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Authors

Okur, Mustafa Nazir

Djalilian, Hamid R

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Review

Approaches to Mitigate Mitochondrial Dysfunction in Sensorineural Hearing Loss

Mustafa Nazir Okur[1] and Hamid R. Djalilian[1,2]

[1]Department of Otolaryngology, School of Medicine, University of California Irvine, Irvine, CA 92697, USA; and [2]Departments of Biomedical Engineering, University of California, Irvine, CA 92617, USA

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Abstract—Mitochondria are highly dynamic multifaceted organelles with various functions including cellular energy metabolism, reactive oxygen species (ROS) generation, calcium homeostasis, and apoptosis. Because of these diverse functions, mitochondria are key regulators of cell survival and death, and their dysfunction is implicated in numerous diseases, particularly neurodegenerative disorders such as Alzheimer's Disease, Parkinson's Disease, and Huntington's Disease. One of the most common neurodegenerative disorders is sensorineural hearing loss (SNHL). SNHL primarily originates from the degenerative changes in the cochlea, which is the auditory portion of the inner ear. Many cochlear cells contain an abundance of mitochondria and are metabolically highly active, rendering them susceptible to mitochondrial dysfunction. Indeed, the causal role of mitochondrial dysfunction in SNHL progression is well established, and therefore, targeted for treatment. In this review, we aim to compile the emerging findings in the literature indicating the role of mitochondrial dysfunction in the progression of sensorineural hearing loss and highlight potential therapeutics targeting mitochondrial dysfunction for hearing loss treatment.

Keywords—Mitochondria, Mitochondrial dysfunction, Hearing loss, Sensorineural hearing loss.

INTRODUCTION

Hearing loss is one of the most disabling and prevalent conditions in the world. According to World Health Organization, 1.57 billion people suffer from some degree of hearing loss, and this prevalence is projected to reach 2.45 billion by 2050 as populations constantly age and recreational noise levels are on the rise with emerging technology.[46] The incidence of hearing loss is associated with other neurodegenerative diseases as it triggers social isolation and reduces cognitive ability, which in many individuals can lead to depression and increase the risk of dementia. Presently, hearing loss is a high-impact and costly health issue and requires pressing attention.

Hearing loss mostly arises from sensorineural deficits that occur in the cochlea, an auditory portion of the inner ear. Age-related decline in function, exposure to noise, and drug-induced ototoxicity are the main known considerations that lead to hearing loss. Decades of research have shown that certain cochlear cells are highly associated with hearing loss due to their key role in hearing. These cells are inner hair cells (IHC), outer hair cells (OHC), and innervating auditory neurons. IHC transduces sound vibrations into electrical signals that are relayed to the brain through auditory neurons while OHC amplifies sound stimulus. Interestingly, those cells contain an abundance of mitochondria whose dysfunction plays an important role in the progression of hearing loss.[13,63]

The primary function of mitochondria is to generate energy through oxidative phosphorylation (OXPHOS). Mitochondria comprise a double membrane system (outer and inner membrane) creating two distinct compartments of intermembrane space (an area between inner and outer membrane) and matrix (inside inner membrane). The outer membrane includes pore-forming membrane proteins that allow for the free transfer of ions or small uncharged proteins into the intermembrane space whereas the inner membrane is highly selective only allowing certain types of ions and

Address correspondence to Mustafa Nazir Okur, Department of Otolaryngology, School of Medicine, University of California Irvine, Irvine, CA 92697, USA. Electronic mail: mokur@hs.uci.edu

molecules through its specific membrane transport proteins. Mitochondrial DNA (mtDNA) in mammals is a 16.6-kb circular DNA, which encodes two rRNAs, 22 tRNAs, and 13 subunits of the OXPHOS components crucial to its function.[11] Most of the OXPHOS process occurs on the inner membrane through the protein complexes of the electron transport chain (ETC) and ATP synthase that are embedded across the membrane. ETC mediates the transfer of electrons to oxygen molecules through a series of oxidation–reduction events. The electron flow through ETC is coupled with the generation of a proton gradient across the inner membrane leading to inner mitochondrial membrane potential. The energy accumulated in the protein gradient is then converted into energy in the form of ATP through ATP synthase.

