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Hearing Loss: Mechanisms, Prevention and Cure

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Chapter 1
Hair Cell Regeneration

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Abstract Cochlear hair cells are mechanoreceptors of the auditory system and cannot spontaneously regenerate in adult mammals; thus hearing loss due to hair cell damage is permanent. In contrast, hair cells in nonmammalian vertebrates such as birds and in the zebrafish lateral line have the ability to regenerate after hair cell loss. Many regulatory factors, including signaling pathways, transcription factors, and epigenetic regulators, play roles in hair cell regeneration in various species. In this chapter, we review the history of hair cell regeneration research, the methods

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used in the study of hair cell regeneration, the properties and modulating factors of inner ear stem cells, and the re-formation of cochlear ribbon synapses and hearing function recovery.

Keywords Auditory system · Cochlea · Hair cell regeneration · Transcription factors · Signaling pathways · Epigenetic regulation

1.1 History of Hair Cell Regeneration

Aging, loud noise, environmental chemical toxins, aminoglycosides, and innate genetics can damage hair cells and cause hearing impairment [1]. The mammalian cochlea has a very limited ability to regenerate only during embryonic development and the early neonatal period, and adult mammalian hair cells lack regenerative ability resulting in permanent hearing impairment after injury [2]. The ideal method for the treatment of sensorineural deafness would be to restore hearing at a fundamental level by repairing the structure and function of the cochlea through the regeneration of hair cells from stem cells or cochlear precursor cells. Therefore, finding ways to make hair cells regenerate after injury is the focus of much recent hearing research [3].

Current research suggests that nonmammalian vertebrates can regenerate new hair cells from adult stem cells or precursor cells when the hair cells in the cochlea are damaged by noise, drugs, or aging [4], but the mammalian cochlea cannot spontaneously produce new mature hair cells [5]. Forge et al. and Warchol et al. (1993) found that mammalian cochleae can regenerate cells with an immature hair cell phenotype after injury by gentamycin [6]. White et al. discovered the potential of hair cells to be transformed and regenerated from supporting cells during the in vitro culture of postnatal mouse supporting cells [7]. However, recent studies have shown that new hair cells can be regenerated after cochlear damage in neonatal mice [8, 9].

Li et al. [10] and Oshima et al. [11, 12] found that the sensory epithelium of the adult mouse utricle contains inner ear stem cells and that these stem cells can self-renew and can be subcultured multiple times. These cells express key transcriptional regulators required for differentiation into hair cells, such as Atoh1 and Brn3.1, and hair cell structural proteins, such as unconventional myosin VIIA, microalbumin 3, and espin, and they can grow in bundles. Cochlear Lgr5-positive cells and tympanic border cells can act as postnatal mouse cochlear progenitor cells [8, 9, 13, 14]. Oshima et al. successfully induced mouse embryonic stem cells and induced pluripotent stem cells (IPS) to differentiate into otic progenitor cells. Some of the embryonic stem cells or IPS-derived inner ear precursor cells could differentiate into cilia-like hair cells. When these hair cells were stimulated mechanically, they also had the ability to emit signals similar to those of immature hair cells. This showed that these induced hair cells had at least some level of hair cell function [15].

1.2 Methods Used in Hair Cell Regeneration

1.2.1 Method for Lineage Tracing of Specific Gene (e.g., X) Positive Inner Ear Stem Cells

In X-EGFP-CreERT2, Rosa26-tdTomato mice, there is a stop codon surrounded by two Loxp sites in front of the tdTomato gene, so tdTomato do not express without tamoxifen treatment. Injection with tamoxifen activates the Cre recombinase so that the stop codon is excised and the X positive cells and their daughter cells are labeled with the red fluorescent tdTomato protein.

1.2.2 Method for Sorting EGFP-Positive Cochlear Stem Cells Using Flow Cytometry

EGFP reporter mice are helpful for investigating whether one gene can be used as a marker of inner stem cells. In EGFP mice, the *GFP* gene is added after the promoter of the gene of interest, and thus the cells that express the gene of interest also express GFP. The cochleae of EGFP mice are then isolated, digested with trypsin into single cells, and subjected to flow cytometry where the GFP green fluorescence signal is used to separate the cells expressing the gene of interest.

1.2.3 Culture of Cochlear Stem Cells In Vitro and Methods for Detecting Their Proliferation and Differentiation

Sorted putative stem cells are cultured on laminin-coated plates using DMEM/F12 medium supplemented with N2, B27, heparin sulfate, and the growth factors bFGF, EGF, and IGF-1 for 4 days. EdU is then added to the medium for 3 consecutive days as an indicator of cell proliferation. On day 10 the cells are stained for EdU to measure the proliferative ability of cochlear stem cells cultured in vitro.

1.2.4 Method for In Vitro Culture of Cell Spheres After Flow Cytometry Sorting of Cochlear Stem Cells

After flow cytometry sorting, cells are added to a culture dish without laminin coating, and the cells are cultured in DMEM/F12 medium with N2, B27, bFGF, EGF, IGF-1, and heparan sulfate. Cellular spheres are passaged to the next generation after trypsinization, and the growth and proliferation of stem cells can be determined by observing the number of cell spheres and measuring their diameters. Renjie Chai's team used this method to sort Lgr5-positive cochlear stem cells and found that these cells have the ability to proliferate and differentiate into hair cells.

1.3 Inner Ear Stem Cells

1.3.1 Inner Ear Supporting Cells as Precursor Cells

There are two ways for supporting cells to serve as precursor cells for hair cell regeneration. The first is for supporting cells to become activated and reenter the cell cycle and to begin to proliferate through mitosis to form new supporting cells and to further differentiate into hair cells. The second way is through direct transdifferentiation into hair cells. White et al. screened GFP(+) P27-transgenic neonatal mouse cochlear supporting cells and tested the ability of the dividing cells to reenter the cell cycle and generate hair cells [7]. Co-cultured purified supporting cells and auricular mesenchymal cells were found. The supporting cells aggregated into small epithelial islands, and this was accompanied by the downregulation of P27 expression and the appearance of BrdU labeling, suggesting that the mitotic supporting cells had entered the cell cycle and proliferated. The expression of the hair cell marker myosin VI was measured, and 20% of the cells expressed myosin VI. The cells expressing myosin VI also showed positive BrdU staining. The presence of BrdU(+) and BrdU(−) regenerated hair cells indicates that the supporting cells can generate new hair cells through direct differentiation or through mitotic pathways [7, 16].

1.3.2 Supporting Cell Subtypes

White et al. detected the hair cell-specific surface antigens p75 and Math1-GFP(+) in pillar cells and Hensen's cells and found that these two subtypes of supporting cells have the capacity to regenerate hair cells and more supporting cells [7]. Lgr5 was detected in the neonatal mammalian cochlea and was found in non-sensory epithelial cells, limbic cells, columnar cells, finger cells, and Deiter's cells in the cochlear epithelium [8, 13].

Chai et al. used the Wnt signaling pathway downstream gene *Lgr5* reporter mice to show that *Lgr5*-positive cells are an enriched hair cell precursor cell population in in vitro cell culture. The self-proliferation and differentiation of these *Lgr5*-positive cells can also be regulated by exogenous Wnt inhibitors and enhancers, suggesting that *Lgr5*-positive cells can also be potential cell sources for stem cells to treat hearing disorders [8, 17]. In another study, Chai et al. used *Axin2* reporter mice, which are a downstream negative feedback gene of the Wnt signaling pathway. In vitro cell culture and in vivo animal experiments demonstrated that Axin2-positive cells also have similar characteristics as cochlear stem cells. These cells can self-proliferate into single cells, and colonies can be cloned and can be differentiated into cochlear supporting cells and hair cells. The highly proliferative capacity of these cochlear stem cells allows them to be passaged many times after forming monoclonal populations. At the same time, the ability of these Axin2-positive cells to self-proliferate and differentiate can be regulated by exogenous Wnt inhibitors

and enhancers. Therefore, it is suggested that Axin2-positive cells might also be a potential source of stem cells for treating hearing disorders. In summary, the discovery of stem cells in the inner ear has provided a new approach for cell transplantation therapy [14].

Li et al. studied whether cotransfection of *Pax2* and *Atoh1* promotes in situ cochlear hair cell regeneration after neomycin insult. The ideal strategy for hair cell regeneration is to promote residual supporting cell proliferation and then to induce hair cell differentiation. In this study, cultured newborn mouse organs of Corti were treated with neomycin to damage the hair cells and then incubated with recombinant adenovirus expressing *Pax2* and/or *Atoh1*. Overexpression of *Pax2* significantly promoted the proliferation of supporting cells. Compared with the Ad-Pax2 and Ad-Atoh1 groups, the number of BrdU1/myosin VIIA1 cells in the pre-existing hair cell region was significantly increased 2 weeks after adenovirus infection in the Ad-Pax2-IRES-Atoh1 group. This indicates that cotransfection of *Pax2* and *Atoh1* induces in situ proliferation and differentiation into hair cells. Most new hair cells are labeled with FM1–43, indicating that they maintain their mechanotransduction functions. The results also indicate that differentiation of proliferating cells, rather than quiescent cells, into hair cells by forced expression of *Atoh1* is a feasible method for mammalian hair cell regeneration [18].

1.4 Transcription Factor Regulation of Inner Ear Stem Cells

Atho1 is an indispensable transcription factor with a helix-loop-helix structure and is essential for hair cell differentiation. In mice lacking the *Atoh1* gene, the sensory epithelium of the cochlea only differentiates into supporting cells and fails to form hair cells. The *Atoh1* gene is thus considered to be a necessary transcription factor for hair cell production. Studies have shown that ectopic expression of Atho1 in the cochlear basilar membrane or vestibular sensory epithelium induces the regeneration of hair cells in tissue culture and in in vitro experiments. In vivo and in vitro experiments have shown that the expression of Atoh1 in prenatal rats induces the differentiation of the supporting cells into sensory hair cells, but the regenerated hair cells do not mature. This suggests that in addition to the expression of Atoh1, other regulatory factors are still needed [19].

The hairy and enhancer of split homolog genes, *Hes1* and *Hes5*, are Notch downstream transcription factors. The Notch target gene hairy-related basic helix-loop-helix transcription factor *Hey2* is expressed in the cochlear epithelium prior to terminal differentiation. Notch signaling has been shown to regulate inner ear development and hair cell regeneration. Knockdown of *Hes1* and *Hes5* leads to increased numbers of hair cells, and in combination with the loss of *Hes1* or *Hes5*, genetic inactivation of *Hey2* also leads to increased numbers of mis-patterned inner and outer hair cells.

Storkhead box 1 (STOX1) belongs to the forkhead family of transcription factors, and Nie et al. reported that STOX1 plays an important role in regulating the

proliferation of inner ear epithelial cells. STOX1 is selectively expressed in epithelial cells, but not in stromal cells of the inner ear. Its overexpression enhances cell proliferation and sphere formation, while STOX1 knockdown inhibits cell proliferation and sphere formation in purified utricular epithelial cells in culture. Consistent with this, several cell cycle regulatory genes such as PCNA, cyclin A, and cyclin E are upregulated by STOX1 overexpression. STOX1 is a novel stimulatory factor for inner ear epithelial cell proliferation and might be an important therapeutic target for regeneration or repair of inner ear epithelium [20].

Cochlear hair cells proliferate and regenerate rapidly after neonatal hair cell-specific conditional knockout of *p27Kip1* (p27CKO), a tumor suppressor gene upstream of the *Rb* gene. Hair cell-specific p27CKO results in the proliferation of these cells without upregulation of the supporting cell or progenitor cell proteins Sox2 or Prox1, indicating that they are still hair cells. Furthermore, p27CKO results in the significant addition of postnatal-derived hair cells expressing characteristic synaptic and steric fibrosis markers that survive to adulthood, although some of the newly derived new hair cells lack Vglut3 expression. Nonetheless, p27CKO mice show normal hearing according to evoked auditory brainstem response measurements, suggesting that the newly produced hair cells might contribute to, or at least not significantly detract from, hearing function. These results indicate that p27Kip1 actively maintains hair cell quiescence in postnatal mice and suggests that inhibition of p27Kip1 in residual hair cells represents a potential strategy for auditory hair cell regeneration [21].

In the inner ear, the biochemical and molecular pathways involved in retinoblastoma (pRB) family, particularly p107 and p130, are relatively unexplored, and their therapeutic suitability has not been determined. In Rocha-Sanchez et al.'s study, they analyzed the cochleae of adult *p130* knockout (*p130−/−*) mice and showed that lack of the *p130* gene results in extra rows of hair cells and supporting cells in the more apical regions of the cochlea. No evidence of transdifferentiation of these supernumerary supporting cells into hair cells was observed in the *p130−/−* mouse. However, the proliferation of supporting cells in the adult *p130−/−* cochlea combined with the downregulation of cell cycle inhibitors provides a mechanism for the role of p130 in the apical region of the cochlea as a regulator of supporting cell and hair cell mitosis. Interestingly, *p130−/−* mice exhibit nearly normal peripheral auditory sensitivity [22].

Myc and Sox2 are expressed in the developing inner ear, and Kwan et al. created immortalized multipotent otic progenitor (iMOP) cells, a fate-restricted cell type, by transient expression of Myc in Sox2-expressing otic progenitor cells. This activates endogenous Myc and amplifies existing Sox2-dependent transcripts to promote self-renewal. RNA-seq and ChIP-seq analyses showed that Myc and Sox2 occupy over 85% of the same promoters. Myc and Sox2 target genes include cyclin-dependent kinases that regulate cell cycle progression. iMOP cells continue to divide but retain the ability to differentiate into functional hair cells and neurons. Their group verified that Sox2 and Myc regulate the cell cycle progression of these cells and downregulate Myc expression as a molecular switch after growth factor withdrawal [23].

1.5 Signaling Pathways That Regulate Inner Ear Stem Cells and Hair Cell Regeneration

Generally speaking, there are two mechanisms of mammalian hair cell regeneration (Fig. 1.1). One is transdifferentiation in which the surrounding supporting cells switch fates to become hair cells. The other is mitotic regeneration in which inner ear progenitors or supporting cells proliferate and differentiate into new hair cells. Many signaling pathways are involved in hair cell regeneration, including the Wnt, Notch, BMP, FGF, IGF, and Hedgehog signaling pathways.

1.5.1 The Wnt Signaling Pathway in Auditory Hair Cell Development and Regeneration

During cochlear development, the canonical Wnt/β-catenin signaling pathway regulates cell proliferation, cell fate decision, and hair cell differentiation. The inhibition of Wnt/β-catenin signaling by small molecules or in transgenic mice in the embryonic cochlea reduces the proliferation of prosensory cells [24]. Conversely, the activation of Wnt/β-catenin signaling promotes the formation of the prosensory domain and increases the number of hair cells [25]. The Wnt targets Lgr5 and Lgr6 are expressed in embryonic and neonatal cochlear progenitors [13, 26]. Lgr5+ cells can act as hair cell progenitors both in vivo and in vitro because of their ability to

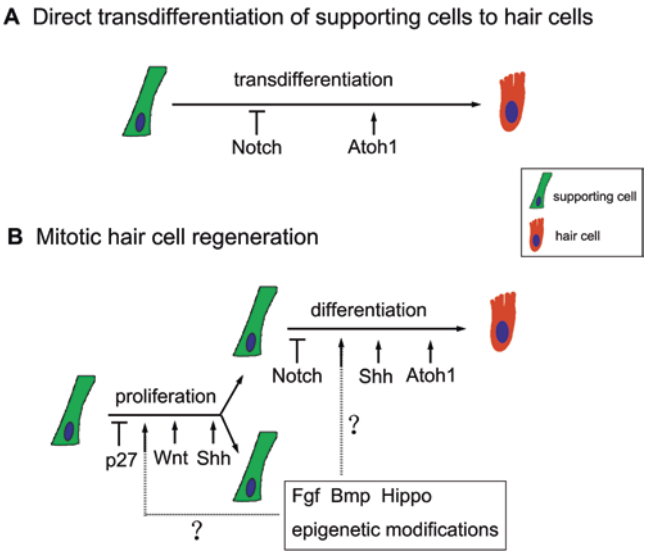

Fig. 1.1 Two mechanisms of mammalian hair cell regeneration. Red cells represent hair cells; green cells represent supporting cells, respectively

self-renew, proliferate, and regenerate hair cells [8, 9, 27, 28], and treatment with Wnt agonists in the neonatal cochlea enhances the proliferation of Lgr5+ progenitor cells and hair cell differentiation [8].

Enhancement of canonical Wnt signaling facilitates cell proliferation and hair cell regeneration in both mammalian and nonmammalian vertebrates [29, 30]. Wnt agonists or β-catenin overexpression promotes the proliferative capacity of Lgr5+ progenitor cells and hair cell formation, whereas Wnt antagonists reduce the ability of Lgr5+ cells to proliferate and to regenerate hair cells [8, 28, 31]. In newborn mice, Wnt activation also causes the Axin2-positive tympanic border cells to proliferate and differentiate into hair cells and supporting cells [14]. The combined expression of β-catenin and Atoh1 in Lgr5+ cells can enhance the hair cell regeneration capacities of the postnatal cochlea by tenfold, and these newly regenerated hair cells can survive until adulthood [32]. However, in the adult mammalian cochlea, the combined expression of β-catenin and Atoh1 cannot induce hair cell regeneration.

1.5.2 The Notch Signaling Pathway in Auditory Hair Cell Regeneration

The role of the Notch signaling pathway in hair cell regeneration has been examined because of its participation in hair cell differentiation during inner ear development. In both the zebrafish lateral line and mature avian basilar papilla, Notch inhibition increases the regeneration of hair cells at the expense of supporting cells through mitotic division and direct transdifferentiation. In contrast, the constitutive activation of the Notch pathway in supporting cells maintains these cells in a quiescent state, thereby inhibiting hair cell regeneration [33, 34]. In the mammalian postnatal cochlea, the blockade of Notch signaling by a γ-secretase inhibitor upregulates the expression of the Atoh1 transcription factor and results in the transdifferentiation of adjacent supporting cells into hair cells, although the new hair cells possess the characteristics of immature hair cells [35, 36]. Li et al. reported the direct interaction between the Wnt and Notch signaling pathways during hair cell regeneration [37]. The conditional inhibition of Notch signaling in the postnatal cochlea accelerates the formation of new hair cells, and the majority of new hair cells are derived from Wnt-responsive Lgr5+ supporting cells. In addition, the combined inhibition of Wnt and Notch signaling decreases mitotic hair cell generation, indicating that the proliferation of progenitor cells induced by Notch inhibition is dependent on the Wnt pathway [37].

1.5.3 The Hedgehog Signaling Pathway in Auditory Hair Cell Regeneration

Hedgehog signaling, together with other signals from the hindbrain, is important for the formation of the dorsoventral axis of the inner ear during development. Hedgehog signaling also plays important roles in the formation of the prosensory domain [38], the proliferation of progenitor cells, and hair cell differentiation during inner ear development [39]. A balance between Wnt and Shh signaling activities is crucial in determining whether progenitor cells will differentiate into vestibular or auditory cell types [40, 41].

A few studies have reported the effect of Hedgehog signaling on mammalian hair cell regeneration. Shh signaling promotes renewed proliferation of supporting cells and hair cell regeneration in the postnatal rat cochlea after neomycin exposure [42], and recombinant Shh protein effectively promotes sphere formation, proliferation, and differentiation of Lgr5+ progenitor cells isolated from the neonatal mouse cochlea. Using transgenic R26-SmoM2 mice that constitutively activate Hedgehog signaling in the supporting cells of the cochlea, Chen et al. reported that the activation of Hedgehog signaling leads to significant supporting cell proliferation and hair cell regeneration in neomycin-injured cochlear epithelium explants [43].

1.5.4 The FGF Signaling Pathway in Hair Cell Regeneration

Many FGF receptors and ligands are expressed in a spatially coordinated pattern in the embryonic and neonatal mouse inner ear [44, 45]. FGF10 and FGF3, in addition to FGF8, are necessary for the earliest induction stage of the otic placode and otic vesicle in the mouse, zebrafish, and chicken inner ear [46–54]. FGF signaling plays dosage-sensitive roles in the differentiation of the auditory sensory epithelium and is critical to the fate determination of cochlear supporting cells. FGF23 deficiency leads to mixed hearing loss and middle ear malformations in mice [55], and FGFR1-Frs2/3 signaling through the activation of MAP kinase is necessary for the maintenance of sensory progenitors and commits precursor cells to sensory cell differentiation in the mammalian cochlea [56].

However, no studies on FGF signaling in mammalian hair cell regeneration have been reported. In the chick inner ear, b-FGF is related to the proliferation of supporting cells, the formation of new hair cells, and the extension of nerve fibers after acoustic trauma [57]. FGF2 stimulates proliferation of utricular epithelial cells cultured in vitro, and this effect is enhanced when FGF2 is added in combination with

IGF-1 or TGFα [58]. Myc and FGF are required for zebrafish neuromast hair cell regeneration, and using a zebrafish lateral line neuromast hair cell regeneration model, Lee et al. showed that the specific inhibition of Myc or FGF suppresses hair cell regeneration, demonstrating that both pathways are essential to the process. Manipulation of FGF pathways should be explored for mammalian hair cell regeneration in future studies [59].

1.6 Epigenetic Regulation in Hair Cell Regeneration

Recently, epigenetic factors have emerged as important gene regulators in otic induction, patterning, and hair cell formation both in inner ear development and hair cell regeneration. In the developing zebrafish larvae, pharmacological inhibition of the histone-modifying enzyme lysine-specific demethylase 1 (LSD1) using trans-2-phenylcyclopropylamine (2-PCPA) disrupts cell proliferation, induces apoptosis, and reduces the numbers of sensory hair cells and supporting cells in the neuromasts [60]. Stojanova et al. reported that epigenetic regulation of Atoh1 guides hair cell development in the developing mouse cochlea [7]. Dynamic changes in the histone modifications H3K4me3/H3K27me3, H3K9ac, and H3K9me3 represent a progression from poised to active to repressive markers, respectively, and correlate with the onset of Atoh1 expression and its subsequent silencing during the perinatal period. Inhibition of histone acetyltransferase activity reduces H3K9 acetylation at the *Atoh1* locus and prevents the increase in *Atoh1* mRNA, suggesting a central role for histone acetyltransferases in *Atoh1* transcription and hair cell differentiation. Interestingly, the H3K4me3/H3K27me3 bivalent chromatin structure that is observed in progenitor cells persists at the *Atoh1* locus in perinatal supporting cells [7], highlighting the potential of such structures as therapeutic targets in hair cell regeneration.

Treatment of hair cell-damaged chicken utricles with broad-spectrum histone deacetylase (HDAC) inhibitors or class I selective HDAC inhibitors results in a decrease in supporting cell proliferation but does not affect the formation of new hair cells [9]. Similarly, in hair cell-damaged zebrafish larvae, inhibition of HDAC activity with broad-spectrum HDAC inhibitors reduces supporting cell proliferation and subsequent hair cell regeneration [8]. The Polycomb group protein Bmi1, a component of the Polycomb repressive complex 1, maintains the proliferation ability of supporting cells by sustaining high levels of canonical Wnt signaling in the neonatal mouse cochlea. In neonatal *Bmi1*-deficient cochlear explants, supporting cells fail to reenter the cell cycle in response to hair cell damage, and the sphere-forming ability of cochlear progenitor cells is reduced [10].

1.7 Ribbon Synapse Re-formation in Regenerated Mammalian Hair Cells

The formation of synaptic connections between newly generated hair cells and neurons is critical for the recovery of hearing and balance function. In the mammalian cochlea, the inner hair cells are a key component in sound perception, while the outer hair cells are related to the amplification of auditory signals. The neurotransmissions between inner hair cells and spiral ganglion neurons are conveyed by the ribbon synapses, which are crucial for the accurate encoding of auditory information [11]. Multiple factors and signaling pathways play roles in the establishment of inner hair cell ribbon synapses during cochlear development, including neurotrophin-3 and brain-derived neurotrophic factor (BDNF) [12, 15].

