 kHz of sound (17), were observed 7 DAT (Fig. 2: A – J). Animals treated with 0.5 ng/μL tunicamycin showed the organ of Corti (OC) with a single row of inner hair cells (IHCs) and three rows of outer hair cells (OHCs) supported by Deiter’s cells (Fig. 2: A, C), comparable to the vehicle-treated animals (Fig. 2: I, J), indicating no apparent degeneration. This is in agreement with normal hearing thresholds at 8 and 20 kHz following this tunicamycin dose (Fig. 1A). Treatment with 5 ng/μL tunicamycin (Fig. 2: B, D) induced the loss of OHCs in the OC (Fig. 2D), whereas the morphology of other cochlear tissues remained normal. With 50 ng/μL tunicamycin (Fig. 2: E, G), loss of LW fibrocytes (Fig. 2E, arrow) and the OHCs (Fig. 2G, asterisks) were evident. With 500 ng/μL tunicamycin, most of the spiral ganglion cells (SGCs) were lost (Fig. 2F, arrowhead). In addition, degeneration of the LW fibrocytes was severe compared with rats treated with 50 ng/μL tunicamycin, and degeneration of the Deiter’s cells in addition to the HCs in the OC was observed (Fig. 2: F, H). Visualization of actin-rich stereocilia, critical structures in hair cells that transduce sound-induced mechanical vibrations, can be achieved by staining the cells with fluorescent dye-conjugated phalloidin. Gross degeneration of the OHCs by 5 ng/μL tunicamycin was confirmed by significant loss of stereocilia in the entire auditory epithelia (Fig. 2I). Vehicle treatment did not affect the stereocilia, which consist of three rows of OHCs and one row IHCs (Fig. 2L).

Ultrastructural changes of IHC synapses and SGCs

The frequent loss of OHCs in serial sections as observed by light microscopy was the most obvious pathological feature of hearing loss in animals treated with 5 ng/μL tunicamycin. OHCs are known to amplify sound sensitivities for roughly 50 – 60 dB (18). We set to ascertain whether loss of the OHCs alone account for the approximately 55 – 65 dB threshold shift at 7 DAT (Fig. 1B) by electron microscopic analysis of the IHCs and the SGCs in these animals.

Animals treated with 5 ng/μL tunicamycin showed normal morphology of the nucleus of the IHCs 7 DAT (Fig. 3A). However, the number of ERs appeared to be decreased compared with the IHCs in vehicle-treated animals (Fig. 3: B, E). In the synaptic region of the IHCs, vacuolar changes (Fig. 3C, open triangles) and multiple electron-dense granules (Fig. 3C, small arrows) were observed, indicating degeneration of the nerve endings. IHCs from animals treated with vehicle (Fig. 3: D – F) showed numerous subcellular organelles in the cytoplasm including mitochondria and ERs (Fig. 3: D, E, mt and asterisks). In the synaptic region of the IHCs, there appeared to be multiple nerve endings that descended from the SGCs (Fig. 3: D, F). Some of the nerve endings proximal to the IHCs formed electron-dense membranes, one of the features of postsynaptic membranes (19) (Fig. 3F, closed arrowheads). In tunicamycin-treated animals, numerous vacuoles and swollen mitochondria were observed in the cell bodies of SGCs 7 DAT, indicating degeneration of the SGCs (Fig. 3: G, H). In vehicle-treated animals, the SGCs contained multiple organelles including mitochondria and ERs without evidence of degeneration (Fig. 3: I, J).

Long-term effect of mild ER stress in the cochlea and early expression of ER stress marker genes

To study the long-term effects of tunicamycin-induced mild ER stress in the cochlea, rats treated with 5 ng/μL tunicamycin, which induced subacute and gradually progressive hearing loss, were selected to examine the ABR threshold shifts and morphological changes of the cochlea for up to 4 weeks. The ABR thresholds at 8, 20, and 40 kHz gradually and significantly increased to almost reach or exceed the maximum output 7 DAT (8 kHz), 3 DAT (20 kHz), and 2 DAT (40 kHz), respectively, and remained at this level up to 28 DAT (Fig. 4: A – C). The ABR threshold at 40 kHz increased more acutely than other frequencies of sound, the feature consistent with the primary experiment (Fig. 1B). The ABR threshold shifts in vehicle-treated animals did not show any significant difference between untreated animals in any frequencies of sound during experiment (Fig. 4: A – C).