Besides energy production, mitochondria are also involved in a range of cellular functions including intracellular signaling, fatty acids β-oxidation, calcium regulation, senescence, and death. However, the concept of mitochondrial dysfunction is widely attributed to abnormalities in ATP synthesis by oxidative phosphorylation. In this review, we define mitochondrial dysfunction in the context of bioenergetics and describe it as a condition in which mitochondria fail to meet the cell energy need. Damaged mitochondria, dysregulated mitochondrial homeostasis, and aberrant nuclear-to-mitochondria signaling are among the conditions that lead to mitochondrial dysfunction. Here, we expand on these biological abnormalities in the context of hearing loss and then summarize the potentially-promising therapeutic approaches to mitigate mitochondrial dysfunction and prevent hearing loss.

PROTECTING MITOCHONDRIA

Despite their critical role in energy generation, mitochondria are the major source of reactive oxygen species (ROS) due to electron leakage as a byproduct of oxidative phosphorylation. These ROS include superoxide ions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH). ROS are highly unstable molecules with an affinity to react with and damage almost all macromolecules (e.g., DNA, protein, and lipids). ROS generation is a natural consequence of oxidative phosphorylation. In fact, ROS plays an important role in cell signaling.[27] However, external insults or internal abnormalities such as dysregulation in cellular pathways may exacerbate ROS production, eventually leading to oxidative stress, mitochondrial damage, and ultimately hearing loss.[24,32] Controlling ROS generation, enhancing ROS removal; or repairing ROS-induced damage to mitochondria are the three lines of defense mechanism protecting mitochondria against ROS overproduction and hence targeted for hearing loss therapy as discussed below (Fig. 1).

Mitochondria's first line of defense is lowering ROS production to avoid oxidative stress. This is usually addressed by depolarizing the membrane potential of the inner mitochondrial membrane while still generating adequate ATP to fulfill the energy need.[75] For instance, uncoupling protein 2 (UCP2) residing on the inner membrane mediates free proton flow across the inner membrane and thus lowers the membrane potential, thereby reducing the resistance of electrons to flow through the ETC and minimizing the likelihood of superoxide formation. Indeed, UCP2 deficiency is associated with mitochondrial abnormalities and is implicated in hearing loss.[38,48,60] A study comparing 83 patients suffering from sudden sensorineural hearing loss to 2,048 controls in a Japanese population showed a significant association between UCP2 polymorphisms and sensorineural hearing loss.[38] In a separate study, UCP2 (G-866 A) polymorphism was found to contribute significant risk to age-related hearing loss.[48] Vegetable compounds (e.g., berberine, curcumin, and capsaicin) or pharmaceutical drugs (e.g., fenofibrate) are shown to induce UCP2 expression in a variety of cell types.[60] Interestingly, administration of many of those compounds also showed benefits on hearing loss in animal or cell models. For example, fenofibrate treatment protected mouse cochlear cells against cisplatin-induced ototoxicity by lowering ROS generation and maintaining mitochondrial number and function.[36] Similarly, berberine treatment also decreased mitochondrial ROS generation and prevented cisplatin- or noise-induced hair cell

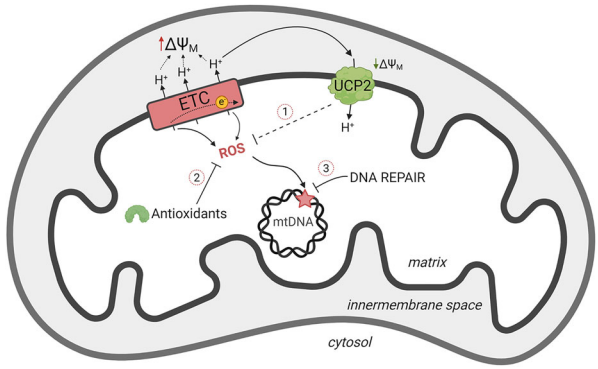

FIGURE 1. Protection of mitochondria. The major lines of defense mechanisms protecting mitochondria from ROS-mediated damage are depicted in the diagram. 1. The uncoupling proteins such as UCP2 allow a free H^+ flow across the membrane and thus lowers the membrane potential, thereby allowing the flow of electrons in ETC with less resistance and decreasing the spurious formation of ROS. 2. Antioxidants mechanism prevents ROS production or neutralizes existing ROS. 3. DNA repair protects mtDNA from ROS-mediated damage.

death.[37,89] Similar benefits were observed with curcumin or capsaicin treatments.[3,81]