In recent years, research into hair cell regeneration has reached certain milestones, but the inner hair cell marker vesicular glutamate transporter (Vglut3) is not detected in these newly regenerated hair cells [27]. *p27Kip1* deletion-induced new hair cells express characteristic synaptic (Ctbp2) and stereociliary (espin) markers, re-forming the "synaptic structure" to some extent, and the new hair cells survive to adulthood. However, a portion of the postnatally derived inner hair cells lack Vglut3 expression [8]. The ectopic expression of Atoh1-induced ectopic hair cells express the synaptic markers CSP, synaptophysin, and synaptotagmin 1, but although some synaptic markers and neuron terminals are found at the base of the new hair cells, the normal synaptic ribbons are still absent [8].

Many factors have been reported to play roles in promoting axonal regeneration and synapse reformation after neuron damage [61]. Members of the neurotrophin family, such as nerve growth factor, BDNF, and neurotrophin-3, are reported to promote the re-formation of ribbon synapses after brain or spinal cord injury [62–64]. In the inner ear, the addition of BDNF and neurotrophin-3 promotes the reinnervation of spiral ganglion neurons and the expression of postsynaptic markers in cultured cochleae after ototoxic drug insult [65]. Supporting cell-derived neurotrophin-3 promotes the regeneration of ribbon synapses and the recovery of hearing function after acoustic trauma [66], indicating that the neurotrophins are important for the re-formation of cochlear ribbon synapses after injury. Whether these neural growth factors can support the re-formation of ribbon synapses of newly regenerated hair cells needs further investigation in the future.

1.8 Hearing Function Recovery

1.8.1 Hearing Function Recovery in the Avian Vestibular and Cochlear System

Noise-induced or ototoxic aminoglycoside-induced trauma to the inner ear in birds leads to hair cell loss followed by hair cell regeneration. These processes are paralleled by hearing loss followed by significant functional recovery, and after acoustic

trauma, functional recovery is rapid and nearly complete [67]. Carey et al. examined the relation between hair cell regeneration and recovery of the vestibuloocular reflex (VOR) in the avian ear and showed that at 8–9 weeks after streptomycin sulfate administration, the gain and phase of the VOR had returned to normal values, which was accompanied by the recovery of vestibular hair cells [68].

1.8.2 Hearing Function Recovery in the Mammalian Inner Ear

The effect of hair cell regeneration on mammalian vestibular and hearing function restoration has been evaluated by electrophysiological tests such as the VOR or auditory brainstem response, but the current results are still controversial [36, 69–72]. Using an adenovector delivery system that expresses Atoh1, mouse vestibular hair cells damaged from aminoglycoside ototoxicity can be regenerated, and their vestibular function can be improved [70]. Izumikawa et al. reported that Atoh1 overexpression induces auditory hair cell regeneration and leads to significant restoration of hearing function in the damaged mature inner ear after *Atoh1* gene delivery to nonsensory cells through adenovectors [72]. However, another study showed that deaf guinea pigs treated with *Atoh1* gene therapy had a significant increase of hair cell number, but there was no improvement in hearing thresholds or the formation of synaptic ribbons [69]. Du et al. reported that *Hes1* downregulation with siRNA results in significant hair cell restoration and hearing recovery at 3–9 weeks after noise exposure in rodent cochleae [71]. Similarly, Notch signaling inhibition by a γ-secretase inhibitor stimulates hair cell regeneration and leads to partial recovery of hearing in ears damaged by noise trauma, indicating that manipulating the cell fate of cochlear sensory cells through pharmacological inhibition of Notch signaling is a potential therapeutic approach to the treatment of hair cell loss-induced deafness [36].

1.9 Brake and Future Directions

Since the 1980s, researchers have made significant advancements in the study of hair cell regeneration. However, current results are still quite far from restoring hearing function in the damaged mammalian inner ear. Some problems still exist: (1) new hair cells are immature and lack functional stereocilia; (2) new hair cells do not survive for long; (3) the majority of new hair cells are prestin-positive outer hair cells, and very few are Vglut3-positive inner hair cells; and (4) reinnervation of new hair cells does not take place.

Considering the above problems in the field, future research should focus on the following topics. Firstly, more pathway and factors, including those that might regulate the proliferation and differentiation of stem cells and precursor cells, such as TGFβ, BMP4, and Hippo, should be explored in the study of hair cell regeneration.

Moreover, the interaction of multiple pathways in cell proliferation and hair cell differentiation should be explored. Secondly, measures should be taken to promote the maturation and survival of new hair cells. Some genes that are key to hair cell maturation, including *Helios* [73] and *Eps8* [74], might play roles in the maturation of newly generated hair cells. Thirdly, more inner hair cells are required for hearing function recovery because these are the "true" hair cells that convert mechanical signals into electrical signals. Recently, it has been reported that *Insm1* is transiently expressed in nascent outer hair cells and consolidates their fate by preventing trans-differentiation into inner hair cells [75], and the absence of *Insm1* switches outer hair cell fate to become mature inner hair cells. Manipulation of these kinds of genes might help to generate more new inner hair cells. Fourthly, more effort should be put into the re-formation of inner hair cell ribbon synapses and hair cell reinnervation. Lastly, the formation and effective arrangement of cilia, as well as the contact between the stereocilium and the tectorial membrane, are also critical to hearing recovery, and factors that can support these processes should be developed in the future.

References

1. Johnsson LG, Hawkins JE Jr (1972) Sensory and neural degeneration with aging, as seen in microdissections of the human inner ear. Ann Otol Rhinol Laryngol 81(2):179–193
2. Henley CM et al (1996) Sensitive developmental periods for kanamycin ototoxic effects on distortion-product otoacoustic emissions. Hear Res 98(1–2):93–103
3. Murillo-Cuesta S et al (2015) Corrigendum: Transforming growth factor beta1 inhibition protects from noise-induced hearing loss. Front Aging Neurosci 7:72
4. Lambert PR, Gu R, Corwin JT (1997) Analysis of small hair bundles in the utricles of mature guinea pigs. Am J Otolaryngol 18(5):637–643
5. Bermingham-McDonogh O, Rubel EW (2003) Hair cell regeneration: winging our way towards a sound future. Curr Opin Neurobiol 13(1):119–126
6. Warchol ME et al (1993) Regenerative proliferation in inner ear sensory epithelia from adult guinea pigs and humans. Science 259(5101):1619–1622
7. White PM et al (2006) Mammalian cochlear supporting cells can divide and trans-differentiate into hair cells. Nature 441(7096):984–987
8. Chai RJ et al (2012) Wnt signaling induces proliferation of sensory precursors in the postnatal mouse cochlea. Proc Natl Acad Sci U S A 109(21):8167–8172
9. Wang T et al (2015) Lgr5+ cells regenerate hair cells via proliferation and direct transdifferentiation in damaged neonatal mouse utricle. Nat Commun 6:6613
10. Li H, Liu H, Heller S (2003) Pluripotent stem cells from the adult mouse inner ear. Nat Med 9(10):1293–1299
11. Oshima K et al (2007) Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. J Assoc Res Otolaryngol 8(1):18–31
12. Oshima K, Senn P, Heller S (2009) Isolation of sphere-forming stem cells from the mouse inner ear. Methods Mol Biol 493:141–162
13. Chai RJ et al (2011) Dynamic expression of Lgr5, a Wnt target gene, in the developing and mature mouse cochlea. Jaro-J Assoc Res Otolaryngol 12(4):455–469
14. Jan TA et al (2013) Tympanic border cells are Wnt-responsive and can act as progenitors for postnatal mouse cochlear cells. Development 140(6):1196–1206

15. Oshima K et al (2010) Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells. Cell 141(4):704–716
16. Savoy-Burke G et al (2014) Activated notch causes deafness by promoting a supporting cell phenotype in developing auditory hair cells. PLoS One 9(9):e108160
17. Lu X et al (2017) Bmi1 regulates the proliferation of cochlear supporting cells via the canonical Wnt signaling pathway. Mol Neurobiol 54(2):1326–1339
18. Chen Y et al (2013) Cotransfection of Pax2 and Math1 promote in situ cochlear hair cell regeneration after neomycin insult. Sci Rep 3:2996
19. Cheng YF (2017) Atoh1 regulation in the cochlea: more than just transcription. J Zhejiang Univ Sci B
20. Nie X et al (2015) Transcription factor STOX1 regulates proliferation of inner ear epithelial cells via the AKT pathway. Cell Prolif 48(2):209–220
21. Walters BJ et al (2014) Auditory hair cell-specific deletion of p27Kip1 in postnatal mice promotes cell-autonomous generation of new hair cells and normal hearing. J Neurosci 34(47):15751–15763
22. Rocha-Sanchez SM et al (2011) Mature mice lacking Rbl2/p130 gene have supernumerary inner ear hair cells and supporting cells. J Neurosci 31(24):8883–8893
23. Kwan KY, Shen J, Corey DP (2015) C-MYC transcriptionally amplifies SOX2 target genes to regulate self-renewal in multipotent otic progenitor cells. Stem Cell Rep 4(1):47–60
24. Jacques BE et al (2012) A dual function for canonical Wnt/beta-catenin signaling in the developing mammalian cochlea. Development 139(23):4395–4404
25. Shi F et al (2014) beta-Catenin is required for hair-cell differentiation in the cochlea. J Neurosci 34(19):6470–6479
26. Zhang YP et al (2015) Dynamic expression of Lgr6 in the developing and mature mouse cochlea. Front Cell Neurosci 9:165
27. Cox BC et al (2014) Spontaneous hair cell regeneration in the neonatal mouse cochlea in vivo. Development 141(4):816–829
28. Shi F, Kempfle JS, Edge AS (2012) Wnt-responsive Lgr5-expressing stem cells are hair cell progenitors in the cochlea. J Neurosci 32(28):9639–9648
29. Romero-Carvajal A et al (2015) Regeneration of sensory hair cells requires localized interactions between the Notch and Wnt pathways. Dev Cell 34(3):267–282
30. Jacques BE et al (2014) The role of Wnt/beta-catenin signaling in proliferation and regeneration of the developing basilar papilla and lateral line. Dev Neurobiol 74(4):438–456
31. Shi F, Hu L, Edge AS (2013) Generation of hair cells in neonatal mice by beta-catenin overexpression in Lgr5-positive cochlear progenitors. Proc Natl Acad Sci U S A 110(34):13851–13856
32. Kuo BR et al (2015) In vivo cochlear hair cell generation and survival by coactivation of beta-Catenin and Atoh1. J Neurosci 35(30):10786–10798
33. Ma EY, Rubel EW, Raible DW (2008) Notch signaling regulates the extent of hair cell regeneration in the zebrafish lateral line. J Neurosci 28(9):2261–2273
34. Daudet N et al (2009) Notch regulation of progenitor cell behavior in quiescent and regenerating auditory epithelium of mature birds. Dev Biol 326(1):86–100
35. Korrapati S et al (2013) Notch signaling limits supporting cell plasticity in the hair cell-damaged early postnatal murine cochlea. PLoS One 8(8):e73276
36. Mizutari K et al (2013) Notch inhibition induces cochlear hair cell regeneration and recovery of hearing after acoustic trauma. Neuron 77(1):58–69
37. Li WY et al (2015) Notch inhibition induces mitotically generated hair cells in mammalian cochleae via activating the Wnt pathway. Proc Natl Acad Sci U S A 112(1):166–171
38. Driver EC et al (2008) Hedgehog signaling regulates sensory cell formation and auditory function in mice and humans. J Neurosci 28(29):7350–7358
39. Zarei S et al (2017) Sonic hedgehog antagonists reduce size and alter patterning of the frog inner ear. Dev Neurobiol 77(12):1385–1400
40. Riccomagno MM, Takada S, Epstein DJ (2005) Wnt-dependent regulation of inner ear morphogenesis is balanced by the opposing and supporting roles of Shh. Genes Dev 19(13):1612–1623

41. Brown AS, Epstein DJ (2011) Otic ablation of smoothened reveals direct and indirect requirements for Hedgehog signaling in inner ear development. Development 138(18):3967–3976
42. Lu N et al (2013) Sonic hedgehog initiates cochlear hair cell regeneration through downregulation of retinoblastoma protein. Biochem Biophys Res Commun 430(2):700–705
43. Chen Y et al (2017) Hedgehog signaling promotes the proliferation and subsequent hair cell formation of progenitor cells in the neonatal mouse cochlea. Front Mol Neurosci 10:426
44. Pickles JO (2001) The expression of fibroblast growth factors and their receptors in the embryonic and neonatal mouse inner ear. Hear Res 155(1–2):54–62
45. Wright TJ et al (2003) Expression of mouse fibroblast growth factor and fibroblast growth factor receptor genes during early inner ear development. Dev Dyn 228(2):267–272
46. Alvarez Y et al (2003) Requirements for FGF3 and FGF10 during inner ear formation. Development 130(25):6329–6338
47. Wright TJ, Mansour SL (2003) Fgf3 and Fgf10 are required for mouse otic placode induction. Development 130(15):3379–3390
48. Represa J et al (1991) The int-2 proto-oncogene is responsible for induction of the inner ear. Nature 353(6344):561–563
49. Mansour SL, Goddard JM, Capecchi MR (1993) Mice homozygous for a targeted disruption of the proto-oncogene int-2 have developmental defects in the tail and inner ear. Development 117(1):13–28
50. Mansour SL (1994) Targeted disruption of int-2 (fgf-3) causes developmental defects in the tail and inner ear. Mol Reprod Dev 39(1):62–67. discussion 67-8
51. McKay IJ, Lewis J, Lumsden A (1996) The role of FGF-3 in early inner ear development: an analysis in normal and kreisler mutant mice. Dev Biol 174(2):370–378
52. Vendrell V et al (2000) Induction of inner ear fate by FGF3. Development 127(10):2011–2019
53. Leger S, Brand M (2002) Fgf8 and Fgf3 are required for zebrafish ear placode induction, maintenance and inner ear patterning. Mech Dev 119(1):91–108
54. Maroon H et al (2002) Fgf3 and Fgf8 are required together for formation of the otic placode and vesicle. Development 129(9):2099–2108
55. Lysaght AC et al (2014) FGF23 deficiency leads to mixed hearing loss and middle ear malformation in mice. PLoS One 9(9):e107681
56. Ono K et al (2014) FGFR1-Frs2/3 signalling maintains sensory progenitors during inner ear hair cell formation. PLoS Genet 10(1):e1004118
57. Umemoto M et al (1995) Hair cell regeneration in the chick inner ear following acoustic trauma: ultrastructural and immunohistochemical studies. Cell Tissue Res 281(3):435–443
58. Zheng JL, Helbig C, Gao WQ (1997) Induction of cell proliferation by fibroblast and insulin-like growth factors in pure rat inner ear epithelial cell cultures. J Neurosci 17(1):216–226
59. Lee SG et al (2016) Myc and Fgf are required for zebrafish neuromast hair cell regeneration. PLoS One 11(6):e0157768
60. Kawamoto K et al (2009) Spontaneous hair cell regeneration in the mouse utricle following gentamicin ototoxicity. Hear Res 247(1):17–26
61. Deyst KA, Ma J, Fallon JR (1995) Agrin: toward a molecular understanding of synapse regeneration. Neurosurgery 37(1):71–77
62. Deng LX et al (2013) A novel growth-promoting pathway formed by GDNF-overexpressing Schwann cells promotes propriospinal axonal regeneration, synapse formation, and partial recovery of function after spinal cord injury. J Neurosci 33(13):5655–5667
63. Marler KJ et al (2008) A TrkB/EphrinA interaction controls retinal axon branching and synaptogenesis. J Neurosci 28(48):12700–12712
64. Alto LT et al (2009) Chemotropic guidance facilitates axonal regeneration and synapse formation after spinal cord injury. Nat Neurosci 12(9):1106–11U8
65. Tong MJ, Brugeaud A, Edge ASB (2013) Regenerated synapses between postnatal hair cells and auditory neurons. Jaro-J Assoc Res Otolaryngol 14(3):321–329
66. Wan GQ et al (2014) Neurotrophin-3 regulates ribbon synapse density in the cochlea and induces synapse regeneration after acoustic trauma. elife 20:3

67. Smolders JWT (1999) Functional recovery in the avian ear after hair cell regeneration. Audiol Neuro Otol 4(6):286–302
68. Carey JP, Fuchs AF, Rubel EW (1996) Hair cell regeneration and recovery of the vestibulooocular reflex in the avian vestibular system. J Neurophysiol 76(5):3301–3312
69. Atkinson PJ et al (2014) Hair cell regeneration after ATOH1 gene therapy in the cochlea of profoundly deaf adult guinea pigs. PLoS One 9(7):e102077
70. Baker K, Brough DE, Staecker H (2009) Repair of the vestibular system via adenovector delivery of Atoh1: a potential treatment for balance disorders. Adv Otorhinolaryngol 66:52–63
71. Du X et al (2018) Regeneration of cochlear hair cells and hearing recovery through Hes1 modulation with siRNA nanoparticles in adult guinea pigs. Mol Ther 26(5):1313–1326
72. Izumikawa M et al (2005) Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nat Med 11(3):271–276
73. Chessum L et al (2018) Helios is a key transcriptional regulator of outer hair cell maturation. Nature 563(7733):696–700
74. Zampini V et al (2011) Eps8 regulates hair bundle length and functional maturation of mammalian auditory hair cells. PLoS Biol 9(4):e1001048
75. Wiwatpanit T et al (2018) Trans-differentiation of outer hair cells into inner hair cells in the absence of INSM1. Nature 563(7733):691–695

Chapter 2
Protection of Hair Cells from Ototoxic Drug-Induced Hearing Loss

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Abstract Hair cells are specialized sensory epithelia cells that receive mechanical sound waves and convert them into neural signals for hearing, and these cells can be killed or damaged by ototoxic drugs, including many aminoglycoside antibiotics, platinum-based anticancer agents, and loop diuretics, leading to drug-induced hearing loss. Studies of therapeutic approaches to drug-induced hearing loss have been hampered by the limited understanding of the biological mechanisms that protect and regenerate hair cells. This review briefly discusses some of the most common ototoxic drugs and describes recent research concerning the mechanisms of ototoxic drug-induced hearing loss. It also highlights current developments in potential therapies and explores current clinical treatments for patients with hearing impairments.

Keywords Hair cell · Ototoxicity · Aminoglycosides · Reactive oxygen species · Mitochondrial DNA

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2.1 Introduction

Hearing loss is the most frequent sensory impairment worldwide. According to global estimates, hearing loss of greater than 20 dB is the second most common impairment after anemia, affecting 1.33 billion individuals in 2015 [1] . In 2018, the WHO estimated that there were 466 million people (6.1% of the global population) living with disabling hearing loss, which is defined as hearing loss greater than 40 dB or 30 dB in the better-hearing ear in adults and children, respectively. In addition, 93% of these people are adults, with children accounting for the remaining 7%. Moreover, it is estimated that the number of people with disabling hearing loss is predicted to grow to over 900 million by 2050. Although hearing loss is a non-life-threatening disability, it can compromise the individual's quality of life and is a significant burden on families and society in general. Developmental delays in language acquisition and a low level of education are significant problems for children with hearing loss in low- and middle-income regions.

Aside from congenital factors, hearing loss can also be caused by ear infections, noise, and chemical exposure. Notably, drug ototoxicity is one of the major preventable factors that contribute to hearing loss. A growing body of evidence indicates that ototoxic drugs mainly affect hair cells, which are surrounded by supporting cells within the organ of Corti [2, 3]. Hair cells in the cochlea play an essential role in converting mechanical sound waves into neural signals for hearing, and because hair cells are terminally differentiated in adult mammals, they have limited ability to spontaneously regenerate if they are damaged or killed [4].

It has become clear that ototoxic drugs can be transported from the strial vessels or can diffuse through the round window into the cochlear tissues after systemic or intratympanic administration [5, 6]. After entering the inner ear, different drugs can damage different cells and tissues, including hair cells, supporting cells, spiral ganglion cells, and the auditory nerve, but damage to hair cells is the primary effect of ototoxicity. Thus a great deal of research has focused on the mechanisms and possible therapeutic approaches of hair cell loss caused by ototoxic drugs. For example, an older study established the period of sensitivity to ototoxic drugs [7], while a recent study showed that overexpression of the X-linked inhibitor of apoptosis protein gene can prevent hair cell loss during this sensitive period [8]. We believe that a deeper understanding of ototoxicity will ultimately provide new ideas for the prevention and treatment of drug-induced hearing loss.

2.2 Definitions of Ototoxic Drugs

It is well documented that some therapeutic drugs, such as aminoglycosides and antineoplastic drugs used against life-threatening conditions, can result in ototoxicity, which is defined as causing auditory and/or vestibular dysfunction that can lead to hearing loss and/or balance problems. The administration or application of ototoxic drugs or chemicals might either directly or indirectly damage or kill inner ear cells. Symptoms of ototoxicity include temporary or permanent hearing loss,

tinnitus, and/or vertigo. The elderly, children, and adolescents, and patients with chronic kidney disease (in whom renal dysfunction can reduce the excretion of drugs in the urine), are at the greatest risk of ototoxicity and require needs-specific approaches for clinical management when using ototoxic drugs.

Ototoxic drugs initially affect the highest frequencies (above 8000 Hz), progressing to lower frequencies with prolonged treatment [9]. The low speech frequencies (125–4000 Hz) used in conversation are rarely the first to be affected. The full development of both high- and low-frequency hearing loss is invariably found to be delayed with respect to the time of drug removal, with smaller losses occurring during later time intervals. Extended high-frequency audiometry can be used to identify the earliest ototoxic changes and can be used to modify treatment protocols to minimize further toxicity. By interrupting drug administration the moment any impairment is noted, it is hoped that damage can be prevented or minimized [10].

Previous studies have shown that drug-induced hearing loss can be the result of damage to hair cells, spiral ganglion cells, the auditory nerve, or the stria vascularis. In most cases, hair cells are the primary target of ototoxic drugs. In addition, hair cells in the adult mammalian inner ear cannot regenerate spontaneously after damage, although vestibular supporting cells retain a limited capacity for regeneration. Despite extensive research in recent decades, the issue of iatrogenic ototoxicity remains a pressing concern.

2.3 Genetic Mechanisms of Ototoxic Drug-Induced Hearing Loss

Aminoglycosides are one of the most common causes of acquired hearing loss when used in large amounts or for a long time, but lower drug levels can still lead to ototoxicity in particularly vulnerable people. High susceptibility to aminoglycosides has also been shown to be a maternally inherited trait and to potentially be caused by mutations in mitochondrial DNA (mtDNA) [11, 12].

Mitochondria are found in all nucleated cells and are the principal generators of cellular ATP through oxidative phosphorylation, and they control apoptosis. Mitochondria are the only source of extrachromosomal DNA within the mammalian cell, and they are under dual genetic control [13]. Each of these mtDNA molecules, which are double-stranded closed circles, is about 16,569 bp in length in humans and contains 37 known genes encoding 22 tRNAs, 13 mRNAs, and 1 each of 12S rRNA and 16S rRNA [14].

The mitochondrial 12S rRNA is a hot spot for mutations associated with aminoglycoside-induced hearing loss, and it has been shown that mutations in mitochondrial 12S rRNA are the primary risk factor for aminoglycoside antibiotic-induced deafness (AAID). Many studies have provided further support for the important role of the A1555G and C1494T mutations for AAID and have provided clear evidence for the mechanisms behind these effects.

In 1993, Fischel-Ghodsian's research team described a mitochondrial 12S rRNA A1555G mutation that is related to aminoglycoside-induced and nonsyndromic hearing loss. This was the first genetic and molecular study of AAID [15]. Since

then a great deal of inherited mtDNA mutations implicated in AAID have been reported.