ER stress is characterized by upregulation of several marker genes such as chop, Atf4, grp78, and grp94. To study early expression of ER stress markers in the co-
Fig. 2. Dose-dependent cochlear damage by tunicamycin. A, B, E, F, I) Morphology of the middle turn of the cochlea treated with various doses of tunicamycin or vehicle at 7 DAT. Magnified images of the organ of Corti are shown in panels C, D, G, H, and J, K, L. Surface preparations of organ of Corti in the corresponding turn with stereocilia visualized with rhodamine-phalloidin 7 DAT. Asterisks and plus indicate positions of OHCs and IHCs, respectively. Arrows, small arrows, arrowheads, and small arrowheads indicate LW, OC, SG, and Deiter’s cells, respectively. Scale bar = 100 μm.
Fig. 3. Subcellular damage of IHCs and SGCs by tunicamycin. Electron microscopic images of IHCs (A – F) and SGCs (G – J) following treatment with 5 ng/μL tunicamycin (A – C, G, H) or vehicle (D – F, I, J) at 7 DAT. Panels B, C, E, and F are magnified images of the supranuclear or synaptic region of the IHCs, and panels H and J are the perinuclear region of the SGCs (arrows). In panels C and H, electron-dense granules and vacuoles caused by tunicamycin are indicated by small arrows and open triangles. In panels E and J, endoplasmic reticulum is indicated by asterisks. In panel F, electron-dense synapse membrane–like structures are indicated by closed arrowheads. IHC, inner hair cell; mt, mitochondrion; n, nucleus; ne, nerve ending; SGC, spiral ganglion cell.

Scale bars in panels A, D, G, and I = 5 μm; scale bars in panels B, C, E, F, H, and J = 1 μm.
Hearing Loss Caused by Acute ER Stress

chlea, expression of these marker genes was evaluated with qRT-PCR in the whole cochlea (including LW, modiolus, OC, and surrounding connective tissues) from untreated animals, animals treated with vehicle, or those treated with 5 ng/μL tunicamycin at 3 DAT (Fig. 4D). As expected, expression levels of chop, Atf4, and grp94 were significantly increased by tunicamycin treatment compared with vehicle-treated or untreated rats, confirming the tunicamycin-mediated ER stress in the cochlea. However, expression of grp78 did not change significantly.

At 28 DAT, in addition to OHCs, the IHCs and Deiter’s cells were severely degraded (Fig. 5: A, C) in tunicamycin-treated animals, whereas no significant morphological changes were evident in the LW and SGCs (Fig. 5A). The SGCs, LW, together with OHCs, IHCs, and Deiter’s cells in vehicle-treated animals did not show morphological changes at 28 DAT (Fig. 5: B, D). Time-dependent, cell-specific morphological damage by tunicamycin is summarized in Table 1.

Discussion

We established a novel animal model of subacute and gradually progressive hearing loss with perilymphatic perfusion of tunicamycin, which inhibits the first step of N-linked glycosylation in the ER (10), into the inner ear. Induction of ER stress marker genes in the cochlea and morphological changes after tunicamycin treatment indicated that the hearing loss was mediated by ER stress induced by tunicamycin.

We previously reported expression of ER stress marker genes in the cochlea in an animal model of acute cochlear mitochondrial dysfunction by mitochondrial toxin 3-NP (3). In the present model of subacute hearing loss by tunicamycin, a series of distinct features were identified, reflecting the different mechanisms involved in each model. Induction of chop in the cochlea by both acute mitochondrial dysfunction and acute ER stress indicates that the molecular pathways underlying the pathological process of the two types of hearing loss partially overlap. The mitochondrial vacuolation detected in SGCs in the tunicamycin-treated animals also appeared morphologically similar to the vacuolation observed in 3-NP-treated conditions.
However, although SGCs in tunicamycin-treated animals demonstrated morphological changes 7 DAT, SGCs in 3-NP-treated animals showed a similar abnormality 3 h after treatment but returned to normal by 7 DAT (2). This difference is considered to reflect sub-acute and long-lasting effects of tunicamycin-induced ER stress (20) on SGCs and an acute and transient effect of 3-NP-induced mitochondrial dysfunction including ER stress on SGCs (3).