It is now well established that external insults such as noise exposure or certain ototoxic drugs can lead to excessive ROS production, damaging hair cells and causing temporary or permanent hearing loss.[32] Once ROS is formed, endogenous antioxidants provide an additional internal defense mechanism by scavenging free radicals and eliminating ROS. Superoxide dismutases (SOD), catalase, glutathione S-transferase (GST), and glutathione peroxidase (GPX) are some of the primary antioxidant enzymes that play a critical role in ROS clearance. Glutathione (GSH) is the most abundant non-enzymatic antioxidant that functions as a cofactor with GST and GPX to eliminate ROS.[79] Coenzyme Q10 and melatonin are also other known non-enzymatic antioxidants produced endogenously.[51] Pharmacological approaches using small-molecule antioxidants to eliminate excessive ROS products mitigate mitochondrial dysfunction and have been successfully used for noise and drug-induced hearing loss. N-acetylcysteine (NAC), an L-cysteine precursor, scavenges oxygen radicals and its administration protects against noise-induced hearing loss in animal models.[4,21,50] There are also clinical studies supporting the protective effect of NAC. Oral NAC administration exhibited reduced noise-induced hearing loss among textile workers compared to ones who received a placebo.[16] However, a clinical study on military personnel did not lead to any significant benefits of NAC administration on hearing, suggesting that NAC's clinical efficacy is still controversial. D-methionine, a sulfur-containing antioxidant, has also shown some promising results in laboratory and clinical studies.[7,39,76] Additionally, various other antioxidants such as Ebselen are indicated in hearing loss prevention.[22,35]

Repairing or controlling ROS-induced damage is the third line of defense protecting mitochondria against ROS. Due to its proximity, the mtDNA is particularly exposed to ROS, making it prone to oxidative DNA damage. If not repaired efficiently, the damage to mtDNA accumulates and could eventually deteriorate mtDNA integrity and lead to mitochondrial dysfunction.[62] Base excision repair (BER) is considered the primary repair pathway in mitochondria, responsible for the removal of oxidized bases through the following core steps: (i) the damaged base is recognized and removed by DNA glycosylases, leaving an abasic site which is then processed by endonucleases; (ii) the resulting gap is then filled by a DNA polymerase and; (iii) sealed by a DNA ligase[41,62]. BER activity declines with age in various tissues in mice and its deficiency is linked to mitochondrial abnormalities such as reduced oxygen consumption rate.[6,30,44,62] The expression of 8-Oxoguanine DNA Glycosylase (OGG1), a crucial enzyme having a role in the first step of BER, decreases in the auditory tissues in D-galactose-induced aging rats, suggesting an age-dependent decrease in BER activity in the auditory system as well.[9] In line with this, mtDNA integrity declines in an age-dependent manner in both mouse cochlea and auditory cortex and is associated with ARHL.[26,54,83] However, BER's impact on the age-dependent decline in mtDNA integrity is still under debate and thus warrants further investigation.[34] The impact of noise or drug exposure on BER activity is not explored in detail in the cochlea yet. However, a recent study showed that noise-exposed workers with single nucleotide polymorphisms in two important components of BER (XRCC1 and APE1) are more susceptible to noise-induced hearing loss, linking BER to hearing loss.[14]

MITOCHONDRIAL HOMEOSTASIS AND HEARING LOSS

Mitochondria are plastic and dynamic organelles. Depending on energy needs and other cues, they can adapt their numbers and morphology through the following three processes: mitochondrial biogenesis, mitochondrial dynamics (fission and fusion), and mitochondrial degradation (mitophagy). The coordinated actions of these processes not only help cells regulate metabolism but also ensure the health of mitochondria. Disruption in their function, on the other hand, causes mitochondrial dysfunction and is associated with hearing loss. Below, we will dive into each of these processes in more detail and describe how they are targeted to ameliorate mitochondrial dysfunction and hearing loss.