The antibacterial effects of aminoglycosides work by binding to the decoding region of bacterial ribosomal RNA (rRNA) (e.g., *Escherichia coli* 16S rRNA) and causing further errors in protein translation. The interaction between rRNA molecules and aminoglycosides is potentially highly specific. In humans, the nucleotide at position 1555 in the 12S rRNA gene in wild-type cells is A, and when this A is mutated to G, it pairs with C at position 1494. This transition makes the secondary structure of 12S rRNA more similar to the corresponding region of *E. coli* 16S rRNA. Thus, these mutations promote the combination of aminoglycosides and mitochondrial 12S rRNA and consequently cause defects in mtDNA translation and protein synthesis [16, 17]. These findings convincingly demonstrate that mutations in mtDNA can interfere with mitochondrial protein synthesis and thus make cochlear cells more susceptible to drug-induced ototoxicity [18].

Studies by Guan et al. found that the C1494T mutation is another primarily pathogenic mtDNA mutation that causes a genetic predisposition to aminoglycoside ototoxicity and nonsyndromic hearing loss. These studies also strongly suggested that the nuclear background plays a role in aminoglycoside ototoxicity and in the development of the hearing loss phenotype associated with the C1494T mutation in the mitochondrial 12S rRNA gene. In addition, their experiments also showed a significant decrease in the rate of mitochondrial protein synthesis due to the C1494T mutation, which is probably the primary contributor to the respiratory phenotype seen in these mutant cells, and such a reduction in protein synthesis consequently results in a decline in the ATP production in the cochlear cells that is essential for hearing function [19, 20].

Dozens of mtDNA mutations have been identified in humans [17], but the mitochondrial 12S rRNA A1555G and C1494T mutations are the most significant in terms of susceptibility to ototoxicity, and the effects of other mutations await further investigation. The eventual identification of the mechanisms of these modifier genes will likely contribute to determining the cause of AAID and will provide targeted genetic counseling and guidance for patients and their families. At the very least, when it comes to aminoglycoside antibiotic treatment, every individual with potentially inherited mutations should be asked for such a family history in order to avoid using such drugs. Advances in DNA sequencing will continue to lead to the identification of novel mitochondrial proteins and pathways, and such sequencing will also be helpful in prenatal hearing diagnosis in at-risk families as well as in preventing transmission of mutations to future generations.

In addition to aminoglycoside ototoxicity, genetic risk factors for cisplatin-induced ototoxicity are another research focus. Multiple genes related to susceptibility to cisplatin-induced hearing loss have been reported, including variants in glutathione s-transferase genes, the megalin gene (*LRP2*), methyltransferase genes (*TPMT* and *COMT*), cisplatin transporter genes (*ABCC3* and *CTR1*), and DNA repair genes (*XPC*). Colin et al. found two loss-of-function variants in the gene encoding TPMT, a phase II drug-metabolizing enzyme responsible for catalyzing the methylation of thiopurine compounds, which are strongly associated with

cisplatin-induced hearing loss in children [21]. However, clinical research indicated that *TPMT* and *COMT* variations are not related to cisplatin ototoxicity in children with cancer and do not influence cisplatin-induced hearing damage in laboratory models [22]. Additionally, recent genome-wide association studies identified common variants in the superoxide dismutase 2 (*SOD2*) and acylphosphatase-2 (*ACYP2*) genes that are associated with cisplatin-induced hearing loss. In general, the evidence supports the hypothesis that variation in the response to oxidative stress influences the susceptibility to cisplatin ototoxicity [23, 24].

In summary, the precise role that these candidate genes play in cisplatin ototoxicity remains undetermined. Furthermore, by means of different methods, different research teams have come to inconsistent conclusions regarding the association between genetic variants and cisplatin-induced hearing loss [21, 22, 25–27].

2.4 Classification of Ototoxic Drugs

2.4.1 Antibiotics

1. *Aminoglycosides*

According to their source, aminoglycosides can be divided into two categories. Streptomycin was the first aminoglycoside to be isolated from *Streptomyces*, and neomycin, kanamycin, tobramycin, and amikacin are in the same class of drugs as streptomycin. Additionally, the *Micromonospora* produce aminoglycosides including gentamycin, sisomicin, and netilmicin. The aminoglycoside antibiotics are widely used to treat gram-negative and some gram-positive bacterial infections, including tuberculosis, sepsis, respiratory infections in cystic fibrosis, complex urinary tract infections, and endocarditis, and aminoglycoside antibiotics are often used in combination with other antibiotics for broad-spectrum coverage. Unfortunately, the clinical benefits of these drugs can be outweighed by their toxicity, including acute dose-dependent kidney failure (nephrotoxicity) and permanent hearing loss and/or balance disorders (ototoxicity). In recent years, their importance has waned due to the emergence of other broad-spectrum antibiotics with fewer side effects, and since 1990 there have been no new aminoglycosides entering the market, and their market share has become less and less. As the global incidence of drug-resistant gram-negative bacterial infections has increased, however, new research is desperately needed regarding this old class of antibiotics. New clinical candidates, including “third-generation” drugs with fewer side effects, have already been produced against many drug-resistant strains [28].

2. *Macrolides*

In addition to aminoglycosides, many other classes of antibiotics have been reported to be ototoxic. Erythromycin is a macrolide antibiotic that was discovered in 1952, and with its expansive use in the clinic, adverse effects have been frequently

reported. For example, Karmody and Weinstein reported three cases of reversible sensorineural hearing loss during treatment with intravenous erythromycin lactobionate [29], and a subsequent study showed that the reversible ototoxicity of erythromycin is dependent on the dose and the serum concentration [30]. There was also an animal experiment demonstrating that erythromycin-induced ototoxicity likely stems from transient dysfunction of the stria vascularis [31].

3. *Chloramphenicols*

Topical applications of chloramphenicol to the middle ear in Guinea pigs produced severe toxic effects in the cochlea [32]. Furthermore, Henley et al. published an experiment showing the ototoxic interaction between chloramphenicol and noise, and they suggested that the combination of chloramphenicol and noise can cause permanent hearing loss [33].

4. *Vancomycin*

Vancomycin is a glycopeptide antibiotic that is used extensively in the treatment of serious infections caused by methicillin-resistant *Staphylococcus aureus*. Many animal studies confirmed the ototoxicity of vancomycin and norvancomycin [34, 35], and a clinical study showed a significant rate of high-frequency hearing loss in older patients receiving vancomycin monotherapy [36].

5. *Polymyxins*

Topical application of eardrops containing polymyxin B is commonly used for treatment of middle ear diseases. Although experimental data confirmed the cochlear damage due to polymyxin B [37], the clinical relevance of these results remains debatable. Rakover et al. reported that topical eardrops of ototoxic drugs are clinically safe for a short period of time [38].

2.4.2 Nonantibiotics

1. *Antineoplastic drugs*

Platinum derivatives are cytotoxic chemotherapeutic drugs used to treat various adult and childhood malignancies, including head and neck, bladder, lung, and germ cell malignancies. Cisplatin, carboplatin, and oxaliplatin are the only FDA-approved platinum compounds. Nevertheless, most patients treated with cisplatin are at a high risk of ototoxicity, neurotoxicity, and nephrotoxicity. The ototoxicity induced by platinum derivatives is characterized by the loss of cochlear hair cells and cells of the spiral ganglion and by degeneration of the stria vascularis [3]. Cisplatin-induced ototoxicity includes permanent and irreversible hearing loss and tinnitus [39, 40], and clinical studies have demonstrated that children show greater risk for developing hearing loss following cisplatin treatment than adults [41–43]. Currently, there are no established methods to avoid or reverse cisplatin-induced ototoxicity, and the

ototoxicity risk must be weighed against oncological efficacy. Early ototoxicity can be detected by standardized audiologic monitoring protocols, and these might serve as a basis for early intervention in cancer patients [44].

2. *Loop Diuretics*

Loop diuretics, including ethacrynic acid, furosemide, and bumetanide, are commonly used to treat acute pulmonary edema and edema associated with congestive heart failure, cirrhosis, and certain kidney diseases by increasing the volume of urine excretion. Loop diuretics act in the thick ascending limb of Henle and inhibit sodium and chloride ion reabsorption in the kidney. These drugs also inhibit a sodium–potassium–chloride transporter found in the inner ear, thus disturbing the ionic concentration of the endolymph. Thus, these drugs can cause temporary hearing loss and reversible dysfunctions in the stria vascularis [45]. When co-administered with other known ototoxic drugs, such as aminoglycosides or cisplatin, loop diuretics can potentiate the ototoxicity of these agents [46, 47].

3. *Acetyl Salicylic Acid*

Salicylates have been widely used to treat chronic inflammatory diseases. High doses of acetyl salicylic acid can induce a reversible bilateral mild to moderate auditory threshold shift and tinnitus [48]. While the exact mechanism behind salicylate-induced hearing impairments is unclear, there is evidence to suggest that salicylate mainly eliminates outer hair cell electromotility and influences cochlear blood flow. Typically, recovery of normal auditory function occurs within 24–72 h after stopping salicylate administration [49, 50].

4. *Antimalarials*

An extensive body of literature suggests that antimalarials (quinine, chloroquine, and hydroxychloroquine) have potential ototoxic side effects [51–53]. Most of the ototoxic reactions attributed to antimalarial drugs are reversible both in healthy people and in malaria patients [54, 55].

We summarized the classification and cellular target of ototoxic drugs in Table 2.1.

2.5 Metabolism of Ototoxic Drugs in the Inner Ear

Clinically, drugs can be delivered into the inner ear either systemically or through topical routes. In systemic administration, drugs rapidly pass the blood-labyrinth barrier (BLB) and mix with the inner ear fluids through the stria vascularis, while topical administration might enable drugs to bypass the BLB and gain direct access to the inner ear; thus topical administration increases the drug concentrations at the site of application and enhances absorption and toxicity [5, 58] (Fig. 2.1). It is documented that drugs such as aminoglycosides and cisplatin can be more readily transported into the cochlear tissues from the strial vessels after noise exposure after

Table 2.1 The classification and cellular target of ototoxic drugs

Drugs		Cellular target	References
Antibiotics			
Aminoglycosides	Streptomycin	Hair cell	[56, 57]
	Neomycin	Supporting cell	
	Kanamycin	Stria vascularis	
	Tobramycin	Spiral ganglion	
	Amikacin		
	Gentamycin		
	Sisomicin		
	Netilmicin		
Macrolides	Erythromycin	Stria vascularis	[31]
Chloramphenicols	Chloramphenicol	Unclear	[32, 33]
Vancomycin	Vancomycin	Unclear	[34]
Polymyxins	Polymyxin B	Hair cell	[37]
		Stria vascularis	
Nonantibiotics			
Antineoplastic drugs	Cisplatin,	Hair cell	[3]
	Carboplatin	Stria vascularis	
	Oxaliplatin	Spiral ganglion	
Loop diuretics	Ethacrynic acid	Stria vascularis	[45]
	Furosemide		
	Bumetanide		
Acetyl salicylic acid	Acetyl salicylic acid	Hair cell	[49, 50]
		Stria vascularis	
Antimalarials	Quinine	Stria vascularis	[52]
	Chloroquine Hydroxychloroquine		

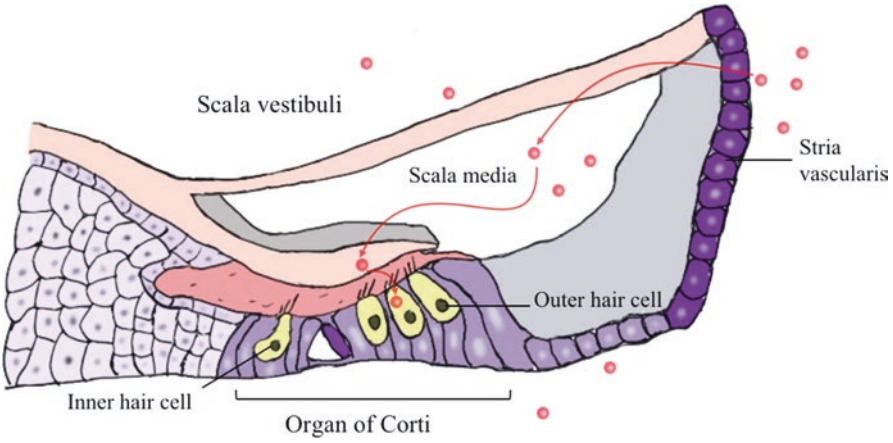

Fig. 2.1 The main trafficking route of aminoglycosides. In systemic administration, the aminoglycosides (red round) pass the BLB and mix with the inner ear fluids through the stria vascularis. After that, they enter into hair cells and contribute to hair cell death

systemic administration [59]. In contrast, the main route of local drug delivery is intratympanic administration, which involves injecting or perfusing drugs into the middle ear with the aim of achieving drug diffusion through the round window [3, 6]. When the drug mixes with the inner ear fluids, which do not flow appreciably compared with most other body fluids, drug concentrations in the inner ear fluids depend on dispersal by diffusion [60]. Issues of metabolism and distribution of drugs are specific for each drug, but the elimination of most drugs involves absorption by epithelial cells in the stria vascularis and by dark cells in the cochlea and vestibule followed by entrance into the bloodstream [61].

The plasma protein binding rate of aminoglycosides is low, and the majority of aminoglycosides entering the bloodstream are primarily excreted by the kidney in the form of protolymph [62, 63]. However, the remaining aminoglycosides can bind to tissue proteins and accumulate in the cortex region of the kidney, especially in the renal proximal tubular cells [64, 65]. Consequently, the selective accumulations lead to reversible damage to renal structure and function [66].

Most cisplatin irreversibly binds to erythrocytes and plasma proteins like albumin and gammaglobulins after injection into the blood [67, 68]. Similar to aminoglycosides, being excreted into the urine without being metabolized by the kidneys is the main mechanism of platinum clearance [69], and the biotransformation of oxaliplatin in vitro is realized by nonenzymatic reactions [70]. Meanwhile, the kidney proximal tubule achieves high local concentrations by transporting cisplatin via CTR1 (copper transporter 1) and OCT2 (organic cation transporter 2) [71], and long-term exposure to cisplatin is associated with the loss of renal function such as a permanent reduction in the glomerular filtration rate [72].

Aminoglycosides can cross the placenta and enter the amniotic fluid and can thus enter the fetal circulatory system. Severe intrauterine otological damage and congenital hearing loss after birth have been reported after the administration of aminoglycosides to mothers. Thus, aminoglycoside antibiotics should be avoided during pregnancy.

2.6 Research Progress and the Mechanisms of Ototoxic Drugs

The targets of ototoxic drugs differ for each drug. After determining the main sites of action of these drugs, investigating the mechanisms of drug damage is essential. Many researchers believe that the following theories are related to each other, and according to the site of action, the mechanism can be classified as follows.

2.6.1 Membrane-Dependent Ototoxicity

1. *Phosphoinositide*

Phosphoinositide is a negatively charged phospholipid located in the cell membranes and is rich in arachidonic acid, and it plays an important role in regulating

signal transduction at the membrane surface [73]. Aminoglycosides can bind to phosphoinositides and induce the release of arachidonic acid, which acts as an electron donor in FeII–aminoglycoside complexes and leads to lipid peroxidation and the creation of reactive oxygen species (ROS) [74]. Excessive ROS production can lead to oxidation of macromolecules and has been implicated in cell death [75].

2. *Iron Chelators*

It has been proposed that the ototoxic effects of aminoglycosides are linked to the formation of iron–aminoglycoside complexes, which can catalyze free radical reactions and generate superoxide radicals. A study suggested that gentamycin acts as an iron chelator, and the iron–gentamycin complex is capable of catalyzing free radical formation [76]. Such complexes can cause metabolic disorders and functional disturbances in hair cells. Iron chelators have also been reported to significantly attenuate cisplatin-induced cytotoxicity [77, 78] suggesting that cisplatin-induced ototoxicity might act through an iron-dependent pathway.

3. *Copper Transporter 1*

The transport of cisplatin across the cytomembrane is accomplished by membrane transporters, such as the mammalian CTR1. Under physiological conditions, the substrate of CTR1 is monovalent copper (Cu+), which is essential for various enzymatic reactions [79]. Cisplatin seems to bind to the same methionine-rich motifs of the extracellular domain as Cu+, thus allowing the entrance of cisplatin into the cell [80]. In addition, a study showed that intratympanic administration of copper sulfate, a CTR1 inhibitor, can prevent hearing loss caused by cisplatin [81].

2.6.2 Cytoplasm-Dependent Ototoxicity

1. *Mitochondrial Malfunction*

The mitochondrion is the main organelle responsible for the production of energy in mammalian cells through a series of oxidative phosphorylation reactions. mtDNA mutations (described in Sect. 2.2) and mitochondrial respiratory chain dysfunction can cause mitochondrial malfunctions, and these are related to various pathological phenotypes due to energy crisis that often induces autophagy or cell death [82]. Mitochondria are the main sites of oxidative phosphorylation and ATP production in nucleated cells, and mutations in mtDNA can cause mitochondrial dysfunction and the production of large amounts of ROS [83]. Many mutations have been found mainly in the mitochondrial 12S rRNA, and the A1555G and C1494T mutations are the most significant changes.

2. *Reactive Oxygen Species*

ROS such as hydroxyl radicals, superoxide anions, hydrogen peroxide, and singlet oxygen are mainly generated by the mitochondria in mammalian cells [76, 84].

Under both physiological and pathological conditions, ROS play an important role in the induction of apoptosis. All mechanisms mentioned above appear to be triggered through elevated ROS formation. Iron chelators consume an electron, activate molecular oxygen, and then form ROS [76]. NADPH oxidase 3 (NOX3) is a relevant source of ROS generation, and cisplatin can stimulate the activity of NOX3 and produce high levels of ROS [85]. Moreover, the JNK signaling pathway and caspase cascades are activated by an increase in ROS [85–87]. ROS have also have been found to play important roles in causing mtDNA defects within cells and tissues. When a mitochondrion fails to completely metabolize oxygen, it generates excessive ROS that consequently accelerate the induction of mtDNA mutations. In general, ROS play a critical role in drug-induced hearing loss.

2.6.3 Nucleus-Dependent Ototoxicity

Early reports indicated that drug-induced ototoxicity might predominantly trigger apoptotic cascades [88]. The c-Jun N-terminal kinases (JNKs) and extracellular signal-regulated kinases are key members of the mitogen-activated protein kinase (MAPK) family and act as key modulators of apoptosis [86, 89]. The intrinsic apoptotic pathways are triggered by cytokine deprivation, DNA damage, and cytotoxic stress and are the major pathways initiated by aminoglycoside or other chemical-induced ototoxicities [90]. In vivo experiments show that activation of the JNK pathway after administration of aminoglycosides is due to the generation of ROS, and inhibitors of the JNK pathway such as CEP-1347, estradiol, and X-linked inhibitor of apoptosis protein attenuate hair cell loss following aminoglycoside application [8, 91, 92]. The activated gene products involved in the apoptotic pathway then translocate to the mitochondria, leading to the release of cytochrome c that triggers caspase-dependent apoptosis [93]. As for cisplatin, the ototoxic mechanism has been shown to be associated with several factors. In p53 and caspase-dependent apoptosis, exposure to high doses of cisplatin can cause high levels of ROS [85, 87], which activate the mitochondrial apoptosis signaling pathway that involves the release of cytochrome c from mitochondria and the activation of caspases 3, 8, and 9 [94–96].

2.7 Approaches for Minimizing Ototoxicity

Minimizing ototoxicity without inhibiting the therapeutic efficacy of these drugs is an ongoing research challenge. Currently, there is no ideal therapy to treat ototoxicity after the administration of these drugs. Therefore, prevention might be a better strategy than trying to find a cure.

2.7.1 Prevention

(a) Strictly monitor indications of ototoxicity, and avoid using these agents when not absolutely necessary.
(b) For infants, the elderly, pregnant women, people with hepatic and/or renal dysfunction, and sensitive populations (the hearing disabled, people with a family history of hearing loss, and those with A1555G or C1494T mtDNA mutations), genetic screening for hearing loss and education on preventing hearing loss should be done prior to the clinical use of the medication.
(c) Increase awareness of early symptoms prior to the onset of hearing loss (such as headache, disorientation, and tinnitus) in order to discontinue the drug in time and to get treatment as soon as possible.
(d) Ototoxic drug administration in conjunction with simultaneous exposure to noise has been associated with enhanced ototoxicity. This makes the removal of sound stimulation prior to and during drug application a reasonable preventive measure [59, 83, 97].

2.7.2 Treatment

(a) *Inhibiting the Transport of Drugs*

One way to reduce ototoxicity might be the use of iron chelators, such as deferoxamine. Iron chelators can compete with aminoglycoside for iron binding and can inhibit drugs from entering into the cell [77]. In addition, the intratympanic administration of copper sulfate, a CTR1 inhibitor, can prevent hearing loss caused by cisplatin [81] (Fig. 2.2).

(b) *Reducing ROS Level*

The administration of ototoxic drugs is known to increase oxidative stress and the production of ROS [85]. Several antioxidants have been shown to be effective in vitro and in vivo in preventing drug-induced ototoxicity, including coenzyme Q10 [98], D- and L-methionine [99–101], thiourea [102], and vitamins B, C, and E [103]. In addition, hyperbaric oxygen therapy (HBOT) is an alternative choice to prevent and treat sudden hearing loss caused by ototoxic drugs. The main effect of HBOT is to increase tissue oxygenation through plasma-dissolved oxygen [104].

(c) *Modulating Cell Death Signaling*

In order to block the functions of specific proteins in cell death signaling pathways, pharmacological inhibitors or RNAi-based approaches might be useful. The B-cell lymphoma-2 (Bcl-2) family can be anti- or pro-apoptotic, and anti-apoptotic Bcl-2 proteins include Bcl-2 and Bcl-X_L. Both in vitro and in vivo studies indicate that overexpression of Bcl-2 in transgenic mice can decrease hair cell loss and pre-

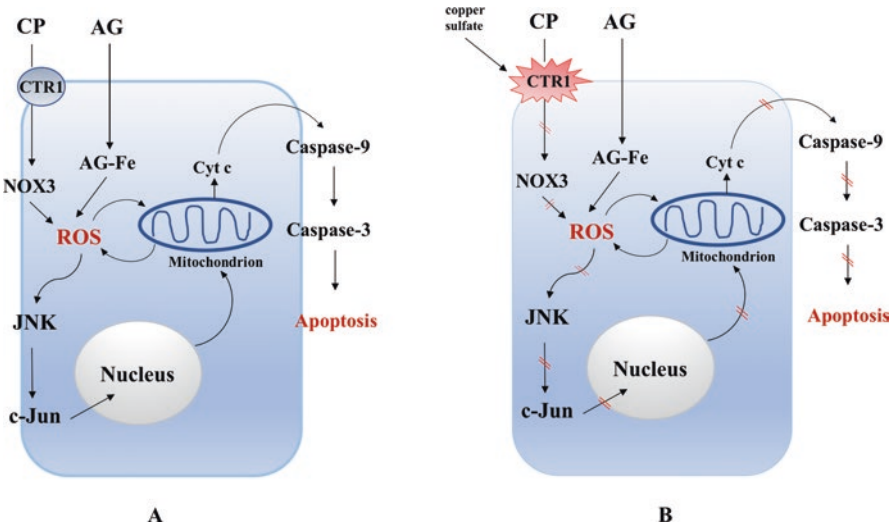

Fig. 2.2 Cell damage mechanisms induced by cisplatin and aminoglycosides. (**a**) (1) Cisplatin and aminoglycosides can enter the cell by binding to CTR1 and iron, respectively. (2) The activation of NOX3 by cisplatin and iron/aminoglycosides can produce high levels of ROS. (3) Mutations in mtDNA can produce large amounts of oxygen free radicals and the generation of ROS that consequently accelerates the induction of mtDNA mutations. (4) The JNK and caspase-dependent apoptosis signaling pathway can be activated by ROS and by Cyt c released from mitochondrion. (**b**) Copper sulfate, a CTR1 inhibitor, can block CTR1 and the subsequent apoptosis pathway caused by cisplatin. *CP* cisplatin, *AG* aminoglycosides, *CTR1* copper transporter 1, *NOX3* NADPH oxidase 3, *ROS* reactive oxygen species, *JNK* the c-Jun N-terminal kinase; *Cyt c* cytochrome c

serve hearing function after exposure to aminoglycosides [105, 106]. Alternatively, inhibition of the MAP-JNK pathway by application of D-JNKI-1, which is a cell-permeable peptide that blocks the MAPK-JNK signaling pathway, before neomycin exposure can promote hair cell survival and prevent hearing loss in vivo [107].