Hearing loss from tunicamycin progressed gradually and irreversibly even with the lowest dose of the drug, whereas low-dose treatment with 3-NP induces acute and transient hearing loss (1). The different time course of hearing loss between tunicamycin and 3-NP are not attributable to the distinct technical procedures between two animal models; tunicamycin was perfused into the perilymph through the semicircular canal to affect cochlear cells more directly and immediately than 3-NP, which was administered to the inner ear by permeation through the round window membrane (1). Progressive hearing loss by tunicamycin, however, occurred much more slowly than that by 3-NP. Onset of hearing loss by tunicamycin can be explained by inhibition of protein maturation in the ER of the cochlear cells that results in depletion of various functional molecules, depending on their turnover rates. The subsequent gradual progressive hearing loss by tunicamycin could be due to the time course of the entire molecular pathway of ER stress that is stimulated by tunicamycin and that leads to degeneration of cochlear cells (11). The irreversible increase in ABR thresholds with tunicamycin-induced hearing loss 28 DAT is consistent with loss of OHCs and loss of IHCs following degenerative changes of the subcellular organelles, both of which usually do not regenerate in mammals (21).

Histochemical study in tunicamycin-treated animals indicated that OHCs were the cell type in the cochlea most susceptible to the toxicity of ER stress. Because

Table 1. Morphological damage in the middle turn of cochlear cells by tunicamycin treatment

<table>
<thead>
<tr>
<th>Loss of cell type</th>
<th>7 DAT</th>
<th>28 DAT</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>0.5 ng/μL</td>
</tr>
<tr>
<td>OHC</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>IHC</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Deiter’s cell</td>
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<td>−</td>
</tr>
<tr>
<td>SGC</td>
<td>−</td>
<td>−</td>
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<tr>
<td>LW fibrocyte</td>
<td>−</td>
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−, no morphological damage; +, moderate loss of cells; ++, severe loss of cells; *, degeneration detected under electron microscopy.

Fig. 5. Morphological damage of the OC by ER stress 28 DAT. Middle turn of the OC in animals treated with 5 ng/μL tunicamycin or vehicle is shown with paraffin sections and HE staining. The structure of the OC appeared markedly deteriorated in tunicamycin-treated animals (A, C) compared with the normal structure of the OC in vehicle-treated animals (B, D). As for symbols, please refer to Fig. 2. Scale bar = 100 μm.
hearing was primarily affected at high frequencies in tunicamycin-treated animals, OHCs in the basal portion of the cochlea are considered to be more susceptible to ER stress than those in the apical portion specialized to respond to lower frequencies of sound. Degeneration of OHCs in the basal portion of the cochlea is a frequent pathological feature in various types of sensorineural hearing loss, including drug-induced hearing loss, age-related hearing loss, and noise-induced hearing loss (22). It would be interesting to study whether ER stress may also be involved as a pathological mechanism in some of these types of sensorineural hearing loss. Predominant degenerative changes of the LW fibrocytes with induction of ER stress marker genes in 3-NP-treated animals (3) may be attributed to the differential susceptibility to mitochondrial dysfunction, a major toxic pathway in cochlear cells, in addition to ER stress following 3-NP treatment.

Induced expression of ER stress markers such as chop, Atf4, and grp94 in the cochlea treated with tunicamycin is a direct evidence for ER stress. Interestingly, grp78, another major ER stress marker gene (23), was not up-regulated in the cochlea of tunicamycin-treated animals or in those of 3-NP-treated animals (3). Thus, the response to ER stress in the cochlea may not involve grp78. The association of ER stress with auditory function has also been suggested in Wolfram syndrome involving high-frequency hearing loss (24, 25) or hereditary nonsyndromic low-frequency hearing loss (26, 27), both of which are caused by WFS1 mutations. WFS1 encodes an ER protein, wolframin. An in vitro experiment has shown that wolframin controls ER stress response through degradation of ATF6α in the normal state, thereby suppressing the downstream ER stress marker genes such as grp78 and chop (28). Dysfunction of wolframin could result in increased expression of the ER stress marker genes and apoptosis. Wolframin is expressed in OHCs, SGCs, and LW fibrocytes (ref. 29 and data not shown). It is of our future interest whether the animal model of ER stress–induced hearing loss established in this study is helpful for considering molecular mechanisms of hereditary hearing loss caused by WFS1 mutations.

In summary, we studied the physiological, morphological, and molecular features of a newly established animal model of hearing loss caused by tunicamycin-induced ER stress. This model is useful for elucidating the mechanisms of some types of hearing disorders that involve ER stress, and it may lead to identification of drug targets to treat such types of hearing loss.

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