Mitochondrial Biogenesis is the generation of new mitochondria, a sophisticated process mediated by the orchestrated action of various transcription factors in response to diverse stimuli, such as nutrient availability, hormonal cues, and temperature fluctuations. Its impairment is accompanied by reduced ATP synthesis, hence potentially contributing to mitochondrial dysfunction.[61] Peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) is considered the master regulator of the biogenesis process (Fig. 2).[20] The upstream energy-sensing proteins such as AMPK and Sirtuin1 activate PGC1α by post-translational modifications, leading to its translocation to the nucleus and association with various transcription factors/proteins such as Nuclear respiratory factors (NRF1 and NRF2), Estrogen-related receptors (ERRs), and Peroxisome proliferator-activated receptors (PPARs) (Fig. 2).[73] NRF activation increases TFAM expres-

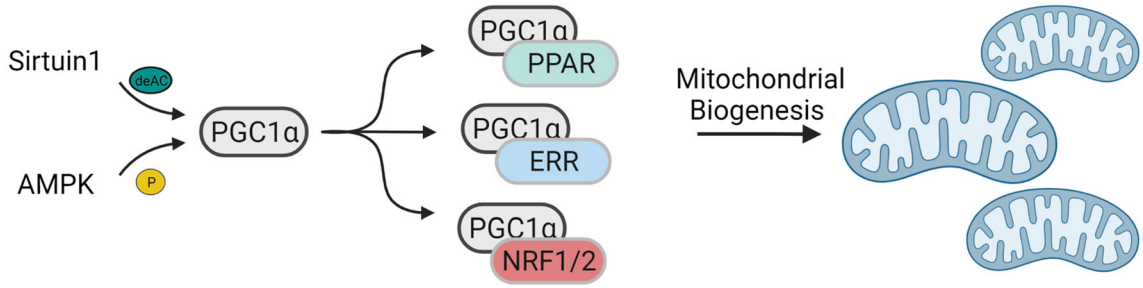

FIGURE 2. Schematic diagram of mitochondrial biogenesis. PGC1α is the main regulator of mitochondrial biogenesis. It is modified by Sirtuin1 and AMPK through deacetylation or phosphorylation respectively, which leads to its translocation to the nucleus. PGC1α in the nucleus associates with various transcription factors/proteins including Nuclear respiratory factors (NRF1 and NRF2), Estrogen-related receptors (ERRs), and Peroxisome proliferator-activated receptors (PPARs), inducing the key events of mitochondrial biogenesis such as mtDNA synthesis.

sion, which then induces mtDNA transcription and replication.[74] ERR also induces mtDNA synthesis while PPARs control mitochondrial function and biogenesis.[23,66] Not surprisingly, many of these abovementioned factors that play an essential role in mitochondrial biogenesis are strongly associated with hearing loss. PGC-1α protein levels are shown to decline after noise exposure.[47] PGC-1α overexpression, on the other hand, induced the NRF-1 and TFAM levels and reduced the cellular senescence and apoptosis in a rat cochlear marginal cell senescence model harboring mtDNA4834 deletion.[88] In accord with this, Zhang et al. showed that the expression of PGC1α, NRF1, NRF2, and TFAM decreased in mouse immortalized cochlear cells (HEI-OC1) following exposure to cisplatin, causing impairment in mitochondrial biogenesis.[77] Inducing mitochondrial biogenesis using drugs that act on the upstream or downstream members of the PGC1α pathway has shown benefits for hearing. For instance, Sirtuin1 agonist resveratrol protects against age-related, cisplatin-induced, aminoglycoside-induced, and noise-induced hearing losses in various animal models including mice, rats, and guinea pigs.[52,67,84] Notably, a small molecule (ZLN005) that selectively increases PGC1α expression activates mitochondrial biogenesis and attenuated the cisplatin-induced loss of immortalized cochlear cells (HEI-OC1).[85] Another example is that the activation of PPAR using pioglitazone is shown to promote outer hair cells survival and protect against noise-induced hearing loss in Wistar rats.[58]

Mitochondria are highly dynamic organelles that can modify their morphology from fragmented states to continuous networks based on the need of the cell. Mitochondria adapt to these different states by undergoing continuous events of fusion (forming larger mitochondria) and fission (breaking up mitochondria into smaller bodies), also referred to as "mitochondrial dynamics". In healthy cells, the balance of these two opposite events contributes to