(d) *Other Therapies*

Some neurotrophins (such as neurotrophin-3 and brain-derived neurotrophic factor) and vasodilators have been shown to be effective against drug ototoxicity in spiral ganglion cells and the stria vascularis by nurturing nerve cells and increasing blood flow, respectively [108, 109]. Interestingly, researchers have developed a sound exposure protocol that induces heat shock proteins indicating that sound preconditioning might induce a generalized stress response that has the potential to protect hearing in patients receiving ototoxic drugs [110]. Small-molecule drugs and gene therapy are the most challenging and difficult, but most promising, research topics in drug-induced sensorineural hearing loss, and these will be discussed below.

2.8 Development of Small Molecules for Hair Cell Protection

Hair cells in the inner ear play an essential role in converting mechanical sound waves into neural signals for hearing, and the most common reason for sensorineural hearing loss resulting from ototoxic drugs is degeneration of hair cells. Cochlear hair cells are terminally differentiated sensory cells without the ability to spontaneously regenerate after damage. Thus, finding ways to protect and regenerate hair cells is a significant challenge facing researchers. With the development of modern disciplines such as molecular biology, molecular genetics, and genetic engineering, we can further explore the protection and regeneration of hair cells by means of new tools and methods. Mice and zebra fish are widely used as model organisms to study hair cell loss and regeneration.

The work of Yin et al. has shown significant expansion of cochlear supporting cells expressing and maintaining Lgr5, an epithelial stem cell marker, in response to stimulation of Wnt signaling and transcriptional activation by using a small-molecule approach. The Lgr5-expressing cells differentiate into hair cells in high numbers [111], and a set of genes that might regulate the proliferation and regeneration of hair cells by Lgr5-positive progenitors has been studied. These genes might be potential therapeutic targets for hair cell regeneration [112]. Another in vivo study demonstrated that Bmi1 plays a vital role in regulating the proliferation of cochlear Lgr5-positive progenitor cells through the Wnt signaling pathway after neomycin injury [113]. These studies suggest the potential for using cochlear progenitor cells to generate hair cells after cochlear damage.

As discussed above, by far the most important factor in apoptotic cell death is ROS, but moderate ROS levels can promote autophagy, which in turn can inhibit apoptosis and protect the hair cells by suppressing further ROS accumulation and by inhibiting oxidative stress [114]. As an immunosuppressant drug, rapamycin can prevent transplant rejection of kidney allografts and inhibit the mTOR signaling pathway and activate autophagy. The autophagy pathway is activated by oxidative stress and is essential for maintaining cellular function. It has been shown that rapamycin has otoprotective effects and can attenuate drug-induced ototoxicity by enhancing autophagy and suppressing ROS accumulation [115].

Ototoxicity eventually leads to apoptosis, which involves the active regulation of transcription. Histone methylation is one of the most important epigenetic covalent modifications, particularly H3K9 dimethylation (H3K9me2), and is critical for euchromatin gene silencing. Two highly homologous histone methyltransferases, G9a and G9a-like protein (GLP), are responsible for this modification. Both in vivo and in vitro experiments show a rapid increase in H3K9me2 upon hair cell damage, and the G9a/GLP inhibitors BIX01294, UNC0638, and LSD1 reduce the level of H3K9me2, suppress the caspase-dependent pathway, and prevent apoptosis in hair cells [116, 117].

In addition, gene therapy has made much progress in terms of both theory and application. *Atoh1* (also known as *Math1*) encodes a basic helix–loop–helix transcription factor and is the key gene in regulating precursor cell differentiation.

Results from in vivo experiments suggest a new therapeutic approach based on expressing this crucial developmental gene for the restoration of damaged auditory epithelium. The restoration of sensory epithelium after Atoh1 treatment can be explained either by cell migration from areas flanking the organ of Corti or by proliferation of nonsensory cells [118].

Finally, advances in nanotechnology might provide a solution for delivering small chemicals into the cochlea in an effective manner. Local administration of nanoparticles via the round window membrane can stabilize and carry biomaterials across the round window membrane and into the inner ear for specific targeting of cells [119].

2.9 Conclusion

The aminoglycosides and platinum-based compounds are the two most common ototoxic drugs causing hearing loss, and they do so primarily through the loss of hair cells. Because they affect hearing starting from high frequencies and then moving to lower frequencies, the hearing impairment might go unnoticed because there are no visible symptoms in the early stages. Genetic studies suggest that increased susceptibility to ototoxic drugs can be attributed to mutations in mtDNA, especially the A1555G and C1494T mutations in the mitochondrial 12S rRNA. The primary mechanism of ototoxicity appears to be through the production of ROS, which play an important role in both defects in mtDNA and the induction of apoptosis. Several inhibitors of caspases and the JNK pathway have been shown to prevent apoptosis and thus to be effective in preventing drug-induced ototoxicity. Great progress has also been made in the field of hair cell regeneration. It has been reported that Lgr5-expressing cells can differentiate into hair cells, and several genes have been identified that regulate the proliferation and regeneration of hair cells by Lgr5-positive progenitors and might be potential therapeutic targets for hair cell regeneration. Also, *Atoh1* gene therapy has been shown to restore the sensory epithelium either by cell migration or by cell proliferation. Despite these advances, much work still needs to be done to develop therapeutic interventions to prevent or treat ototoxicity and drug-induced hearing loss.

References

1. Collaborators., G.D.a.I.I.a.P (2016) Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388(10053):1545–1602
2. Wargo KA, Edwards JD (2014) Aminoglycoside-induced nephrotoxicity. J Pharm Pract 27(6):573–577
3. Ding D, Allman BL, Salvi R (2012) Review: ototoxic characteristics of platinum antitumor drugs. Anat Rec (Hoboken) 295(11):1851–1867

4. Forge A et al (1993) Ultrastructural evidence for hair cell regeneration in the mammalian inner ear. Science 259(5101):1616–1619
5. Juhn SK, Rybak LP, Prado S (1981) Nature of blood-labyrinth barrier in experimental conditions. Ann Otol Rhinol Laryngol 90(2 Pt 1):135–141
6. Salt AN, Plontke SK (2005) Local inner-ear drug delivery and pharmacokinetics. Drug Discov Today 10(19):1299–1306
7. Henley CM et al (1996) Sensitive developmental periods for kanamycin ototoxic effects on distortion-product otoacoustic emissions. Hear Res 98(1–2):93–103
8. Sun S et al (2014) In vivo overexpression of X-linked inhibitor of apoptosis protein protects against neomycin-induced hair cell loss in the apical turn of the cochlea during the ototoxic-sensitive period. Front Cell Neurosci 8:248
9. Crundwell G, Gomersall P, Baguley DM (2016) Ototoxicity (cochleotoxicity) classifications: a review. Int J Audiol 55(2):65–74
10. Beaubien AR et al (2009) Delay in hearing loss following drug administration. Acta Otolaryngol 109(5–6):345–352
11. Higashi K (1989) Unique inheritance of streptomycin-induced deafness. Clin Genet 35(6):433–436
12. Hu DN et al (1991) Genetic aspects of antibiotic induced deafness: mitochondrial inheritance. J Med Genet 28(2):79–83
13. Taylor RW, Turnbull DM (2005) Mitochondrial DNA mutations in human disease. Nat Rev Genet 6(5):389–402
14. Anderson S et al (1981) Sequence and organization of the human mitochondrial genome. Nature 290(5806):457–465
15. Prezant TR et al (1993) Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat Genet 4(3):289–294
16. Hamasaki K, Rando RR (1997) Specific binding of aminoglycosides to a human rRNA construct based on a DNA polymorphism which causes aminoglycoside-induced deafness. Biochemistry 36(40):12323–12328
17. Xing G, Chen Z, Cao X (2007) Mitochondrial rRNA and tRNA and hearing function. Cell Res 17(3):227–239
18. Hobbie SN et al (2008) Genetic analysis of interactions with eukaryotic rRNA identify the mitoribosome as target in aminoglycoside ototoxicity. Proc Natl Acad Sci U S A 105(52):20888–20893
19. Zhao H et al (2004) Maternally inherited aminoglycoside-induced and nonsyndromic deafness is associated with the novel C1494T mutation in the mitochondrial 12S rRNA gene in a large Chinese family. Am J Hum Genet 74(1):139–152
20. Zhao H et al (2005) Functional characterization of the mitochondrial 12S rRNA C1494T mutation associated with aminoglycoside-induced and non-syndromic hearing loss. Nucleic Acids Res 33(3):1132–1139
21. Ross CJ et al (2009) Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy. Nat Genet 41(12):1345–1349
22. Yang JJ et al (2013) The role of inherited TPMT and COMT genetic variation in cisplatin-induced ototoxicity in children with cancer. Clin Pharmacol Ther 94(2):252–259
23. Xu H et al (2015) Common variants in ACYP2 influence susceptibility to cisplatin-induced hearing loss. Nat Genet 47(3):263–266
24. Brown AL et al (2015) SOD2 genetic variant associated with treatment-related ototoxicity in cisplatin-treated pediatric medulloblastoma. Cancer Med 4(11):1679–1686
25. Oldenburg J et al (2007) Cisplatin-induced long-term hearing impairment is associated with specific glutathione s-transferase genotypes in testicular cancer survivors. J Clin Oncol 25(6):708–714
26. Rednam S et al (2013) Glutathione S-transferase P1 single nucleotide polymorphism predicts permanent ototoxicity in children with medulloblastoma. Pediatr Blood Cancer 60(4):593–598

27. Pussegoda K et al (2013) Replication of TPMT and ABCC3 genetic variants highly associated with cisplatin-induced hearing loss in children. Clin Pharmacol Ther 94(2):243–251
28. Becker B, Cooper MA (2013) Aminoglycoside antibiotics in the 21st century. ACS Chem Biol 8(1):105–115
29. Karmody CS, Weinstein L (1977) Reversible sensorineural hearing-loss with intravenous erythromycin lactobionate. Ann Otol Rhinol Laryngol 86(1):9–11
30. Swanson DJ et al (1992) Erythromycin ototoxicity: prospective assessment with serum concentrations and audiograms in a study of patients with pneumonia. Am J Med 92(1):61–68
31. Kobayashi T et al (1997) Ototoxic effect of erythromycin on cochlear potentials in the guinea pig. Ann Otol Rhinol Laryngol 106(7 Pt 1):599–603
32. Beaugard ME, Asakuma S, Snow JB Jr (1981) Comparative ototoxicity of chloramphenicol and kanamycin with ethacrynic acid. Arch Otolaryngol 107(2):104–109
33. Henley CM et al (1984) Impairment in cochlear function produced by chloramphenicol and noise. Neuropharmacology 23(2A):197–202
34. Gao WY et al (2004) Ototoxicity of a new glycopeptide, norvancomycin with multiple intravenous administrations in guinea pigs. J Antibiot 57(1):45–51
35. Lutz H et al (1991) Ototoxicity of vancomycin – an experimental-study in Guinea-Pigs. ORL J Otorhinolaryngol Relat Spec 53(5):273–278
36. Forouzesh A, Moise PA, Sakoulas G (2009) Vancomycin ototoxicity: a reevaluation in an era of increasing doses. Antimicrob Agents Chemother 53(2):483–486
37. Wright CG, Meyerhoff WL, Halama AR (1987) Ototoxicity of neomycin and polymyxin-B following middle-ear application in the Chinchilla and Baboon. Am J Otol 8(6):495–499
38. Rakover Y, Keywan K, Rosen G (1997) Safety of topical ear drops containing ototoxic antibiotics. J Otolaryngol 26(3):194–196
39. Knight KR et al (2007) Early changes in auditory function as a result of platinum chemotherapy: use of extended high-frequency audiometry and evoked distortion product otoacoustic emissions. J Clin Oncol 25(10):1190–1195
40. Frisina RD et al (2016) Comprehensive audiometric analysis of hearing impairment and tinnitus after cisplatin-based chemotherapy in survivors of adult-onset cancer. J Clin Oncol 34(23):2712–2720
41. Landier W et al (2014) Ototoxicity in children with high-risk neuroblastoma: prevalence, risk factors, and concordance of grading scales – a report from the Children's Oncology Group. J Clin Oncol 32(6):527–534
42. Qaddoumi I et al (2012) Carboplatin-associated ototoxicity in children with retinoblastoma. J Clin Oncol 30(10):1034–1041
43. Knight KR, Kraemer DF, Neuwelt EA (2005) Ototoxicity in children receiving platinum chemotherapy: underestimating a commonly occurring toxicity that may influence academic and social development. J Clin Oncol 23(34):8588–8596
44. Landier W (2016) Ototoxicity and cancer therapy. Cancer 122(11):1647–1658
45. Ding D et al (2002) Ethacrynic acid rapidly and selectively abolishes blood flow in vessels supplying the lateral wall of the cochlea. Hear Res 173(1–2):1–9
46. Liu H et al (2011) Ototoxic destruction by co-administration of kanamycin and ethacrynic acid in rats. J Zhejiang Univ Sci B 12(10):853–861
47. Ding D et al (2007) Cell death after co-administration of cisplatin and ethacrynic acid. Hear Res 226(1–2):129–139
48. Cazals Y (2000) Auditory sensori-neural alterations induced by salicylate. Prog Neurobiol 62(6):583–631
49. Didier A, Miller JM, Nuttall AL (1993) The vascular component of sodium salicylate ototoxicity in the guinea pig. Hear Res 69(1–2):199–206
50. Huang ZW et al (2005) Paradoxical enhancement of active cochlear mechanics in long-term administration of salicylate. J Neurophysiol 93(4):2053–2061

51. Claessen FAP et al (1998) Quinine pharmacokinetics: ototoxic and cardiotoxic effects in healthy Caucasian subjects and in patients with falciparum malaria. Tropical Med Int Health 3(6):482–489
52. Bortoli R, Santiago M (2007) Chloroquine ototoxicity. Clin Rheumatol 26(11):1809–1810
53. Johansen PB, Gran JT (1998) Ototoxicity due to hydroxychloroquine: report of two cases. Clin Exp Rheumatol 16(4):472–474
54. Tange RA et al (1997) Ototoxic reactions of quinine in healthy persons and patients with Plasmodium falciparum infection. Auris Nasus Larynx 24(2):131–136
55. Jourde-Chiche N et al (2012) Antimalarial ototoxicity: an underdiagnosed complication? a study of spontaneous reports to the French Pharmacovigilance Network. Ann Rheum Dis 71(9):1586–1587
56. Huth ME et al (2015) Designer aminoglycosides prevent cochlear hair cell loss and hearing loss. J Clin Invest 125(2):583–592
57. Heinrich UR et al (2015) Cell-specific accumulation patterns of gentamicin in the guinea pig cochlea. Hear Res 326:40–48
58. Suzuki M, Kaga K (1996) Effect of cisplatin on the negative charge barrier in strial vessels of the Guinea Pig. A transmission electron microscopic study using polyethyleneimine molecules. Eur Arch Otorhinolaryngol 253(6):351–355
59. Suzuki M et al (2002) Effect of noise exposure on blood-labyrinth barrier in guinea pigs. Hear Res 164(1–2):12–18
60. Salt AN, Ma Y (2001) Quantification of solute entry into cochlear perilymph through the round window membrane. Hear Res 154(1–2):88–97
61. Imamura S, Adams JC (2003) Distribution of gentamicin in the guinea pig inner ear after local or systemic application. J Assoc Res Otolaryngol 4(2):176–195
62. Rosario MC et al (1998) Population pharmacokinetics of gentamicin in patients with cancer. Br J Clin Pharmacol 46(3):229–236
63. Gyselynck AM, Forrey A, Cutler R (1971) Pharmacokinetics of gentamicin – distribution and plasma and renal clearance. J Infect Dis 124:S70–S76
64. Posyniak A, Zmudzki J, Niedzielska J (2001) Sample preparation for residue determination of gentamicin and neomycin by liquid chromatography. J Chromatogr A 914(1–2):59–66
65. Alfthan O, Renkonen OV, Sivonen A (1973) Concentration of gentamicin in serum, urine and urogenital tissue in man. Acta Pathol Microbiol Scand B-Microbiol 81:92–94
66. Mingeot-Leclercq MP, Tulkens PM (1999) Aminoglycosides: nephrotoxicity. Antimicrob Agents Chemother 43(5):1003–1012
67. Deconti RC et al (1973) Clinical and pharmacological studies with Cis-Diamminedichloroplatinum(Ii). Cancer Res 33(6):1310–1315
68. Pendyala L, Creaven PJ (1993) In-vitro cytotoxicity, protein-binding, red-blood-cell partitioning, and biotransformation of oxaliplatin. Cancer Res 53(24):5970–5976
69. Jacobs C et al (1980) Renal handling of Cis-Diamminedichloroplatinum(Ii). Cancer Treat Rep 64(12):1223–1226
70. Graham MA et al (2000) Clinical pharmacokinetics of oxaliplatin: a critical review. Clin Cancer Res 6(4):1205–1218
71. Pabla N, Dong Z (2008) Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int 73(9):994–1007
72. Brillet G et al (1994) Long-term renal effect of cisplatin in man. Am J Nephrol 14(2):81–84
73. Cho W (2006) Building signaling complexes at the membrane. Sci STKE 2006(321):pe7
74. Lesniak W, Pecoraro VL, Schacht J (2005) Ternary complexes of gentamicin with iron and lipid catalyze formation of reactive oxygen species. Chem Res Toxicol 18(2):357–364
75. Orrenius S (2007) Reactive oxygen species in mitochondria-mediated cell death. Drug Metab Rev 39(2–3):443–455
76. Priuska EM, Schacht J (1995) Formation of free radicals by gentamicin and iron and evidence for an iron/gentamicin complex. Biochem Pharmacol 50(11):1749–1752

77. Baliga R et al (1998) In vitro and in vivo evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. Kidney Int 53(2):394–401
78. Dehne N et al (2001) Cisplatin ototoxicity: involvement of iron and enhanced formation of superoxide anion radicals. Toxicol Appl Pharmacol 174(1):27–34
79. Holzer AK et al (2004) The copper influx transporter human copper transport protein 1 regulates the uptake of cisplatin in human ovarian carcinoma cells. Mol Pharmacol 66(4):817–823
80. Ohrvik H, Thiele DJ (2015) The role of Ctr1 and Ctr2 in mammalian copper homeostasis and platinum-based chemotherapy. J Trace Elem Med Biol 31:178–182
81. More SS et al (2010) Role of the copper transporter, CTR1, in platinum-induced ototoxicity. J Neurosci 30(28):9500–9509
82. Chinnery PF, Hudson G (2013) Mitochondrial genetics. Br Med Bull 106:135–159
83. Li H et al (2015) Local mechanisms for loud sound-enhanced aminoglycoside entry into outer hair cells. Front Cell Neurosci 9:130
84. Turrens JF (2003) Mitochondrial formation of reactive oxygen species. J Physiol 552(Pt 2):335–344
85. Garcia-Berrocal JR et al (2007) The anticancer drug cisplatin induces an intrinsic apoptotic pathway inside the inner ear. Br J Pharmacol 152(7):1012–1020
86. Shen HM, Liu ZG (2006) JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. Free Radic Biol Med 40(6):928–939
87. Clerici WJ, DiMartino DL, Prasad MR (1995) Direct effects of reactive oxygen species on cochlear outer hair cell shape in vitro. Hear Res 84(1–2):30–40
88. Forge A, Li L (2000) Apoptotic death of hair cells in mammalian vestibular sensory epithelia. Hear Res 139(1–2):97–115
89. Mielke K, Herdegen T (2000) JNK and p38 stresskinases – degenerative effectors of signal-transduction-cascades in the nervous system. Prog Neurobiol 61(1):45–60
90. Rybak LP, Whitworth CA (2005) Ototoxicity: therapeutic opportunities. Drug Discov Today 10(19):1313–1321
91. Ylikoski J et al (2002) Blockade of c-Jun N-terminal kinase pathway attenuates gentamicin-induced cochlear and vestibular hair cell death. Hear Res 166(1–2):33–43
92. Nakamagoe M et al (2010) Estradiol protects the cochlea against gentamicin ototoxicity through inhibition of the JNK pathway. Hear Res 261(1–2):67–74
93. Lee JE et al (2004) Signaling pathway for apoptosis of vestibular hair cells of mice due to aminoglycosides. Acta Otolaryngol 124:69–74
94. Ashkenazi A, Dixit VM (1998) Death receptors: signaling and modulation. Science 281(5381):1305–1308
95. Watanabe K et al (2003) Expression of caspase-activated deoxyribonuclease (CAD) and caspase 3 (CPP32) in the cochlea of cisplatin (CDDP)-treated guinea pigs. Auris Nasus Larynx 30(3):219–225
96. Wang J et al (2004) Caspase inhibitors, but not c-Jun NH2-terminal kinase inhibitor treatment, prevent cisplatin-induced hearing loss. Cancer Res 64(24):9217–9224
97. Theneshkumar S et al (2009) Effect of noise conditioning on cisplatin-induced ototoxicity: a pilot study. Med Sci Monit 15(7):BR173–BR177
98. Fetoni AR et al (2012) Antioxidant treatment with coenzyme Q-ter in prevention of gentamycin ototoxicity in an animal model. Acta Otorhinolaryngol Ital 32(2):103–110
99. Campbell KC et al (2016) D-methionine (D-met) significantly reduces kanamycin-induced ototoxicity in pigmented guinea pigs. Int J Audiol 55(5):273–278
100. Campbell KC et al (2007) Prevention of noise-and drug-induced hearing loss with D-methionine. Hear Res 226(1–2):92–103
101. Li G et al (2001) Round window membrane delivery of L-methionine provides protection from cisplatin ototoxicity without compromising chemotherapeutic efficacy. Neurotoxicology 22(2):163–176
102. Ekborn A et al (2003) Intracochlear administration of thiourea protects against cisplatin-induced outer hair cell loss in the guinea pig. Hear Res 181(1–2):109–115

103. Tokgoz SA et al (2012) Protective effects of vitamins E, B and C and L-carnitine in the prevention of cisplatin-induced ototoxicity in rats. J Laryngol Otol 126(5):464–469
104. Yassuda CC et al (2008) The role of hyperbaric oxygen therapy (hot) as an otoprotection agent against cisplatin ototoxicity. Acta Cir Bras 23(Suppl 1):72–76; discussion 76
105. Cunningham LL et al (2004) Overexpression of Bcl-2 prevents neomycin-induced hair cell death and caspase-9 activation in the adult mouse utricle In vitro. J Neurobiol 60(1):89–100
106. Pfannenstiel SC et al (2009) Bcl-2 gene therapy prevents aminoglycoside-induced degeneration of auditory and vestibular hair cells. Audiol Neurootol 14(4):254–266
107. Wang J et al (2003) A peptide inhibitor of c-Jun N-terminal kinase protects against both aminoglycoside and acoustic trauma-induced auditory hair cell death and hearing loss. J Neurosci 23(24):8596–8607
108. Bowers WJ et al (2002) Neurotrophin-3 transduction attenuates cisplatin spiral ganglion neuron ototoxicity in the cochlea. Mol Ther 6(1):12–18
109. Li X et al (2011) Protective role of hydrogen sulfide against noise-induced cochlear damage: a chronic intracochlear infusion model. PLoS One 6(10):e26728
110. Roy S et al (2013) Sound preconditioning therapy inhibits ototoxic hearing loss in mice. J Clin Invest 123(11):4945–4949
111. McLean WJ et al (2017) Clonal expansion of Lgr5-positive cells from mammalian cochlea and high-purity generation of sensory hair cells. Cell Rep 18(8):1917–1929
112. Cheng C et al (2017) Characterization of the transcriptomes of Lgr5+ hair cell progenitors and Lgr5– supporting cells in the mouse cochlea. Front Mol Neurosci 10:122
113. Lu X et al (2017) Bmi1 regulates the proliferation of cochlear supporting cells via the canonical Wnt signaling pathway. Mol Neurobiol 54(2):1326–1339
114. He Z et al (2017) Autophagy protects auditory hair cells against neomycin-induced damage. Autophagy 13(11):1884–1904
115. Fang B, Xiao H (2014) Rapamycin alleviates cisplatin-induced ototoxicity in vivo. Biochem Biophys Res Commun 448(4):443–447
116. Yu H et al (2013) Inhibition of H3K9 methyltransferases G9a/GLP prevents ototoxicity and ongoing hair cell death. Cell Death Dis 4:e506
117. He Y et al (2015) Inhibition of H3K4me2 demethylation protects auditory hair cells from neomycin-induced apoptosis. Mol Neurobiol 52(1):196–205
118. Izumikawa M et al (2005) Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nat Med 11(3):271–276
119. Li L et al (2017) Advances in nano-based inner ear delivery systems for the treatment of sensorineural hearing loss. Adv Drug Deliv Rev 108:2–12

Chapter 3
Noise-Induced Cochlear Synaptopathy and Ribbon Synapse Regeneration: Repair Process and Therapeutic Target

Jian Wang, Shankai Yin, Hengchao Chen, and Lijuan Shi

Abstract The synapse between the inner hair cells (IHCs) and the spiral ganglion neurons (SGNs) in mammalian cochleae is characterized as having presynaptic ribbons and therefore is called ribbon synapse. The special molecular organization is reviewed in this chapter in association with the functional feature of this synapse in signal processing. This is followed by the review on noise-induced damage to this synapse with a focus on recent reports in animal models in which the effect of brief noise exposures is observed without causing significant permanent threshold shift (PTS). In this regard, the potential mechanism of the synaptic damage by noise and the impact of this damage on hearing are summarized to clarify the concept of noise-induced hidden hearing loss, which is defined as the functional deficits in hearing without threshold elevation. A controversial issue is addressed in this review as whether the disrupted synapses can be regenerated. Moreover, the review summarizes the work of therapeutic research to protect the synapses or to promote the regeneration of the synapse after initial disruption. Lastly, several unresolved issues are raised for investigation in the future.