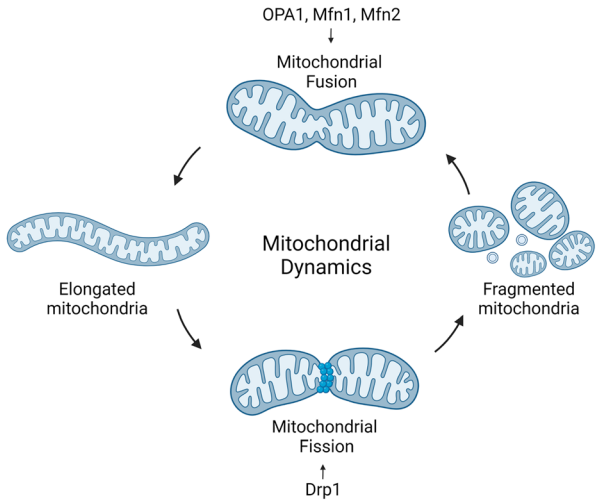

FIGURE 3. Schematic diagram of mitochondrial dynamics. Mitochondria undergo constant fusion and fission processes. Fusion is the process of two mitochondria merging, leading to the formation of elongated mitochondria. OPA1 and MFN1/2 are the key proteins regulating the fusion of the inner and outer membranes, respectively. Fission refers to the division of mitochondria into smaller bodies and is regulated by the Drp1 protein.

maintaining a tubular mitochondrial network to best address the metabolic needs of the cell. Shifting the balance toward fission results in fragmented networking characterized as small round-shape mitochondria while shifting toward fusion leads to a hyper-fused enlarged mitochondrial network. Yet, the same phenomenon is observed in auditory cells under stress.[33] The key proteins regulating the mitochondrial fusion process are Mitofusins (MFN1 and MFN2) on the outer membrane and optic atrophy protein 1 (OPA1) on the inner membrane (Fig. 3). The lack of MFN1 and MFN2 results in the complete loss of mitochondrial fusion, slower cell growth, reduced respiration, and mitochondrial dysfunction.[8] Interestingly, patients with a variant of MFN2 (D414V) suffer from hearing loss and the fibroblasts derived from those patients

exhibit fragmented mitochondrial networks and impaired bioenergetics such as lower basal and maximal oxygen consumption rates.[69] Similarly, patients with a mutation in the OPA1 gene (R445H) displayed elevated hearing thresholds while cochlear outer hair cell receptor potentials were normal.[2,29] Remarkably, skin fibroblasts from these patients exhibited similar phenotypes to patients with MFN (D414V) variant such as elevated fragmentation of the mitochondrial network and impaired bioenergetics.[2] In line with these results, elevated ROS and decreased reduced membrane potential are observed in OPA-1-deficient immortalized cochlear cells.[15] The loss of OPA-1 exacerbated cisplatin-induced mitochondrial dysfunction in mice cochlear explants.[15] Dynamin-related protein 1 (DRP1) is the core protein regulating the mitochondrial fission process. DRP1 is recruited to the outer mitochondrial membrane and oligomerizes to form a spiral that constricts mitochondria, resulting in fission (Fig. 3).[40] Using the single-molecule RNA FISH method, Perkins et al. showed an age-dependent reduction in the number of Drp1 RNA molecules in outer hair cells, indicating downregulation of mitochondrial fission.[59] Administration of mitochondrial division inhibitor 1 (Mdivi1), a small molecule that inhibits DRP1, elevated outer hair cell loss, inhibited mitochondrial degradation, and aggravated ARHL in aged mice.[45] Collectively, these results provide evidence for the importance of mitochondrial dynamics for effective mitochondrial function and hearing. Therefore, factors controlling the imbalance in fusion and fission processes can be the candidates of therapeutic targets for preventing mitochondrial dysfunction and hearing loss.

Mitophagy is a process of selective degradation and removal of damaged or dysfunctional mitochondria through an autophagy-lysosomal pathway. It is one of the crucial events for maintaining mitochondrial homeostasis. A widely accepted mechanism for the selective degradation of damaged mitochondria is based on the notion that dysfunctional mitochondria display a relatively depolarized membrane potential, resulting in the stabilization and accumulation of PINK1 (PTEN-induced putative protein kinase 1) and Parkin on the mitochondrial surface.[49] Parkin is an E3 ubiquitin ligase, and it mediates the ubiquitination of several outer membrane proteins that leads to the recruitment of relevant adapter and autophagy receptor proteins such as p62 and LC3B[42] (Fig. 4). This initiates the mitophagy process and recruits autophagosomal membranes that encapsulate damaged mitochondria and fuse with the lysosome to degrade its contents. Alternatively, there is a Parkin-independent mechanism of mitophagy that particularly occurs under hypoxia conditions and includes Bnip3 protein directly interacting with LC3B, which in turn initiates the mitophagy process (Fig. 4). Many members of mitophagy including PINK1, Parkin, LC3B are shown to decrease in the mouse auditory cortex upon aging.[83] Similarly, Oh et al. showed that Pink1, Parkin, BNIP3 as well as LC3B declines in the cochlea of aged mice, suggesting that age-related decline in mitophagy contributes to ARHL.[54] Mitophagy is also implicated in drug-induced hearing loss. Although not consistently observed, aminoglycoside ototoxicity impairs mitophagy in auditory cells.[28,68,82,87] Regardless, induction of mitophagy through genetic manipulation or using drugs protects murine cochlear hair cells from aminoglycoside-induced damage.[82,87]