Keywords Noise-induced hidden hearing loss · Ribbon synapses · Synapse regeneration · Neurotrophins

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3.1 Introduction

According to the current standard (ISO1999: 2013(E)), noise-induced hearing loss (NIHL) is defined by sustaining a permanent threshold shift (PTS). However, this definition has been challenged by the fact that noise exposure can cause massive damage to the synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) in the cochleae of laboratory animals without a significant PTS [24, 43, 48, 50, 88, 90, 94]. The synaptic damage and the associated functional deficits in signal coding by auditory nerve fibers (ANFs) have been labelled as noise-induced cochlear synaptopathy. Since coding deficits in the absence of a PTS cannot be detected by routine audiological evaluations that are currently focused on seeking thresholds, they are umbrellaed under the concept of noise-induced hidden hearing loss (NIHHL) [42, 47, 51, 57, 64, 94]. This chapter will review the current knowledge of the potential mechanisms of noise-induced synaptic damage and the following repair processes after a brief review on the special anatomy of the synapse between IHCs and SGNs. The chapter will then shift its focus to the therapeutic methods promoting the regeneration of the synapses.

3.2 Anatomic and Functional Features of Cochlear Ribbon Synapse

The synapse between IHCs and SGNs is characterized by the presence of an electron-dense, ribbon-like structure and therefore called a ribbon synapse. It is mainly found in the retina, the inner ear, and in the pinealocytes. The structural features of the ribbon synapses between IHCs and SGNs are summarized in Fig. 3.1. The synaptic ribbons found in mature hair cells are anchored to the plasma membrane, one ribbon per active zone (AZ). A small number of "floating" ribbons (<5%) were observed and probably reflected the turnover of these subcellular organelles [40, 114]. The synaptic ribbons in the IHC are shaped like an American football, with a tee structure underneath that is formed by a protein named Bassoon. This bar structure anchors the ribbon to the AZ [59].

The main protein forming the framework of the ribbon is called the Ribeye, which consists of two domains: the A-domain is located inside and appears to have a structural role, whereas the B-domain points to the cytoplasmic face of the ribbon, where it interacts with other proteins and tethers synaptic vesicles [2]. The amino-terminal A-domain is not homologous with any other protein in the public databases and therefore specific to the ribbon synapse, whereas the carboxyterminal B-domain is largely identical to the nuclear corepressor protein, C-terminal binding protein 2 (CtBP2). The gene encoding the Ribeye is called the CtBP2 gene, which encodes two proteins: the unique Ribeye(A + B) in the ribbon synapses and the CtBP2, which is also expressed in the cellular nucleus [52, 100, 106, 114]. The Ribeye in photoreceptor cells contains CtBP1 [102], which has not been verified in IHC ribbons.

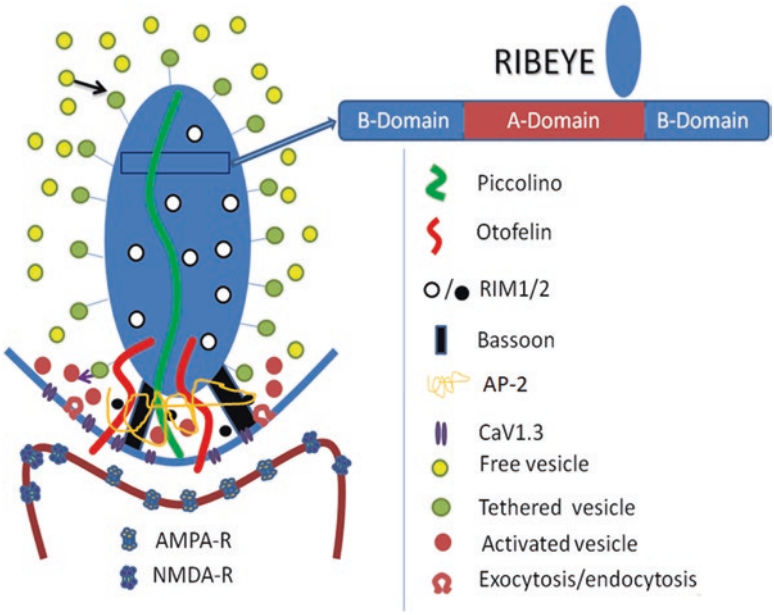

Fig. 3.1 Schematic view of IHC ribbon synapses

The scaffold of synaptic ribbons is built up from multiple Ribeyes [52]. The Ribeye A-domain has three interaction docking sites that mediate homotypic interactions with other RIBEYE(A)-domains. In addition, homotypic B-domain interactions can be formed as well as heterotypic interactions between the RIBEYE A- and B-domains, which are regulated (inhibited) by nicotinamide adenine dinucleotide hydride (NADH). In the photoreceptors, ribbon size dynamically changes in response to light: ribbons are disassembled in bright light and reassembled in dark [1, 75, 84, 95, 103]. It is not clear if the ribbons in the IHCs are dynamically disassembled/reassembled.

The most striking functional characteristic of the IHC ribbon synapse is its ability to make fast response to transient signals in the meantime to keep its long-lasting response to persistent stimuli. These features require special mechanisms to enable fast neurotransmitter release (exocytosis) and replenishment, as well as fast recycling of neurotransmitters via endocytosis. It is not entirely clear how these processes are realized. However, they must be related to the special protein compositions and the structure of the cytomatrix of the active zone (CAZ). Several proteins that are important for transmission across conventional synapses are not seen in IHCs. Those include synaptotagmins 1 and 2, synapsins, synaptophysins, synaptogyrin complexins, neuronal SNAREs as well as priming factors of the Munc13 and CAPS families (see reviews [56, 81]). Instead, the function of those proteins seems to be replaced by a single protein, otoferlin, which is located between the ribbon and the presynaptic membrane and strongly interacts with adaptor protein 2 (AP-2) [17, 38, 61].

Bassoon and Piccolo are two big proteins (>400 kDa) that are seen in conventional synapses. Their function in synaptic transmission is not clear. In ribbon synapses, Bassoon is responsible for anchoring the ribbons to the CAZ. The knockout of this protein in the cochlea of mice results in the loss of ribbons and the deterioration of temporal resolution of the auditory nerve fibers (ANFs), without significant change in hearing sensitivity [7, 37]. Piccolo is present in ribbon synapses as a shortened variant, called Piccolino, which is distributed over the entirety of the ribbon. Knockdown of this protein resulted in a lack of dynamic ribbon assembly in the retina of mice [23, 76]. However, it is not clear what role the Piccolino plays in IHC ribbon synapses.

The postsynaptic terminal of the ribbon synapses exhibits similarities with the conventional excitatory synapses. Glutamate has been confirmed as the neurotransmitter in the IHC ribbon synapse [27, 28, 58]. Once the neurotransmitter is released into the synaptic cleft, it activates an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) residing within a receptor cluster at the postsynaptic density of afferent ANFs [27]. Glutamate receptor subtypes (GluR) 2, 3, and 4 are abundant in IHC ribbon synapses [53]. GluR2 is not expressed until the onset of hearing, while GluR3 and GluR4 are present earlier during development. N-methyl-d-aspartate (NMDA) receptors (NR1, NR2A/B) are also present at the afferent synapses in the cochlea [58, 69, 80]. They are not activated for fast transmission as they are blocked by magnesium at resting membrane potential [31]. However, they modulate the reaction of AMPAR to glutamate at the type I afferent terminals [9, 28].

3.3 Synaptic Damage by Noise

3.3.1 Potential Mechanisms

The finding that noise induces cochlear synaptopathy reveals a novel locus of cochlear damage for NIHL. The damage to the postsynaptic terminal occurs through a similar mechanism as seen in conventional excitatory synapses: the glutamate-induced excitotoxicity. This mechanism is supported by the fact that cochlear infusion with glutamate or agonists mimics noise-induced damage [68, 71, 78]. Calcium influx and accumulation in the postsynaptic terminal is the initial step toward excitotoxicity [98]. Among the ionotropic glutamate receptors (iGluRs), AMPARs, NMDARs, and kainite-Rs, NMDARs have been considered the major contributors to the calcium influx and accumulation in the postsynaptic terminals. Therefore they are mainly responsible for the excitotoxicity in general [65, 98] and in the mammalian cochlea [55, 98]. While the neurotransmission between IHCs and SGNs is mainly mediated by the AMPAR, it is not considered responsible for the calcium influx. However, this opinion has been challenged by a recent report [85]. In this study, three subunits of AMPARs (GluA2, GluA3, and GluA4) were identified, and

only GluA2 lacks calcium permeability. Moreover, calcium influx to the postsynaptic neurons was found to occur mainly via the Ca-permeable AMPARs (CP-AMPARs), but not NMDARs as previously recognized. This conclusion is supported by the fact that the CP-AMPAR blocker, IEM1460 (N, N, N-trimethyl-5-(tricyclo [3.3.1.13,7] dec-1-ylmethyl) amino-1-pentanaminium bromide hydrobromide), significantly reduces calcium accumulation in the postsynaptic auditory neurons, whereas the NMDAR blocker, APV (DL-2-amino-5-phosphonovaleric acid), shows no effect.

It is not clear if there is/are presynaptic mechanism(s) for the synaptic damage by noise or other toxic factors other than the Ca^{2+}-mediated glutamate release. The presynaptic ribbons within photoreceptors are dynamic: they are dissembled in bright light and reassembled in dark [1, 75, 84, 95, 103]. This dynamic change serves as a mechanism of adaptation to stimulation and results in the change of neurotransmitters released. However, there is no evidence to date that supports the ribbons in the IHCs being dynamic in their response to acoustic overstimulation. Based upon the immunohistochemistry observation, the noise-induced reduction of the presynaptic ribbons is parallel with the breakdown of the postsynaptic terminals [89, 94]. In the photoreceptors, ribbons are dissembled when the cells are hyperpolarized by light that causes a reduction of $[Ca^{2+}]_i$. In the IHCs, the response to acoustic stimulation is a depolarization of membrane potential (not hyperpolarization) and an increase of $[Ca^{2+}]_i$. Therefore, if there is a disassembly/reassembly process in the IHCs, it must undergo a different mechanism. It is possible that the presynaptic ribbons in the IHCs are broken down after the postsynaptic terminals are destroyed. More research is needed to identify the fate of the ribbon protein after they are broken down by noise.

3.3.2 Selective Damage to Synapses with Low Spontaneous Rate Units

One IHC synapses with more than ten SGNs, and the synapses are distributed around the bottom of the IHC. The susceptibility of the synapses to noise damage appears to be location dependent: the synapses at the modiolar side of the IHC are more easily damaged. Although, the underlying mechanism is not entirely clear, this bias has been linked to the morphological variation around IHCs when identified in immunohistochemistry against CtBP2 and AMPAR (Fig. 3.2). The synapses close to the modiolar side of an IHC have larger ribbons and smaller postsynaptic terminals, whereas the synapses distributed toward the pillar side are the opposite [46]. This difference is functionally important because the synapses located at the modiolar side of IHCs innervate auditory nerve fibers (ANFs) that have lower spontaneous spike rates (SR), higher thresholds, and larger dynamic ranges. These ANFs are considered critical for hearing in noisy backgrounds, where only the high spontaneous SR units are saturated [10, 18, 33, 63, 112].

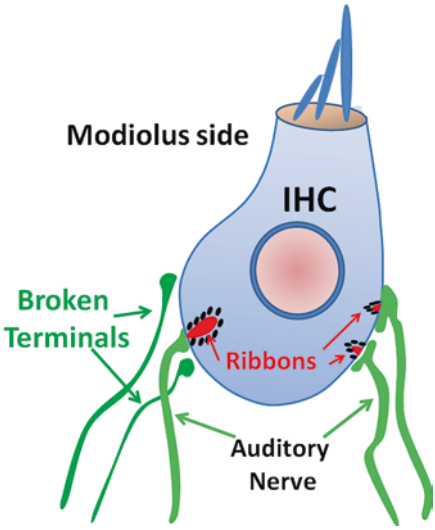

Fig. 3.2 Spatial variations of ribbon synapses around the IHC. The synapses on the modiolar side appear to have a larger ribbon but a smaller postsynaptic terminal. The synapses on this side are also more sensitive to noise-induced damage

Several potential mechanisms have been proposed to explain the difference in the noise susceptibility between the synapses around IHCs. Firstly, there is a heterogeneity of Ca^{2+} channels around IHCs: synapses at modiolar side appear to have more Ca^{2+} channels per CAZ and display a higher Ca^{2+} influx and potentially a larger neurotransmitter release [20, 54]. The activation of the Ca^{2+}channels at the modiolar side requires a larger degree of depolarization [54, 109, 110]. This heterogeneity has been linked to the spatial variations in the threshold and dynamic range of ANFs and to synaptic damage by noise around the IHCs [56]. Secondly, the larger amount of neurotransmitter release may be related to the larger ribbon size. The larger ribbons at the modiolar side can harbor more neurotransmitter vesicles close to the CAZ [27, 45, 93]. However, it is not clear if the vesicular priming and replenishment occurs faster for the ribbon synapses at the modiolar side of IHCs. Thirdly, the clearance of the released glutamate likely occurs slower at the modiolar side due to the lower amount of glutamate-aspartate transporters (GLAST) [25, 26, 74]. Fourthly, iGluRs (including AMPARs and NMDARs) are responsible for the glutamate-induced excitotoxic cell death in many neurologic diseases [29, 104]. Previously, NMDARs were thought to play a major role in noise-induced postsynaptic cochlear damage [4, 41], but more recently, this role has been attributed to Ca^{2+}-permeable AMPAR as discussed above [85]. Nevertheless, it is not clear if the NMDARs are selectively distributed to the synapses at the modiolar side of the IHCs [83]. Interestingly, heterogeneity in the relative distribution of both Ca^{2+}-permeable and Ca^{2+}-impermeable subunits of AMPARs has been demonstrated across the IHC-SGN synapses. However, it is not clear how the heterogeneity is related to the synaptic distribution around the IHCs.

The functional significance of the selective damage to ANF synapses with the low-SR units remains to be confirmed. Theoretically, the selective loss of synapses with the low-SR ANFs will impair signal coding in strong background noise, one of the major problems seen in aging subjects [42, 56, 57, 88]. However, this coding deficit remains a speculation and has not been confirmed in single-unit data.

3.3.3 Can the Disrupted Ribbon Synapse Be Rebuilt?

It is currently debated whether the synaptic disruption by noise is reversible. In a pioneering study with CBA mice, no significant recovery of synapse counts was found after the threshold recovery that occurred in 1 week after the noise exposure [43]. Therefore, a single brief noise exposure caused up to 50% loss of synapses, permanently. However, studies from our labs in both Canada and China found that the decrease in synapse count was largely reversible in guinea pigs [50, 89, 94]. This reversibility was also reported in mice other than the CBA strain [90, 91]. It is worthy to notice that the concept of synaptic repair may involve two different phenotypes: (1) the rebuild of the synapses that are destroyed (by synaptogenesis) and (2) the repair of survived synapses that are damaged but not disconnected. The synaptic repair of the second type was reported after the initial noise-induced damage by the group of scientists who first reported noise-induced synaptic damage in the cochlea [67, 70, 71, 77]. In those early reports, the synaptic damage was observed using transmission electron microscopy (TEM). This technique limited the observation on the synapses that were partially damaged, but not destroyed. More importantly, the observation was not quantitative for the counting of total synapses. More recently, the dynamic changes of the number of ribbon synapses were reported in a study using AMPA infusion [79]. In our labs, the rebuild of the disrupted synapses was demonstrated by recovery of synapse counts. Functional data supported the synaptogenesis in that the recovery of the synapse count was matched by the recovery of compound action potential (CAP) measures (Fig. 3.3 a and b) [50, 89, 94]. In addition, the repair is also supported by the morphological changes of the synapses in the noise-damaged cochlea. Shortly after noise-induced damage, some synapses were found to be located up to the level of IHC nuclei and with extremely large ribbons, seen in immunohistochemical observation [89]. The synapse distribution returned to normal several weeks after the noise exposure, suggesting the re-established synapses were formed at a location close to the protein synthesis organelle. Furthermore, the repaired presynaptic ribbons appeared to have uneven sizes, with bigger hollow cores. In addition, many synapses observed weeks after the noise exposure had multiple ribbons to one AZ in TEM observation (Fig. 3.3c) [94]. This feature is seen in naïve ribbon synapses during early development [81] and is consistent with the regeneration of the synapses after they are destroyed by AMPA [79].

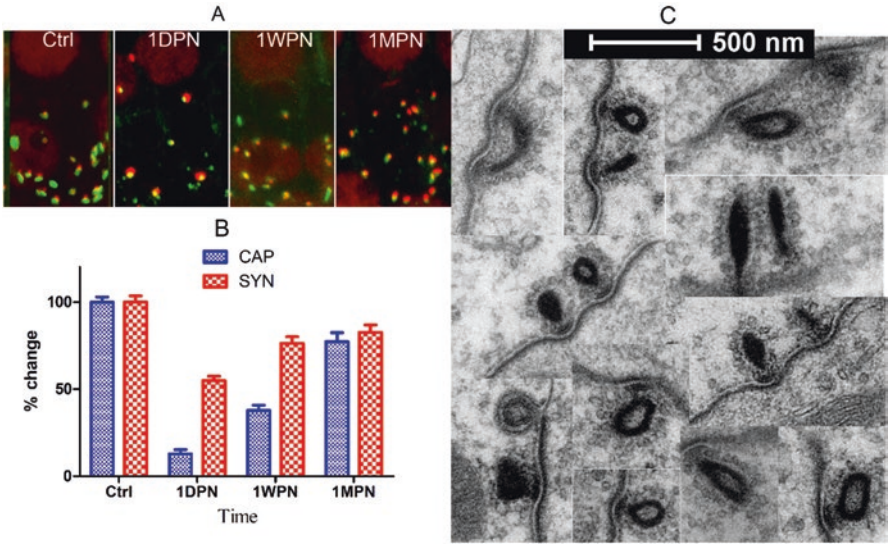

Fig. 3.3 Evidence for synaptic repair after noise-induced damage. (**a**) Immunostaining images of pre- and postsynaptic components in control (ctrl) IHC and those 1 day, 1 week, and 1 month post noise (1DPN, 1WPN, and 1MPN). (**b**) Percentage changes of maximal CAP amplitude and synapse counts after noise. (**c**) TEM images of IHC ribbon synapses taken at 1MPN, showing hollow cores in some ribbons and double ribbons in some synapses

3.4 Synaptic Protection and Regeneration in Noise-Induced Synaptopathy

3.4.1 Synapse Protection

Noise-induced ribbon synapse damage involves the structural breakdown of both presynaptic ribbons and postsynaptic terminals [43, 89]. The mechanisms for the noise-induced damage on the postsynaptic terminal are clear and likely due to the glutamate-mediated excitotoxicity [70–72, 79]. Ca^{2+} overload via GluRs and voltage-gated Ca^{2+} channels (VGCCs) has been recognized as playing a critical role in noise-induced cochlear damage, both on HCs and postsynaptic terminals [5, 62, 65, 85]. Application of VGCC blockers (both L- and N-types) has shown the ability to protect the cochlear HCs from noise damage, consistent with the distribution of those calcium channels on HCs [34, 39, 49, 87, 101, 113]. However, it is not clear if the application of the blocker can prevent noise-induced synaptic damage.

Since noise causes synaptic damage via GluRs, blockage of these receptors may protect the synapses against noise. HC damage has been seen as part of excitotoxicity in zebrafish larvae, in which iGluRs are found to be expressed in the HCs [86]. Several studies have shown that NMDAR blockers can prevent tinnitus induced by salicylate [12, 66] and noise [4, 32]. Further research is needed to verify potential mechanisms [82, 83]. It is also important to note that blocking of iGluRs may have

unforeseen effects. For example, long-term blockage of NMDA has been found to hinder the regeneration of the IHC-SGN synapses after excitotoxic damage [11, 79].

Previously, NMDAR antagonists have been tested for this potential protection [14, 15, 60]. However, the most significant effect of protection was seen on HCs, not on SGNs. Application of the NMDAR antagonists has been reported to reduce the swelling of the afferent dendrites synapsed with the IHCs in guinea pigs [14, 15]. However, the method for synapse quantification in those studies is questionable, since the number of the damaged synapses by noise in those studies was much fewer than that reported more recently using immunohistochemistry staining [43, 50, 89, 94]. Clearer evidence of synaptic protection against noise by the NMDAR antagonist was reported more recently [4]. However, in this study, the antagonist was administered at least 2 days after the noise exposure. Therefore, it is not clear what mechanism is underneath the reduction in synaptic damage. Presumably, the effect of the NMDARs in noise-induced damage to the afferent dendrites is based upon their role as a ligand-gated calcium channel. However, a recent study indicated that the sound-induced calcium entry was not mediated by NMDARs but by Ca^{2+}-permeable AMPARs at the site [85]. This finding has shaken the theoretical basis of using NMDAR antagonists to reduce noise-induced synaptic damage in the cochlea. Previously, one study showed the protective effect of a blocker (caroverine) against both AMPA and NMDA receptors. It reduced the HC loss caused by impulse noise [16]. However, the protective effect on the synapses was not investigated. Therefore, further studies are needed to verify if NMDAR and/or AMPAR antagonists can protect the synapses from noise.

3.4.2 Synapse Regeneration

Since the synaptic damage induced by noise is partially reversible, there exists an endogenous mechanism to maintain the stability of the synaptic connections between SGNs and IHCs. Various studies have indicated the role of neurotrophic factors (NTFs) in synapse formation during development, plasticity, and the maintenance of synaptic stability in the cochlea (see review [21, 22, 73, 111]). Using NTFs appears to be a practical approach to rescue the damaged auditory nerves and their synapses to HCs [3]. Neurotrophins are a subclass of NTFs that are ubiquitously expressed and are very extensively studied. There are four types of neurotrophins in mammals: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). BDNF and NT-3 are the major types of neurotrophins seen in the mammalian cochleae [73]. Within these two, BDNF is highly expressed during early development and declines to undetectable levels in adulthood. NT-3 is the only neurotrophin that exists in the adult cochleae, in addition to glial cell line-derived neurotrophic factor (GDNF) [13, 21, 30]. While the p75 neurotrophin receptor ($p75^{NTR}$) is the receptor shared by BDNF, NT-3, and GDNF, each of the three factors has its own specific binding site(s): NT-3 binds mainly to the receptor C of the tropomyosin-related kinase

(TrkC), BDNF to TrkB, and GDNF to RET-GFRα1 complex [73]. However, TrkA, TrkB, and TrkC are all expressed in the adult cochlea, even though their corresponding ligands (NGF to TrkA and BDNF to TrkB) are not detectable [30]. Therefore, BDNF and NGF can also be used for therapy in addition to NT-3, for the regeneration of the synapses after disruption.

NT-3 has been examined in several studies for its ability to promote the regeneration of the synapse. In the mammalian cochlea, NT-3 is expressed in both HCs and supporting cells [19, 22]. NT-3 overexpression by gene knock-in has been reported to increase the synapse density between IHCs and SGNs and decreased ABR threshold in mice [105]. The study suggested that the supporting cells are a more important source of NT-3 because the selective knock-in of NT-3 in the supporting cells promoted the regeneration of the synapses after disruption by noise exposure. However, the effect of selective knock-in in the HCs on synapses regeneration was not examined in this study.

Two studies showed a rescue effect of exogenous NT-3 to IHC-SGN synapses, when applied through the round window after noise trauma [92, 96]. In the first study done in mice, NT-3 was administered via the round window 24 hours after a 2 h noise exposure at 100 dB SPL, with the synapse count performed days later [96]. The NT-3 was delivered via slow-release gel placed in the round window niche. The protective effect of NT-3 was evaluated in both functional tests of auditory brainstem response (ABR) amplitude and synapse count. However, a large individual variation in the protective outcome made the authors divide the subjects into "effective" versus "ineffective" subgroups. Presumably, the "ineffective" was likely due to the failure in NT-3 application. A weak significance was seen when all of the NT-3 treated subjects were grouped together. In the second study listed above, guinea pigs were used. An equal mixture of NT-3 and BDNF was applied to the round window immediately after the noise exposure, which was given either at 95 or 105 dB SPL for 2 hours [92]. The synapse count observed 2 weeks after the noise exposure showed a significantly greater number in the ear treated with neurotrophins for the subjects receiving the 95 dB noise exposure. Since no data was reported from the subjects receiving the 105 dB noise exposure, and no control subjects were assessed, the interpretation of this data is difficult. Furthermore, the mixture of the two neurotrophins makes it difficult to measure the contribution from each neurotrophin.

Instead of using exogenous neurotrophins, the gene therapy type of approach appears to be more attractive in that it can provide long-term protection against repeated noise exposures. Using the gene knock-in technique, it has been found that overexpression of the NT-3 (but not BDNF) gene in supporting cells could significantly promote the ribbon synapse regeneration after noise-induced damage. However, no such protection was seen if the overexpression was only done in the IHCs [105]. In this study of normal-hearing guinea pigs, the overexpression of NT-3 in supporting cells and IHCs surprisingly increased the synaptic density of IHCs. Furthermore, the increase in synaptic density was accompanied by an increased ABR wave I amplitude and a decreased ABR threshold [105].

Due the ethical considerations, gene knock-in is unlikely to be used on human subjects. Instead, local gene transfection is an approach that can be translated to human clinics. To date, viral vectors appear to be much more effective in gene transfection. Among the viral vectors available, adeno-associated virus (AAV) is the most attractive due to its safety and the ability to cause long-lasting expression of the transfected gene. Several human trials of gene therapy are ongoing using AAV vectors. In a recent report, AAV-mediated NT-3 overexpression was found to cause considerable regeneration of synapses between IHCs and ANFs in guinea pigs that were deafened by aminoglycosides [6]. However, the benefit of NT-3 overexpression in the cochlea has been challenged by a study in which the overexpression was mediated by using (AAV) or adenovirus (Adv) [44]. In this study, subjects receiving the transfection either by AAV or Adv experienced ABR threshold elevations, more with Adv transfection. A decreased synapse count was seen in the subjects receiving Adv, but not AAV. The authors concluded that the elevation of NT-3 levels in the cochlea can disrupt synapses and impair hearing. A comparison between the two studies is impossible because one was done with normal-hearing subjects, while the other was done in subjects deafened by aminoglycosides. Furthermore, neither of the studies provided data for the transfection rate; and the study was done on deafened guinea pigs; no data was reported from a control group [6].

The safety of AAV vectors in cochlear gene transfection has been widely supported in the literature [35, 97, 99] and by our own published work [36, 107]. Recently conducted in our labs, the AAV of serotype 8 that had a surface tyrosine mutation at the residue of 733th amino acid on the capsid (rAAV8-mut773, at the titer of 6.92×10^{13}, provided by the Retinal Gene Therapy Group, University of Florida, USA) was applied to transfect NT-3 into the cochlear cells of guinea pigs [8]. Figure 3.4 shows that the transfections of IHCs reached ~100% at the base and spread up to the second turn of the cochlea (10 mm from the apex or 4 kHz region). Therefore, it is a good model to test if the overexpression of NT-3 by AAV could promote synapse regeneration after noise-induced damage. After baseline ABR tests, transfection with AAV-NT-3 was done in one ear of each of the seven guinea pigs, whereas the other ear was given a sham surgery with the injection of the equivalent amount of saline. The ABR was retested 1 week after the transfection surgery. No threshold differences were seen between the ears (Fig. 3.5a). Then the animals were exposed to a high-pass noise with the cutoff at 4 kHz at 105 dB SPL for 2 h to create synaptopathy. A third ABR was administered 2 weeks after the noise exposure, followed by a near-field test of CAP with a round electrode. After the functional tests, the animals were sacrificed, and their cochleae were harvested for morphological evaluation for ribbon synapse counts. Another six animals were recruited as no-noise blank control.

Consistent with our previous reports, there was no significant difference in ABR thresholds between the baseline and 2 weeks after the noise exposure (Fig. 3.5a). To evaluate the impact of the synaptic damage on cochlear output, CAP was measured with clicks of different levels (Fig. 3.5b). The ears injected with AAV and saline are labelled as the two noise groups. In both groups, the noise exposure reduced CAP amplitude by more than half, and the input-output (I/O) functions from the noise

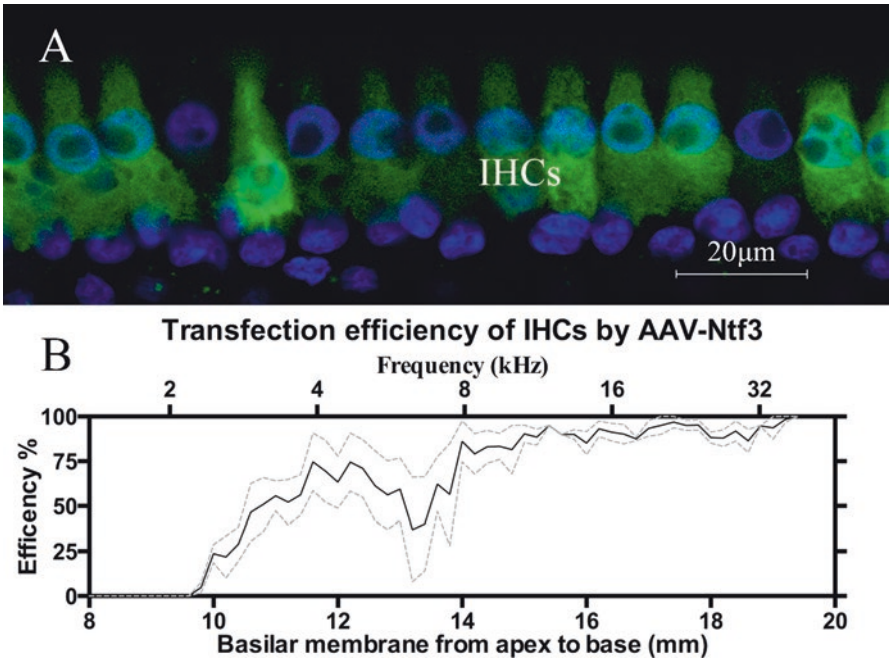

Fig. 3.4 AAV-mediated NT-3 transfection. (**a**) Representative IHCs image from 16 kHz region showing the transfected cells (green) across the cochlea. (**b**) Transfection cochleogram showing the mean (solid line) and +/-SEM (dashed lines) transfection of IHCs from three cochleae. (This figure is adapted from Chen et al. [8] Gene Therapy 25(4): 251–259)

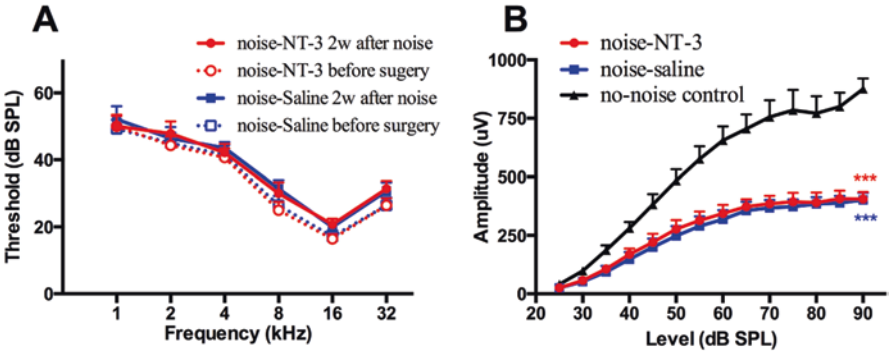

Fig. 3.5 Physiological function. (**a**) ABR threshold of two noise groups tested before surgery and 2 weeks after noise exposure. (**b**) The click-evoked CAP input-output function. (This figure is adapted from Chen et al. [8] Gene Therapy 25(4): 251–259)

groups were overlapped. Significant differences among the noise groups were seen in a one-way ANOVA, performed for the maximal CAP amplitude at 90 dB SPL ($F = 57.6$, $P < 0.001$). Post hoc tests (Bonferroni method) showed that the differences between the no-noise control group and the two noise groups were significant

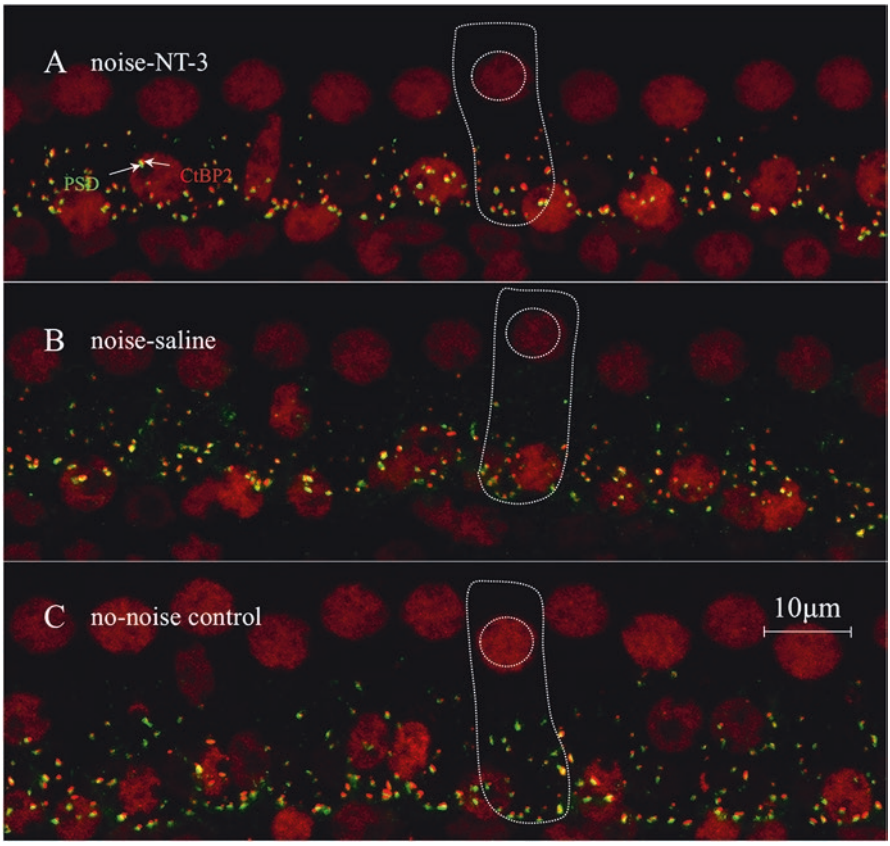

Fig. 3.6 Images of CtBP2 and PSD staining from the three groups at 16 kHz region. (**a**) (**b**), and (**c**) were noise-NT-3, noise-saline, and no-noise control groups, respectively. The dashed lines indicate the outlines of IHCs and their nuclei. Only paired CtBP2 (red) and PSD (green) puncta were counted as synapses

(control vs noise-NT-3: $t = 9.3$, $p < 0.001$; control vs noise-saline $t = 9.3$, $p < 0.001$), but not significant between the two noise groups ($t = 0.1$, $p = 1$).

To evaluate the synaptic loss induced by the noise, the presynaptic ribbons (CtBP2) and postsynaptic densities (PSDs) were examined in immunohistochemistry (Fig. 3.6). The number of synapses was counted with the puncta of CtBP2s and PSDs that were paired. At each frequency point in each ear, the synapses were counted over eight IHCs to calculate the average synapse density (# of synapses per IHC). The noise-induced synaptic loss was mainly seen in the high-frequency region (>8 kHz, Fig. 3.7a). The effect of the NT-3 overexpression was demonstrated by less synaptic loss in the frequency region between 11.3 and 22.6 kHz (Fig. 3.7a). Over the high-frequency region (>8 kHz), the average synapse densities were 16.4 ± 0.2, 15.2 ± 0.2, and 18.4 ± 0.1 per IHC, for the noise-NT-3 group, noise-saline group, and no-noise control group, respectively. When compared to the

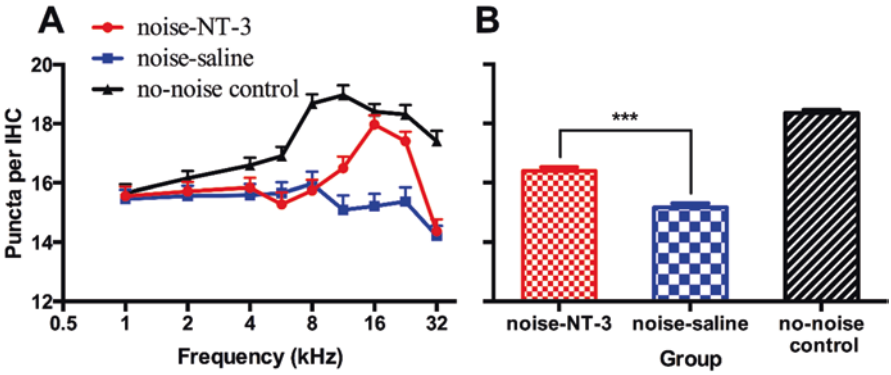

Fig. 3.7 NT-3 overexpression reduced the noise-induced synaptic loss. (**a**) The density-frequency curves of paired CtBP2 and PSD puncta. (**b**) The averaged counts of synapse density in the high-frequency region (between 8 and 32 kHz). One-way ANOVA followed by Bonferroni's post hoc test revealed the significant differences between the no-noise control group and the two noise groups (not shown) and between the two noise groups. (**a**) error bar represent mean±SEM, (**b**) error bar represent mean±SEM. Asterisks indicate a significant level for the comparison between the two noise-exposed ears. ***$p < 0.001$. (This figure is adapted from Chen et al. [8] Gene Therapy 25(4): 251–259)

no-noise control, this finding resulted in a 17.4% synaptic density reduction in the noise-saline group and a 10.9% reduction in the noise-NT-3 group. Compared between the noise-exposed groups, NT-3 overexpression appeared to reduce the synapse loss by ~38.5% in the high-frequency region. A significant effect of grouping was seen in a one-way ANOVA ($F_{2,477} = 81.3$, $p < 0.001$). The Bonferroni's post hoc tests revealed the differences between the no-noise control group and the two noise groups (control vs noise-NT-3: $t = 7.8$, $p < 0.001$; control vs noise-saline $t = 12.6$, $p < 0.001$) and between the two noise groups ($t = 4.9$, $p < 0.001$) (Fig. 3.7b).

The nonsignificant difference result for NT-3, seen in the CAP I/O function, is likely due to the frequency range of the click-evoked CAP being biased to low-frequency regions, where no protection in the synapse count was seen. The power spectrum of clicks of 0.1 ms pulses was below 5 kHz. Even with the up-spread of cochlear vibration at a high intensity (90 dB SPL), the auditory nerves with characteristic frequencies higher than 8 kHz are unlikely to be excited. It is interesting to note that a synapse reduction of less than 5% was seen in the low-frequency region, while the CAP amplitude was reduced more than 50%. This suggests that the surviving/repaired synapses are functionally abnormal at this frequency.

In this study, we did not dynamically track the change in synapse counts at different time points after the noise exposure, nor did we compare the change across groups. Therefore, we do not know if the small reduction of synapse counts in the NT-3 overexpressed group resulted from the reduction of the initial loss of the synapses or the promoting effect of NT-3 on the regeneration of the synapses. However, based upon the working mechanism of NT-3 on synapse formation, and the rescue effect of NT-3 observed after noise exposure [92, 96, 108], we hypothesize that the

major effect of NT-3 overexpression by AAV transfection in the present study is due to its effect on promoting synapse regeneration.

Based upon the study with the knock-in mouse model, NT-3 from supporting cells appear to be more effective than NT-3 from IHCs for promoting synapse regeneration [105]. In this study, the overexpression of the NT-3 was reportedly not effective at all. However in the present study (accepted), a significant protective effect is seen even though the NT-3 overexpression is limited to only IHCs (Fig. 3.4). While the quantitative comparison is impossible between the two studies due to the use of different species and different techniques for the overexpression, the protective effect in our study may have been limited by the confined transfection mediated by rAAV8-mut773 in the IHCs. We are exploring the use of new AAV that will transfect both the HCs and supporting cells for better protection against noise.

3.5 Conclusion and Future Direction

Gene therapy via cochlear gene transfection is an attractive approach to reduce noise-induced synaptopathy. The significance of this therapy is emphasized by the high probability of exposure to noise that can potentially produce such damage. Since NT-3 in both IHCs and supporting cells contributes to the synaptic regeneration, the AAV vector should be improved to transfect both the IHCs and supporting cells.

More research is needed to understand why synapses to the low-SR ANFs are more sensitive to noise damage. Research is also needed to investigate if there is a dis/reassembly mechanism of ribbons that act adaptively to reduce the traumatic glutamate release in response to intense noise. If this occurs, investigation into how this mechanism is regulated should be pursued. Research on gene therapy should be associated with the mechanisms for the neural transmission across this special synapse. Understanding the mechanisms of noise-induced synaptic damage in association with the working mechanism of ribbon synapses will provide insight toward reducing noise-induced damage and then increasing the amount of repair.

References

1. Adly MA, Spiwoks-Becker I, Vollrath L (1999) Ultrastructural changes of photoreceptor synaptic ribbons in relation to time of day and illumination. Invest Ophthalmol Vis Sci 40:2165–2172
2. Alpadi K, Magupalli VG, Kappel S, Koblitz L, Schwarz K, Seigel GM, Sung CH, Schmitz F (2008) RIBEYE recruits Munc119, a mammalian ortholog of the Caenorhabditis elegans protein unc119, to synaptic ribbons of photoreceptor synapses. J Biol Chem 283:26461–26467
3. Bezdjian A, Kraaijenga VJ, Ramekers D, Versnel H, Thomeer HG, Klis SF, Grolman W (2016) Towards clinical application of neurotrophic factors to the auditory nerve; assessment of safety and efficacy by a systematic review of neurotrophic treatments in humans. Int J Mol Sci 17

4. Bing D, Lee SC, Campanelli D, Xiong H, Matsumoto M, Panford-Walsh R, Wolpert S, Praetorius M, Zimmermann U, Chu H, Knipper M, Ruttiger L, Singer W (2015) Cochlear NMDA receptors as a therapeutic target of noise-induced tinnitus. Cell Physiol Biochem 35:1905–1923
5. Brassai A, Suvanjeiev RG, Ban EG, Lakatos M (2015) Role of synaptic and nonsynaptic glutamate receptors in ischaemia induced neurotoxicity. Brain Res Bull 112:1–6
6. Budenz CL, Wong HT, Swiderski DL, Shibata SB, Pfingst BE, Raphael Y (2015) Differential effects of AAV.BDNF and AAV.Ntf3 in the deafened adult guinea pig ear. Sci Rep 5:8619
7. Buran BN, Strenzke N, Neef A, Gundelfinger ED, Moser T, Liberman MC (2010) Onset coding is degraded in auditory nerve fibers from mutant mice lacking synaptic ribbons. J Neurosci 30:7587–7597
8. Chen H, Xing Y, Xia L, Chen Z, Yin S, Wang J (2018) AAV-mediated NT-3 overexpression protects cochleae against noise-induced synaptopathy. Gene Ther 25:251–259
9. Chen Z, Peppi M, Kujawa SG, Sewell WF (2009) Regulated expression of surface AMPA receptors reduces excitotoxicity in auditory neurons. J Neurophysiol 102:1152–1159
10. Costalupes JA (1985) Representation of tones in noise in the responses of auditory nerve fibers in cats. I. Comparison with detection thresholds. J Neurosci 5:3261–3269
11. d'Aldin CG, Ruel J, Assie R, Pujol R, Puel JL (1997) Implication of NMDA type glutamate receptors in neural regeneration and neoformation of synapses after excitotoxic injury in the guinea pig cochlea. Int J Dev Neurosci 15:619–629
12. Deng L, Ding D, Su J, Manohar S, Salvi R (2013) Salicylate selectively kills cochlear spiral ganglion neurons by paradoxically up-regulating superoxide. Neurotox Res 24:307–319
13. Despres G, Romand R (1994) Neurotrophins and the development of cochlear innervation. Life Sci 54:1291–1297
14. Diao M, Zhang Y, Liu H, Han H, Gao W (2005) Observation on the protective effect of MK-801 against hearing loss in acoustic trauma. Lin Chuang Er Bi Yan Hou Ke Za Zhi 19:27–30
15. Duan M, Agerman K, Ernfors P, Canlon B (2000) Complementary roles of neurotrophin 3 and a N-methyl-D-aspartate antagonist in the protection of noise and aminoglycoside-induced ototoxicity. Proc Natl Acad Sci U S A 97:7597–7602
16. Duan M, Chen Z, Qiu J, Ulfendahl M, Laurell G, Borg E, Ruan R (2006) Low-dose, long-term caroverine administration attenuates impulse noise-induced hearing loss in the rat. Acta Otolaryngol 126:1140–1147
17. Duncker SV, Franz C, Kuhn S, Schulte U, Campanelli D, Brandt N, Hirt B, Fakler B, Blin N, Ruth P, Engel J, Marcotti W, Zimmermann U, Knipper M (2013) Otoferlin couples to clathrin-mediated endocytosis in mature cochlear inner hair cells. J Neurosci 33:9508–9519
18. Eggermont JJ (2015) Animal models of auditory temporal processing. Int J Psychophysiol 95:202–215
19. Farinas I, Jones KR, Backus C, Wang XY, Reichardt LF (1994) Severe sensory and sympathetic deficits in mice lacking neurotrophin-3. Nature 369:658–661
20. Frank T, Khimich D, Neef A, Moser T (2009) Mechanisms contributing to synaptic Ca2+ signals and their heterogeneity in hair cells. Proc Natl Acad Sci U S A 106:4483–4488
21. Fritzsch B, Silos-Santiago I, Bianchi LM, Farinas I (1997) The role of neurotrophic factors in regulating the development of inner ear innervation. Trends Neurosci 20:159–164
22. Fritzsch B, Tessarollo L, Coppola E, Reichardt LF (2004) Neurotrophins in the ear: their roles in sensory neuron survival and fiber guidance. Prog Brain Res 146:265–278
23. Fuchs M, Brandstatter JH, Regus-Leidig H (2014) Evidence for a clathrin-independent mode of endocytosis at a continuously active sensory synapse. Front Cell Neurosci 8:60
24. Furman AC, Kujawa SG, Liberman MC (2013) Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. J Neurophysiol 110:577–586
25. Furness DN, Lehre KP (1997) Immunocytochemical localization of a high-affinity glutamate-aspartate transporter, GLAST, in the rat and guinea-pig cochlea. Eur J Neurosci 9:1961–1969