Mitophagy is a crucial process to maintain mitochondrial quality and quantity, and thus its induction ameliorates mitochondrial dysfunction and is targeted for therapeutic approaches against diseases including hearing loss. One potent approach to induce mitophagy is using compounds that lower mitochondrial membrane potential which leads to PINK1 stabilization on the mitochondrial surface and initiates mitophagy. For instance, a prodrug for dinitrophenol (DNP), a small-molecule uncoupler, lowers the protonmotive force (Δp) across the mitochondrial inner membrane and is shown to preserve auditory function after noise exposure.[25] Niclosamide is another mitochondrial uncoupling small molecule that reduces mitochondrial membrane potential in cultured fibroblast cells and prevents noise or cisplatin-induced hair cell loss and restores elevated hearing thresholds in mice.[64,71] Several natural metabolites that induce mitophagy have a beneficial impact on hearing loss. For instance, Urolithin A, a natural food metabolite that is abundant in pomegranate, increases mitochondrial function and attenuated premature senescence in H_2O_2-induced auditory cells.[10] Although their specificity is unclear, these compounds suggest the importance of mitophagy in mitigating mitochondrial dysfunction and preventing hearing loss.

NUCLEAR TO MITOCHONDRIA (NM) SIGNALING AND HEARING LOSS

Emerging evidence shows that signaling from the nucleus to mitochondria (NM signaling) that is triggered by DNA damage has an impact on mitochondrial function. It is postulated that persistent DNA damage due to a lack of DNA repair proteins causes sustained activation of poly-ADP ribose polymerase 1 (PARP1), an enzyme that mediates the initial steps of DNA repair.[19] Activated PARP1 consumes nicotinamide dinucleotide (NAD +) to such an extent to cause depletion of cellular NAD + levels.[19] NAD +