26. Furness DN, Lawton DM (2003) Comparative distribution of glutamate transporters and receptors in relation to afferent innervation density in the mammalian cochlea. J Neurosci 23:11296–11304
27. Glowatzki E, Fuchs PA (2002) Transmitter release at the hair cell ribbon synapse. Nat Neurosci 5:147–154
28. Glowatzki E, Grant L, Fuchs P (2008) Hair cell afferent synapses. Curr Opin Neurobiol 18:389–395
29. Gonzalez J, Jurado-Coronel JC, Avila MF, Sabogal A, Capani F, Barreto GE (2015) NMDARs in neurological diseases: a potential therapeutic target. Int J Neurosci 125:315–327
30. Green SH, Bailey E, Wang Q, Davis RL (2012) The Trk A, B, C's of neurotrophins in the cochlea. Anat Rec (Hoboken) 295:1877–1895
31. Guilarte TR, Chen MK (2007) Manganese inhibits NMDA receptor channel function: implications to psychiatric and cognitive effects. Neurotoxicology 28:1147–1152
32. Guitton MJ, Dudai Y (2007) Blockade of cochlear NMDA receptors prevents long-term tinnitus during a brief consolidation window after acoustic trauma. Neural Plast 2007:80904
33. Heil P, Peterson AJ (2015) Basic response properties of auditory nerve fibers: a review. Cell Tissue Res 361(1):129–158
34. Heinrich UR, Maurer J, Mann W (1999) Ultrastructural evidence for protection of the outer hair cells of the inner ear during intense noise exposure by application of the organic calcium channel blocker diltiazem. ORL J Otorhinolaryngol Relat Spec 61:321–327
35. Iizuka T, Kanzaki S, Mochizuki H, Inoshita A, Narui Y, Furukawa M, Kusunoki T, Saji M, Ogawa K, Ikeda K (2008) Noninvasive in vivo delivery of transgene via adeno-associated virus into supporting cells of the neonatal mouse cochlea. Hum Gene Ther 19:384–390
36. Jie H, Tao S, Liu L, Xia L, Charko A, Yu Z, Bance M, Yin S, Robertson GS, Wang J (2015) Cochlear protection against cisplatin by viral transfection of X-linked inhibitor of apoptosis protein across round window membrane. Gene Ther 22:546–552
37. Jing Z, Rutherford MA, Takago H, Frank T, Fejtova A, Khimich D, Moser T, Strenzke N (2013) Disruption of the presynaptic cytomatrix protein bassoon degrades ribbon anchorage, multiquantal release, and sound encoding at the hair cell afferent synapse. J Neurosci 33:4456–4467
38. Jung S, Maritzen T, Wichmann C, Jing Z, Neef A, Revelo NH, Al-Moyed H, Meese S, Wojcik SM, Panou I, Bulut H, Schu P, Ficner R, Reisinger E, Rizzoli SO, Neef J, Strenzke N, Haucke V, Moser T (2015) Disruption of adaptor protein 2mu (AP-2mu) in cochlear hair cells impairs vesicle reloading of synaptic release sites and hearing. EMBO J 34:2686–2702
39. Kansu L, Ozkarakas H, Efendi H, Okar I (2011) Protective effects of pentoxifylline and nimodipine on acoustic trauma in guinea pig cochlea. Otol Neurotol 32:919–925
40. Khimich D, Nouvian R, Pujol R, Tom Dieck S, Egner A, Gundelfinger ED, Moser T (2005) Hair cell synaptic ribbons are essential for synchronous auditory signalling. Nature 434:889–894
41. Knipper M, Van Dijk P, Nunes I, Ruttiger L, Zimmermann U (2013) Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis. Prog Neurobiol 111:17–33
42. Kobel M, Le Prell CG, Liu J, Hawks JW, Bao J (2017) Noise-induced cochlear synaptopathy: past findings and future studies. Hear Res 349:148–154
43. Kujawa SG, Liberman MC (2009) Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J Neurosci 29:14077–14085
44. Lee MY, Kurioka T, Nelson MM, Prieskorn DM, Swiderski DL, Takada Y, Beyer LA, Raphael Y (2016) Viral-mediated Ntf3 overexpression disrupts innervation and hearing in nondeafened guinea pig cochleae. Mol Ther Methods Clin Dev 3:16052
45. Lenzi D, Crum J, Ellisman MH, Roberts WM (2002) Depolarization redistributes synaptic membrane and creates a gradient of vesicles on the synaptic body at a ribbon synapse. Neuron 36:649–659

46. Liberman LD, Wang H, Liberman MC (2011) Opposing gradients of ribbon size and AMPA receptor expression underlie sensitivity differences among cochlear-nerve/hair-cell synapses. J Neurosci 31:801–808
47. Liberman MC, Kujawa SG (2017) Cochlear synaptopathy in acquired sensorineural hearing loss: manifestations and mechanisms. Hear Res 349:138–147
48. Lin HW, Furman AC, Kujawa SG, Liberman MC (2011) Primary neural degeneration in the guinea pig cochlea after reversible noise-induced threshold shift. J Assoc Res Otolaryngol 12:605–616
49. Liu J, Niu YG, Li WX, Yuan YY, Han WJ, Yu N, Yang SM, Li XQ (2012) Interaction of a calcium channel blocker with noise in cochlear function in guinea pig. Acta Otolaryngol 132:1140–1144
50. Liu L, Wang H, Shi L, Almuklass A, He T, Aiken S, Bance M, Yin S, Wang J (2012) Silent damage of noise on cochlear afferent innervation in guinea pigs and the impact on temporal processing. PLoS One 7:e49550
51. Lobarinas E, Spankovich C, Le Prell CG (2017) Evidence of "hidden hearing loss" following noise exposures that produce robust TTS and ABR wave-I amplitude reductions. Hear Res 349:155–163
52. Magupalli VG, Schwarz K, Alpadi K, Natarajan S, Seigel GM, Schmitz F (2008) Multiple RIBEYE-RIBEYE interactions create a dynamic scaffold for the formation of synaptic ribbons. J Neurosci 28:7954–7967
53. Matsubara A, Laake JH, Davanger S, Usami S, Ottersen OP (1996) Organization of AMPA receptor subunits at a glutamate synapse: a quantitative immunogold analysis of hair cell synapses in the rat organ of Corti. J Neurosci 16:4457–4467
54. Meyer AC, Frank T, Khimich D, Hoch G, Riedel D, Chapochnikov NM, Yarin YM, Harke B, Hell SW, Egner A, Moser T (2009) Tuning of synapse number, structure and function in the cochlea. Nat Neurosci 12:444–453
55. Monaghan DT, Jane DE (2009) Pharmacology of NMDA receptors. In: Van Dongen AM (ed) Biology of the NMDA receptor. CRC Press, Boca Raton
56. Moser T, Vogl C (2016) New insights into cochlear sound encoding. F1000Res 5
57. Moser T, Starr A (2016) Auditory neuropathy—neural and synaptic mechanisms. Nat Rev Neurol 12:135–149
58. Nordang L, Cestreicher E, Arnold W, Anniko M (2000) Glutamate is the afferent neurotransmitter in the human cochlea. Acta Otolaryngol 120:359–362
59. Nouvian R, Beutner D, Parsons TD, Moser T (2006) Structure and function of the hair cell ribbon synapse. J Membr Biol 209:153–165
60. Ohinata Y, Miller JM, Schacht J (2003) Protection from noise-induced lipid peroxidation and hair cell loss in the cochlea. Brain Res 966:265–273
61. Pangrsic T, Reisinger E, Moser T (2012) Otoferlin: a multi-C2 domain protein essential for hearing. Trends Neurosci 35:671–680
62. Paoletti P (2011) Molecular basis of NMDA receptor functional diversity. Eur J Neurosci 33:1351–1365
63. Plack CJ, Barker D, Prendergast G (2014) Perceptual consequences of "hidden" hearing loss. Trends Hear 18
64. Plack CJ, Leger A, Prendergast G, Kluk K, Guest H, Munro KJ (2016) Toward a diagnostic test for hidden hearing loss. Trends Hear 20
65. Prentice H, Modi JP, Wu JY (2015) Mechanisms of neuronal protection against excitotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction in stroke and neurodegenerative diseases. Oxidative Med Cell Longev 2015:964518
66. Puel JL (2007) Cochlear NMDA receptor blockade prevents salicylate-induced tinnitus. B-ENT 3(Suppl 7):19–22
67. Puel JL, Ruel J, Gervais d'Aldin C, Pujol R (1998) Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. Neuroreport 9:2109–2114

68. Puel JL, Ruel J, Guitton M, Pujol R (2002) The inner hair cell afferent/efferent synapses revisited: a basis for new therapeutic strategies. Adv Otorhinolaryngol 59:124–130
69. Puel JL, Ladrech S, Chabert R, Pujol R, Eybalin M (1991) Electrophysiological evidence for the presence of NMDA receptors in the guinea pig cochlea. Hear Res 51:255–264
70. Puel JL, d'Aldin C, Ruel J, Ladrech S, Pujol R (1997) Synaptic repair mechanisms responsible for functional recovery in various cochlear pathologies. Acta Otolaryngol 117:214–218
71. Pujol R, Puel JL (1999) Excitotoxicity, synaptic repair, and functional recovery in the mammalian cochlea: a review of recent findings. Ann N Y Acad Sci 884:249–254
72. Pujol R, Puel JL, Gervais d'Aldin C, Eybalin M (1993) Pathophysiology of the glutamatergic synapses in the cochlea. Acta Otolaryngol 113:330–334
73. Ramekers D, Versnel H, Grolman W, Klis SF (2012) Neurotrophins and their role in the cochlea. Hear Res 288:19–33
74. Rebillard G, Ruel J, Nouvian R, Saleh H, Pujol R, Dehnes Y, Raymond J, Puel JL, Devau G (2003) Glutamate transporters in the guinea-pig cochlea: partial mRNA sequences, cellular expression and functional implications. Eur J Neurosci 17:83–92
75. Regus-Leidig H, Specht D, Tom Dieck S, Brandstatter JH (2010) Stability of active zone components at the photoreceptor ribbon complex. Mol Vis 16:2690–2700
76. Regus-Leidig H, Fuchs M, Lohner M, Leist SR, Leal-Ortiz S, Chiodo VA, Hauswirth WW, Garner CC, Brandstatter JH (2014) In vivo knockdown of Piccolino disrupts presynaptic ribbon morphology in mouse photoreceptor synapses. Front Cell Neurosci 8:259
77. Robertson D (1982) Effects of acoustic trauma on stereocilia structure and spiral ganglion cell tuning properties in the guinea pig. Hear Res 7:55–74
78. Ruel J, Chen C, Pujol R, Bobbin RP, Puel JL (1999) AMPA-preferring glutamate receptors in cochlear physiology of adult guinea-pig. J Physiol 518(Pt 3):667–680
79. Ruel J, Wang J, Rebillard G, Eybalin M, Lloyd R, Pujol R, Puel JL (2007) Physiology, pharmacology and plasticity at the inner hair cell synaptic complex. Hear Res 227:19–27
80. Ruel J, Chabbert C, Nouvian R, Bendris R, Eybalin M, Leger CL, Bourien J, Mersel M, Puel JL (2008) Salicylate enables cochlear arachidonic-acid-sensitive NMDA receptor responses. J Neurosci 28:7313–7323
81. Safieddine S, El-Amraoui A, Petit C (2012) The auditory hair cell ribbon synapse: from assembly to function. Annu Rev Neurosci 35:509–528
82. Sahley TL, Hammonds MD, Musiek FE (2013) Endogenous dynorphins, glutamate and N-methyl-d-aspartate (NMDA) receptors may participate in a stress-mediated Type-I auditory neural exacerbation of tinnitus. Brain Res 1499:80–108
83. Sanchez JT, Ghelani S, Otto-Meyer S (2015) From development to disease: diverse functions of NMDA-type glutamate receptors in the lower auditory pathway. Neuroscience 285:248–259
84. Schmitz F (2009) The making of synaptic ribbons: how they are built and what they do. Neuroscientist 15:611–624
85. Sebe JY, Cho S, Sheets L, Rutherford MA, von Gersdorff H, Raible DW (2017) Ca(2+)-permeable AMPARs mediate glutamatergic transmission and excitotoxic damage at the hair cell ribbon synapse. J Neurosci 37:6162–6175
86. Sheets L (2017) Excessive activation of ionotropic glutamate receptors induces apoptotic hair-cell death independent of afferent and efferent innervation. Sci Rep 7:41102
87. Shen H, Zhang B, Shin JH, Lei D, Du Y, Gao X, Wang Q, Ohlemiller KK, Piccirillo J, Bao J (2007) Prophylactic and therapeutic functions of T-type calcium blockers against noise-induced hearing loss. Hear Res 226:52–60
88. Shi L, Chang Y, Li X, Aiken SJ, Liu L, Wang J (2016) Coding deficits in noise-induced hidden hearing loss may stem from incomplete repair of ribbon synapses in the cochlea. Front Neurosci 10:231
89. Shi L, Liu L, He T, Guo X, Yu Z, Yin S, Wang J (2013) Ribbon synapse plasticity in the cochleae of guinea pigs after noise-induced silent damage. PLoS One 8:e81566

90. Shi L, Liu K, Wang H, Zhang Y, Hong Z, Wang M, Wang X, Jiang X, Yang S (2015) Noise induced reversible changes of cochlear ribbon synapses contribute to temporary hearing loss in mice. Acta Otolaryngol 135(11):1–10
91. Shi L, Guo X, Shen P, Liu L, Tao S, Li X, Song Q, Yu Z, Yin S, Wang J (2015) Noise-induced damage to ribbon synapses without permanent threshold shifts in neonatal mice. Neuroscience 304:368–377
92. Sly DJ, Campbell L, Uschakov A, Saief ST, Lam M, O'Leary SJ (2016) Applying neurotrophins to the round window rescues auditory function and reduces inner hair cell synaptopathy after noise-induced hearing loss. Otol Neurotol 37:1223–1230
93. Sobkowicz HM, Slapnick SM, August BK (2002) Differentiation of spinous synapses in the mouse organ of Corti. Synapse 45:10–24
94. Song Q, Shen P, Li X, Shi L, Liu L, Wang J, Yu Z, Stephen K, Aiken S, Yin S, Wang J (2016) Coding deficits in hidden hearing loss induced by noise: the nature and impacts. Sci Rep 6:25200
95. Spiwoks-Becker I, Glas M, Lasarzik I, Vollrath L (2004) Mouse photoreceptor synaptic ribbons lose and regain material in response to illumination changes. Eur J Neurosci 19:1559–1571
96. Suzuki J, Corfas G, Liberman MC (2016) Round-window delivery of neurotrophin 3 regenerates cochlear synapses after acoustic overexposure. Sci Rep 6:24907
97. Suzuki J, Hashimoto K, Xiao R, Vandenberghe LH, Liberman MC (2017) Cochlear gene therapy with ancestral AAV in adult mice: complete transduction of inner hair cells without cochlear dysfunction. Sci Rep 7:45524
98. Szydlowska K, Tymianski M (2010) Calcium, ischemia and excitotoxicity. Cell Calcium 47:122–129
99. Tao Y, Huang M, Shu Y, Ruprecht A, Wang H, Tang Y, Vandenberghe LH, Wang Q, Gao G, Kong WJ, Chen ZY (2017) Delivery of adeno-associated viral vectors in adult mammalian inner ear cell subtypes without auditory dysfunction. Hum Gene Ther 29(4)
100. tom Dieck S, Altrock WD, Kessels MM, Qualmann B, Regus H, Brauner D, Fejtova A, Bracko O, Gundelfinger ED, Brandstatter JH (2005) Molecular dissection of the photoreceptor ribbon synapse: physical interaction of bassoon and RIBEYE is essential for the assembly of the ribbon complex. J Cell Biol 168:825–836
101. Uemaetomari I, Tabuchi K, Nakamagoe M, Tanaka S, Murashita H, Hara A (2009) L-type voltage-gated calcium channel is involved in the pathogenesis of acoustic injury in the cochlea. Tohoku J Exp Med 218:41–47
102. Valente C, Spano S, Luini A, Corda D (2005) Purification and functional properties of the membrane fissioning protein CtBP3/BARS. Methods Enzymol 404:296–316
103. Vollrath L, Spiwoks-Becker I (1996) Plasticity of retinal ribbon synapses. Microsc Res Tech 35:472–487
104. Vyklicky V, Korinek M, Smejkalova T, Balik A, Krausova B, Kaniakova M, Lichnerova K, Cerny J, Krusek J, Dittert I, Horak M, Vyklicky L (2014) Structure, function, and pharmacology of NMDA receptor channels. Physiol Res 63(Suppl 1):S191–S203
105. Wan G, Gomez-Casati ME, Gigliello AR, Liberman MC, Corfas G (2014) Neurotrophin-3 regulates ribbon synapse density in the cochlea and induces synapse regeneration after acoustic trauma. eLife 3
106. Wan L, Almers W, Chen W (2005) Two ribeye genes in teleosts: the role of ribeye in ribbon formation and bipolar cell development. J Neurosci 25:941–949
107. Wang H, Murphy R, Taaffe D, Yin S, Xia L, Hauswirth WW, Bance M, Robertson GS, Wang J (2012) Efficient cochlear gene transfection in guinea-pigs with adeno-associated viral vectors by partial digestion of round window membrane. Gene Ther 19:255–263
108. Wang Q, Green SH (2011) Functional role of neurotrophin-3 in synapse regeneration by spiral ganglion neurons on inner hair cells after excitotoxic trauma in vitro. J Neurosci 31:7938–7949

109. Wong AB, Jing Z, Rutherford MA, Frank T, Strenzke N, Moser T (2013) Concurrent maturation of inner hair cell synaptic Ca2+ influx and auditory nerve spontaneous activity around hearing onset in mice. J Neurosci 33:10661–10666
110. Wong AB, Rutherford MA, Gabrielaitis M, Pangrsic T, Gottfert F, Frank T, Michanski S, Hell S, Wolf F, Wichmann C, Moser T (2014) Developmental refinement of hair cell synapses tightens the coupling of Ca2+ influx to exocytosis. EMBO J 33:247–264
111. Yang T, Kersigo J, Jahan I, Pan N, Fritzsch B (2011) The molecular basis of making spiral ganglion neurons and connecting them to hair cells of the organ of Corti. Hear Res 278:21–33
112. Young ED, Barta PE (1986) Rate responses of auditory nerve fibers to tones in noise near masked threshold. J Acoust Soc Am 79:426–442
113. Yu YF, Wu WY, Xiao GS, Ling HY, Pan C (2016) Protection of the cochlear hair cells in adult C57BL/6J mice by T-type calcium channel blockers. Exp Ther Med 11:1039–1044
114. Zenisek D, Horst NK, Merrifield C, Sterling P, Matthews G (2004) Visualizing synaptic ribbons in the living cell. J Neurosci 24:9752–9759

Chapter 4
Protection and Prevention of Age-Related Hearing Loss

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Abstract Presbycusis is a sensorineural hearing loss caused by hearing system aging and degeneration. The clinical manifestations are progressive bilateral symmetrical hearing loss, and the hearing curve is mostly slope-shaped with high-frequency reduction, sometimes flat. The results of the second national sample survey of disabled persons (2006) showed that the total number of hearing and speech disability in China was 27.8 million, accounting for 34% of the total number of disabled people in China. Among them are people over 60 years old. There are 20.4541 million people with hearing disabilities. There are 9.49 million senile deaf patients, accounting for 34.1% of the total number of hearing disabilities. As society gradually becomes aging, the incidence of presbycusis is getting higher and higher. The study of its pathogenesis is of great significance for the diagnosis, treatment, and prevention of presbycusis. The rapid progress of molecular biology experimental technology has provided us with a new opportunity to fully understand and reveal the presbycusis. In the near future, early diagnosis of presbycusis-related genes and early prevention or delay of the occurrence and development of presbycusis will become a reality.

Keywords Presbycusis · Etiology · Pathology · Diagnosis · Treatment

4.1 Introduction

Presbycusis, also known as age-related hearing loss (AHL), refers to the progressive deterioration of auditory system and binaural hearing ability (mainly high-frequency hearing) as they age, which belongs to sensorineural hearing loss [1]. Senile deafness has adverse effects on the physical and mental health of the elderly, such as physical,

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cognitive, emotional, behavioral, and social functions. It may also lead to social isolation, depression, and inferiority [2, 3]. As early as 1999, China was already the country with the most elderly population in the world. The elderly population accounted for one-fifth of the world's aging population, marking that China has entered an aging society ahead of schedule. According to the results of China's sixth population census in 2010, China's population aged 60 and over has exceeded 177 million, accounting for 13.31% of China's total population, of which population aged 65 and over accounted for 8.91%, up 1.82% from the fifth census (National Bureau of Statistics of the People's Republic of China, China 2010 Census Data: http://www.stats.gov.cn/tjsj/pcsj/rkpc/6rp/indexch.htm). From these data, we can see that China is in the stage of rapid development of an aging society. According to the WHO, about 360 million people worldwide have varying degrees of hearing loss, and about one-third of people aged 65 and over suffer from varying degrees of disability hearing loss (http://www.who.int/mediacentre/factsheets/fs300/en/). Senile deafness is mainly determined by genetic factors, and it is also affected by noise, ototoxic drugs, heavy metals, lifestyle (smoking, drinking, diet, etc.), metabolic diseases, and other factors [2, 4–7]. At present, there is no way to cure senile deafness which can only improve the auditory function as much as possible. Wearing a hearing aid is the most commonly used and effective treatment, but it cannot meet the needs of all senile deafness patients. In real life, only 20% of people with senile deafness will seek help. Only 11% of them patients have hearing aids, but 24% of people in this population never use their hearing aids [2, 8]. Therefore, an in-depth study of the mechanism of senile deafness development and the search for ways to treat senile deafness is of great significance for improving the quality of life of the elderly.

4.2 The Etiology of Age-Related Hearing Loss

Presbycusis is a multifactor process, and the individual expression of each factor is quite different, which damages the auditory system over time. After age 50, in addition to age, noise exposure, cardiovascular disease, diabetes, and smoking are also risk factors for senile deafness. The cause of presbycusis may be related to the following factors.

4.2.1 External Environmental Factors

Long-term external environmental noise and ototoxic drug damage are an important cause of senile deafness. Some scholars believe that the notched audiogram is related to noise exposure, and that the effect of noise on the pure tone threshold may continue after the noise exposure is stopped [9]. Noise exposure accelerates age-related hearing loss. The elderly consume several drugs every day, and drug exposures may have adverse reactions and drug interactions. The most commonly

used in the elderly are cardiovascular, hypertension, diabetes, gastrointestinal, and central nervous system drugs and analgesics. According to liver metabolism, kidney metabolism, drug absorption distribution, and clearance research data, physiological changes in the elderly often affect their drug response that may have dangerous drug reactions; the dosage of prescription drugs for treating these diseases in the elderly may be somewhat high; physiological changes may make them susceptible to the toxic effects of certain drugs; the adverse effects of ototoxicity may result from a combination of drug and disease.