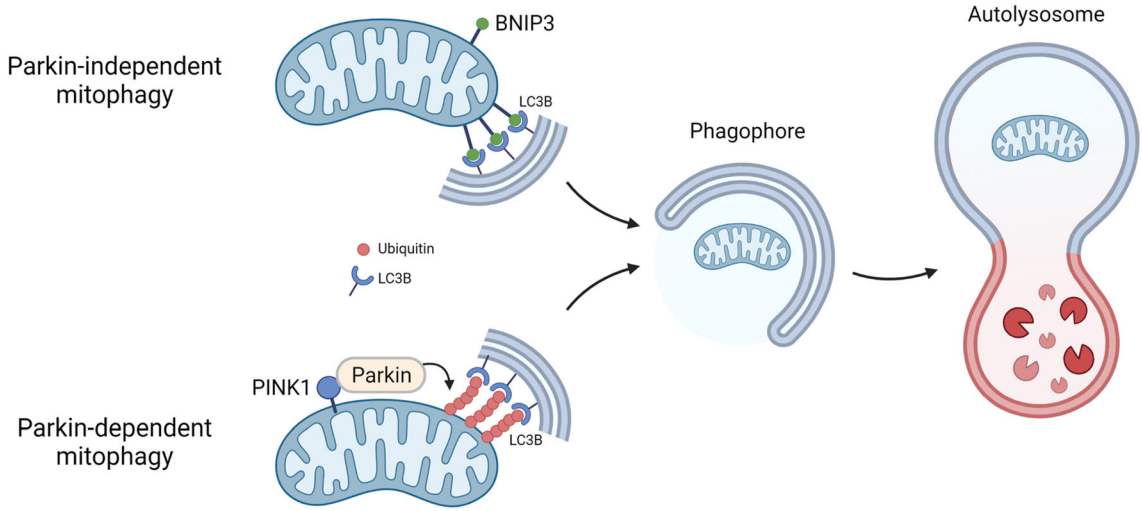

FIGURE 4. Schematic diagram of mitophagy. Mitophagy is a process of selective degradation and removal of damaged or dysfunctional mitochondria in the lysosome. The process is initiated by two different mechanisms. Parkin-dependent mechanisms involve depolarization of mitochondrial membrane potential leading to stabilization and accumulation of PINK1 on the outer membrane which then recruits Parkin protein to the surface of mitochondria. Parkin ubiquitinates several outer membrane proteins which then provides a binding platform for autophagosomal membranes. Autophagosomal membranes eventually encapsulate the entire mitochondrion and mediate the degradation of the organelle in the lysosome. Unlike Parkin-dependent mechanisms, the Parkin-independent mechanism involves Bnip3 directly binding the adaptor proteins of autophagosomal membranes in the initiation process.

is a key molecule for cellular metabolism and its depletion inhibits a range of enzymes including Sirtuin1 which modulates downstream pathways and mitochondrial functions (as mentioned above).[19] Interestingly, the actors in the axis of PARP1/NAD + /Sirtuin1 are implicated in hearing loss progression. For example, PARP1 deficiency protects mice cochlear explants from cisplatin-induced cell death and prevents damage in rat cochlear marginal strial cells under oxidative stress.[72,86] Cellular NAD + levels decline in the cochlea with age or in response to noise exposure.[5,57] Lastly, the age-dependent decline in Sirtuin1 expression is observed in the mouse cochlea.[80] Additionally, many DNA-repair disease models such as Cockayne syndrome, xeroderma pigmentosum group A (XPA), and ataxia-telangiectasia with dysregulated NM signaling exhibited both mitochondrial dysfunction and hearing loss phenotype.[1,17,55,65,70] Remarkably, strategies targeting the components of NM signaling such as PARP inhibition, NAD + supplementation, or Sirtuin1 activation displayed promising benefits on hearing, highlighting the therapeutic potential of this signaling pathway on hearing loss prevention.[5,56,57,72,80]

CONCLUDING REMARKS

Given the central role of mitochondria in cellular metabolism, it is not surprising that mitochondria are tightly regulated and protected to ensure their proper function. Balancing net ROS production, DNA repair, mitochondrial biogenesis, mitochondrial dynamics, and mitophagy are crucial processes that allow cells to ensure the quality and quantity of the mitochondria based on cellular needs. These processes are closely integrated to an extent that one depends on another. For instance, DRP-1 mediated fission of mitochondria is shown to be a prerequisite to mitophagy, perhaps due to small mitochondrial size enabling an easier mitochondrial encapsulation by autophagosomal membranes. In line with this, mitochondrial hyper-fusion inhibits mitophagy.[12,43] A similar but more indirect interaction is seen between mitochondria and other cellular components. For instance, the sustained damage in nuclear DNA impairs mitophagy and causes an increase in mitochondrial content in diseases with hearing loss feature.[17,18,65,78] Therefore, approaches targeting mitochondrial dysfunction should consider the interplay between these processes.

This review intentionally focused on targets of pharmacological applications as a strategy to target mitochondrial dysfunction for hearing loss treatment. Gene therapy techniques, dietary regimens and exercise, and mitochondrial therapies are other approaches shown to improve mitochondria's functions although not elaborated on here as a hearing treatment approach since they are beyond the scope of this review. However, among those approaches, the mitochondrial therapy technique deserves to be briefly discussed as it has never been applied to treating

hearing loss. Mitochondrial therapy (mitotherapy) is a strategy of exogenous transplantation of healthy mitochondria into damaged organs and has shown promising results in treating mitochondria-related diseases.[53] While isolated mitochondria can be directly applied to damaged tissue, alternative delivery methods such as the encapsulation of mitochondria using extracellular vehicles (EVs) were also developed and have shown promising results. Kalinec et al. recently showed that extracellular vehicles (EVs) from auditory cells have particle sizes up to 900 nm and could function as a carrier for intracochlear drug delivery.[31] These EVs could also work as small-size mitochondria carriers and facilitate mitochondrial delivery to the cochlea. Further research can thus shed light on this approach to mitigating mitochondrial dysfunction as a novel treatment for hearing loss.

CONFLICT OF INTEREST

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this review.

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