4.2.2 Vascular Disease in the Inner Ear

Vascular lesions in the inner ear and changes in blood rheology are one of the intrinsic causes of senile deafness. One of the basic manifestations of human aging is that the exchange of oxygen in the blood vessels of the auditory system is affected by arteriosclerosis, which also caused metabolic disorders. Some scholars have used aging C57BL/6J mouse models to study the expression of vascular endothelial growth factor (VEGF) receptors in the inner ear [10]. They found that vascular abnormalities are associated with age-related hearing loss. In the elderly, changes in blood viscosity, erythrocyte stiffness, and erythrocyte filtration capacity are associated with sensorineural hearing loss in the elderly.

4.2.3 Changes of Neurotransmitters and Neuroactive Substances

Glutamate is a central nervous system excitatory synaptic transmitter whose excitotoxicity is used to explain hypoxemia and ischemia-related brain damage during aging. Excessive release of glutamate may act directly or indirectly postsynaptic neuronal receptors which cause ion influx and carry a large amount of water that lead to acute edema of dendrites [11]. The influx of calcium ions also leads to dysregulation of calcium internal environment stability, which may lead to cell death. Glutamate is also a neurotransmitter between the inner hair cells of the cochlea and the dendrites of the auditory nerve. Therefore, this toxic damage also occurs in the acute injury of the cochlear Corti organ, causing edema of the radial nerve fibers and loss of type I neurons.

The changes of GABA (gamma-aminobutyric acid) are as follows: the research of GABA in the inferior colliculus and cochlear nucleus in animal model of senile deafness found that GABA immunoreactive neurons were reduced by 36% compared to normal status. There were also the reduced synthesis and release of GABA and glutamate decarboxylase activity, GABA-bound receptors, presynaptic terminals. GABA receptor binding changed meanwhile [12]. Therefore, senile sensorineural hearing loss occurs due to a decrease or loss of GABA-mediated inhibition.

4.2.4 Diet and Related Factors

There are three mechanisms for hyperlipidemia to cause sensorineural deafness. The first factor is lipid metabolism disorder. Lipid particles are more common in inner and outer hair cells, marginal cells, and vascular striate cells, as a result of hyperlipidemia. Lipid metabolism disorders and lipid deposition can lead to vascular streaks and degeneration of outer hair cells, ultimately leading to hearing impairment. The second factor is increased blood viscosity. Animal experiments and clinical studies have shown that hyperlipidemia elderly blood viscosity increases, which caused microcirculation disturbance in the inner ear, ischemia and hypoxia in the inner ear, atrophy of blood vessels, and hair cell damage. The third factor is hyperplasia of platelet aggregation which caused blood flow in the inner ear was significantly reduced, or even stagnant, forming microthrombus.

4.2.5 Related Gene Mutation

The mitochondrial DNA of the cochlear cells in aged rats and the elderly showed that 4834 bp mtDNA was absent in the aged rats and 4977 bp mtDNA deletion in the elderly caused a decrease in mitochondrial oxidative phosphorylation, which affected the function of the auditory nervous system [13]. Mutations in mtDNA exacerbate ROS-induced cell damage through activating the apoptotic cascade and cell death, which creates a "vicious circle" (mitochondrial clock theory) that ultimately leads to aging of cells and individuals [14, 15] (Fig. 4.1).

4.2.6 Epigenetic Changes

The expression level of connexin 26 in the inner ear of aging rats is decreased. The increased methylation level of the promoter region of Cx26 gene may be the cause of the decrease of Cx26 level in senile deafness. The decrease of Cx26 level leads to the disorder of energy metabolism in the inner ear, which is a possible mechanism leading to the occurrence of senile deafness [16] (Fig. 4.2).

4.2.7 Other Factors

Changes in Na^+-K^+-ATPase activity lead to a decrease in endocochlear potential. The value of the endocochlear potential measured in the round window/turn 1 region of the cochlea is related to the level of lateral wall Na,K-ATPase specific activity [17]. There is a strong relationship between the age-related reductions in enzyme activity and the magnitude of the endocochlear potential.

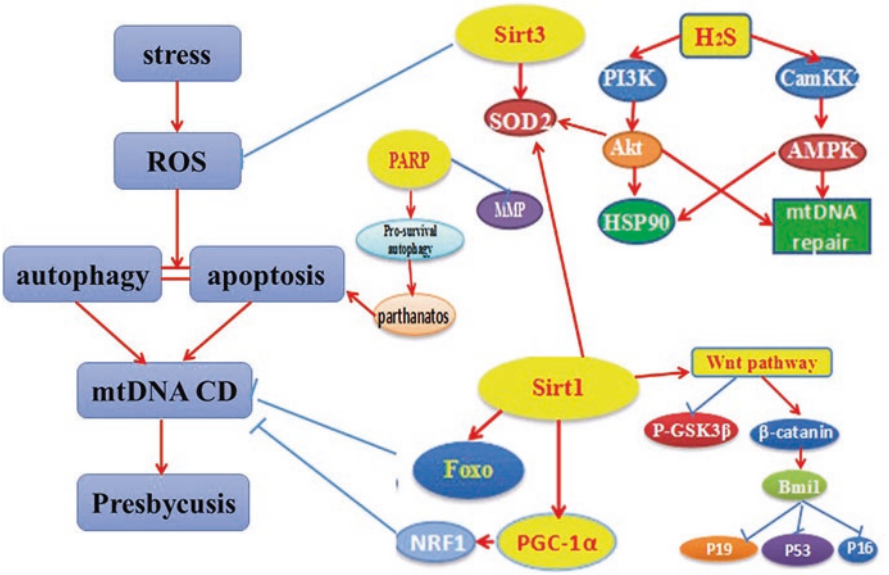

Fig. 4.1 The role of mtDNA and related genes in the development of senile deafness

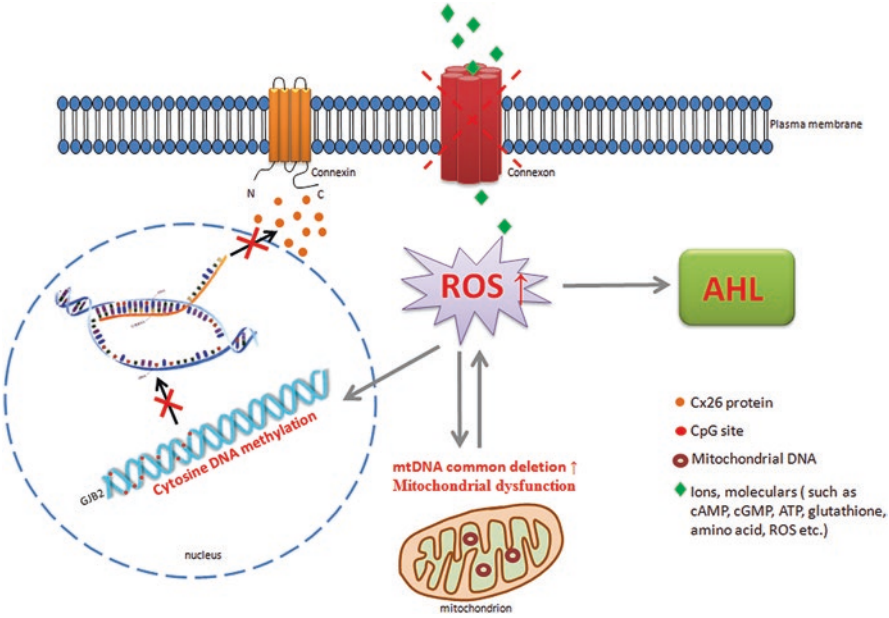

Fig. 4.2 The effect of reduced expression of connexin 26 on senile deafness

4.3 The Pathology of Age-Related Hearing Loss

The cochlea components have their own different specific functions in the dynamic balance of the inner ear, and as the age increased, they are prone to changes. In addition to the organ of Corti, little is known about the neural framework of afferent fibers and efferent fibers, and the brainstem's cochlear nucleus and spiral ganglion show age-related changes [18]. Moreover, Eckert MA proved that the peripheral cochlear organs may be adversely affected by age-related pathologies of the central nervous system [19].

The age-related hearing loss could be classified into four types according to different pathogenesis by Schuknecht [20]. With the advancement of technology and research, some scholars put forward different opinions. In 1993, Schuknecht and Gacek studied the 21 human temporal bone; in addition to verifying the original classification, they added two types [21]. Thus, the age-related hearing loss was divided into six types:

Sensory Presbycusis Sensory presbycusis is mainly caused by loss of outer hair cells of the organ of Corti. The current opinion is that the outer hair cells loss of ARHL is caused by long-term noise exposure and other environmental toxicity. The audiogram shows normal or slight decrease in the low frequency of the bone conduction. From 1000 Hz to the high-frequency region, the audiogram is sharply descending, and the high frequency presents severe hearing loss [21].

Neural Presbycusis When the ratio of cochlear neurons loss exceeded 50%, the term was used. The pathological mechanism is the neuronal degeneration of the cochlear nerve pathway and the auditory system [22]. The audiogram is a curve that gradually decreases from low frequency to high frequency. However, the speech recognition rate decreased significantly, which was not proportional to the decline in pure tone hearing.

Strial Presbycusis (Metabolic Presbycusis) This type is mainly caused by vascular atrophy, which caused the dysfunction of endolymphatic metabolism and biochemical characteristics [23]. The audiogram shows an almost horizontal hearing curve, and the hearing loss in the high-frequency region is slightly obvious. The patient's speech recognition rate is excellent, and there is no loudness reverberation.

Cochlear Conductive Presbycusis (Mechanical Presbycusis) The pathological mechanism of this type is changes in the physical structure and properties of the basement membrane [24]. At least five octaves of gradual decline in pure tone audiograms. The decline is at least 50 dB, and the hearing threshold between two adjacent frequencies does not exceed 25 dB. Some scholars believed that this subtype is only theoretical, which is derived from the purpose of histology. In fact, there is little evidence that the mechanical structure of the organ of Corti is gradually stiffened with age. They believed that the cochlear conductive presbycusis is only extreme case of metabolic presbycusis.

Mixed Presbycusis This type is characterized by more than one significant pathological change in the cochlear structure, involving more than two of the above four classic types.

Intermediate Presbycusis The intermediate presbycusis is characterized by the lack of pathological changes under light microscopy but with submicroscopic structural changes in the cochlea, including changes in organelles of the damage cells metabolism, decrease of HC synapses, and chemical changes in the endolymph. The latter two types are two subtypes added by Schuknecht and Gacek in 1993.

The classic types of presbycusis can be associated with defined audiograms but lack clear pathophysiological explanations or other types of pathological patterns [25]. The clinical manifestations of presbycusis are complicated and varied and do not need to be expressed in a single form. Estimation of hearing loss in the elderly must be comprehensive and individualized to ensure effective intervention strategies.

4.4 Clinical Symptom and Diagnosis of Age-Related Hearing Loss

4.4.1 Clinical Manifestation

Presbycusis mostly shows as a progressive, bilateral, and symmetrical sensorineural hearing impairment [26]. The degree of bilateral deafness may not be completely consistent. A few patients with outer ear or middle ear degenerative diseases can be characterized by mixed hearing loss. The disorder is characterized by high-frequency-dominated hearing loss, reduced speech understanding (particularly in noisy environments), slowed central processing of acoustic information, and impaired localization of sound sources.

With the impairment of hearing, the prevalence of tinnitus in geriatric population is increased [27, 28]. Most patients have high-frequency tinnitus; some of them are pulsatile tinnitus, intermittent, and persistent.

Presbycusis may accompany with vertigo. The pathogeny of vertigo may with the degeneration of vestibular system or the aging-related change of vertebrobasilar artery.

4.4.2 Examination Method and Diagnosis

Otoscopy An excellent practitioner, like a doctor of otolaryngology, uses an otoscope, a visual instrument inserted into ears to make this examination. Some view of the middle ear is also available through the translucent tympanic membrane. The tympanic membrane can be invaginated, atrophied, or has calcified plaques, without other characteristic changes [29].

Pure Tone Audiometry When being checked by pure tone audiometry, the patients are required to response to high and low tones in different volumes [30]. Then the result is shown with the faintest tone that is inaccessible. An auditory threshold curve will be yielded and consequently describes results of this exam. The test result shows as a sensorineural hearing loss, with high-frequency hearing loss firstly, and pure tone audiograms are type of mostly high-frequency descending, high-frequency plunge type or flat type [31]. This type of hearing loss goes beyond the maximum age-associated hearing loss. If the illness has progressed further, deeper tones could also be affected. The binaural hearing threshold is not completely symmetrical. According to Gates and Cooper [32], the left ear is much worse than the right ear, and the peripheral hearing sensitivity has advantage in the right ear. The pure tone hearing threshold has a large difference for different individuals. The pure tone audiometry is critical in diagnosing and evaluating presbycusis. The most important way is to make assessments about the condition of patients undergoing rehabilitation and the effect after wearing hearing aid devices. Conventional pure tone test includes test of air conduction and bone conduction. According to the introduction of NCBI, elderly people generally have difficulty in following the test instructions, and as the test is time-consuming, some may feel exhausted. Hence, it is recommended to give pretest training for elderly checked persons.

Supra Threshold Testing The binaural alternating loudness balance test and the short incremental sensitivity test are mainly used to judge whether there is re-vibration phenomenon and evaluate the components of the cochlear lesions and posterior cochlear lesions. The positive results of these tests suggest that there is re-vibration [29].

Otoacoustic Emissions Otoacoustic emissions can detect early damage to the cochlea during aging and also help to detect cochlear and retrocochlear senile [29]. Otoacoustic emission can be used to screen and monitor cochlear state during aging [33]. According to Bevan Yueh**,** the tone-emitting otoscope (AudioScope, Welch-Allyn Inc., Skaneateles Falls, NY) is a handset which gives out 20, 25, and 40 dB HL tones responsively at 500, 1000, 2000, and 4000 Hz. These are the most important frequencies used for human to listen. An examiner took the device with a probe tip sealing the canal directly in the ear canal and activated some chosen tones. Screened positive for hearing loss would be reported when patients even could not hear a 40-dB tone at 2000 Hz in either ear.

Speech Audiometry It is also termed as "word recognition score." Individuals with normal hearing can correctly identify 90 percent or more of words delivered [34]. It measures subject's ability to process sound, and in cases of neural or central dysfunction, this ability often decreases disproportionately. A higher word recognition score represents a more favorable response to amplification, because they indicate the amplified level of comfort that the patient is able to understand. During a speech audiometry check, the patient is given a certain number of words through the headset, and he/she will need to repeat the words. After the test is completed, checking

the collected information will help the doctor determine whether the patient's language understanding is flawed. If a patient with senile deafness undergoes the language test for, it will help to find his/her language understanding is impaired. Patients with aging-related deafness may find it harder to hear and understand others' remarks. Measuring the hearing and speech skills of patients with presbycusis is important for selecting the appropriate treatment. In addition, test results can be used to develop guidelines for hearing rehabilitation interventions, as well as to assess the difficulties encountered by patients with hearing loss and their ability to adapt in society. Speech audiometry is more complex and extensive than pure tone testing because it examines the physical, linguistic, and psychological aspects of speech, making it essential for presbycusis patients. The speech recognition rate and speech intelligibility index of presbycusis decreased more; especially in speech, the speech test score was lower. Recently, it has been reported in the literature that age-related auditory neuropathy is detected by word recognition score and sharpness index.

Other Methods Laboratory studies may include a blood or other sera test for inflammatory markers such as those for autoinflammatory diseases [35]. Magnetic resonance imaging (MRI) is used for differential diagnosis and can be used to examine vascular abnormalities and tumor and tissue structure problems such as mastoid enlargement [36]. MRI and other types of imaging studies do not directly detect age-related hearing loss.

Diagnosis Because hearing loss in the elderly may be due to systemic or otologic reasons, any patient with hearing loss needs to be thoroughly evaluated before attributing the symptoms to aging-related changes. So diagnosis now has been put forward to a high position. According to Gates, the diagnosis includes history, physical examination, screening, and central auditory testing four steps [37].

Hearing loss is divided into four degrees, namely, mild, moderate, severe, and profound. Typically, pure tone audiometry with 500, 1000, and 2000 Hz air conduction thresholds is used to determine the degree of hearing loss in the ear [38]. The normal hearing threshold is recognized as 25 dB, although it has been proposed that the threshold is too high and 15 dB (about half the size) is more typical. The threshold for mild hearing loss is 25–45 dB, the threshold for moderate hearing loss is 45–65 dB, the threshold for severe hearing loss is 65–85 dB, and the threshold for severe hearing loss is greater than 85 dB.

The high frequency of tinnitus that occurs in a single ear should prompt the clinician to further evaluate the cause [39]. Additionally, hearing impact sounds synchronized with the pulse may require additional imaging studies to rule out vascular disease [40].

Collecting patient history and conducting hearing testing (audiometry) are essential. The examination is usually performed by an audiologist (hearing specialist) [41]. Other procedures may be used to rule out the causes of other possible hearing problems, including blood tests, X-ray, and CT (computed tomography) scans or MRI (magnetic resonance imaging) [42].

4.5 Prevention and Treatment of Age-Related Hearing Loss

Diet The occurrence of AHL is closely related to hyperlipidemia and arteriosclerosis, and it is closely related to the lack of vitamin D, iron, and zinc in the body. Therefore, we make the following suggestions on the diet of the elderly.

1. Reduce the intake of high-fat foods: Lipid metabolism disorders can lead to decreased blood supply to the inner ear and increased peroxidation of lipids in the serum and inner ear tissue, resulting in damage to the inner ear [43].
2. Eat food containing more zinc: The zinc content in the cochlea is much higher than other organs; there are data showed that the serum concentration of zinc in patients with AHL is one-third.
3. Eat more foods with high iron content: Studies have shown that supplementation with iron can dilate microvessels, ensure blood supply to the ear, and give priority to hearing loss [44].
4. Eat foods with vitamin D: AHL is related to abnormal metabolism of vitamin D in the body.

Avoid Noise Studies have shown that a low-intensity noise environment may be more helpful in delaying the onset of AHL [45]. Therefore, earplugs should be worn in noisy environments, and irritating sounds should be avoided to avoid damage to the eardrum and inner ear cells.

Blood Circulation Surgical treatment can improve local blood circulation and restore partial function of reversible damage to the inner ear. Patients with severe deafness can choose to have a surgery of meatomyosynangiosis or endolymphatic.

Proper Exercise Proper exercise not only enhances physical fitness and improves physiological functions but also enhances immune function and reduces free radicals in the body. Study suggests that long-term exercise delays the progression of AHL by reducing age-related loss of strial capillaries associated with inflammation [46].

Drug Treatment First, the diseases closely related to senile sputum such as hypertension, hyperlipidemia, diabetes, and other cardiovascular and cerebrovascular diseases affecting the circulatory system are primarily treated. Secondly, the use of drugs such as vasodilators and nutrient nerves further delays the degeneration of the auditory nerve. Seidman MD reported that antioxidants can effectively delay the hearing decline in aged rats. Kong WJ and other studies found that coenzyme Q10 and vitamin E can prevent mitochondrial DNA deletion mutation in rat inner ear tissue, which provides an experimental basis for the prevention and treatment of senile deafness. In addition, some scholars applied the theory of traditional Chinese medicine to the treatment of AHL and proposed that the pathogenesis of AHL is kidney deficiency. Therefore, acupuncture and Chinese medicine treatment com-

bined with western medicine treatment have achieved good therapeutic effects. After Ma Lei applied acupuncture to patients with senile deafness, the hearing status of the patients improved significantly, and the symptoms such as tinnitus were alleviated. Sun Aihua confirmed that the combination of traditional Chinese and Western medicine is better than western medicine alone in the treatment of AHL.

Hearing Aids A hearing aid is a device that increases the intensity of sound and helps some hearing-impaired patients to make full use of residual hearing to compensate for hearing loss in the ear. In general, moderate hearing loss people benefit most from the use of hearing aids [47]. Hearing aids can be broadly divided into three types: collective, desktop, and portable. The choice of clinical hearing aids is mainly portable hearing aids. Portable hearing aids can be divided into bone conduction hearing aids and air conduction hearing aids according to the acoustic conduction pathway. They are mainly divided into box type, ear type, and in-ear type according to their different placement positions. According to the degree of patients' demand for hearing aids, the corresponding price, appearance and operation requirements, etc., and combined with the results of hearing tests, the doctor should determine the main technical indicators for hearing aids, such as the gain, output or frequency response required by the hearing aids analyzer, and the calculation of relevant formulas, so as to select the hearing aids with appropriate performance.

4.6 Conclusion

Whether in developing or developed countries, AHL has become a public health issue of great concern. Due to the complex pathogenesis and mechanism of AHL, and its vulnerability to genetic, environmental, socioeconomic, and medical factors, the pathogenesis of AHL has not yet been elucidated, but great progress has been made in population and animal research. Studying and elucidating the pathogenesis of senile sputum will lay a theoretical foundation and provide new ideas for the clinical diagnosis and treatment of senile deafness. With the rapid development of molecular biology technology, stem cells and gene therapy will make important breakthroughs. Other interventions for senile deafness will continue to improve, which can effectively prevent senile deafness. The quality of life of patients with senile deafness will be significant improvement.

References

1. Olofsson J (2012) A new phase of European archives of oto-rhino-laryngology and head & neck. Eur Arch Otorhinolaryngol 269:2019–2020
2. Huang Q, Tang J (2010) Age-related hearing loss or presbycusis. Eur Arch Otorhinolaryngol 267:1179–1191

3. Mulrow CD, Aguilar C, Endicott JE, Tuley MR, Velez R, Charlip WS et al (1990) Quality-of-life changes and hearing impairment. A randomized trial. Ann Intern Med 113:188
4. Andrea C, Chiara B, Stefano P, Antonio P (2012) The impact of hearing loss on the quality of life of elderly adults. Clin Interv Aging 2012:159
5. Bovo R, Ciorba A, Martini A (2011) Environmental and genetic factors in age-related hearing impairment. Aging Clin Exp Res 23:3–10
6. Ciorba A, Benatti A, Bianchini C, Aimoni C, Volpato S, Bovo R et al (2011) High frequency hearing loss in the elderly: effect of age and noise exposure in an Italian group. J Laryngol Otol 125:776–780
7. Tremblay K, Ross B (2007) Effects of age and age-related hearing loss on the brain. J Commun Disord 40:305–312
8. Ha-Sheng LK (2012) Age-related hearing loss: quality of care for quality of life. Gerontologist 52:265–271
9. Gates GA, Schmid P, Kujawa SG, Nam BH, D'Agostino R (2000) Longitudinal threshold changes in older men with audiometric notches. Hear Res 141:220–228
10. Clinkard D, Amoodi H, Kandasamy T, Grewal AS, Chen S, Qian W et al (2014) Changes in the cochlear vasculature and vascular endothelial growth factor and its receptors in the aging c57 mouse cochlea. ISRN Otolaryngol 430625:2013
11. Pujol R, Rebillard G, Puel JL, Lenoir M, Eybalin M, Recasens M (1990) Glutamate neurotoxicity in the cochlea: a possible consequence of ischaemic or anoxic conditions occurring in ageing. Acta Otolaryngol 111:32–36
12. Godfrey DA, Chen K, O'Toole TR, Mustapha AIAA (2017) Amino acid and acetylcholine chemistry in the central auditory system of young, middle-aged and old rats. Hear Res 350:173–188
13. Zhong Y, Hu YJ, Chen B, Peng W, Sun Y, Yang Y et al (2011) Mitochondrial transcription factor A overexpression and base excision repair deficiency in the inner ear of rats with D-galactose-induced aging. FEBS J 278:2500–2510
14. Tavanai E, Mohammadkhani G (2017) Role of antioxidants in prevention of age-related hearing loss: a review of literature. Eur Arch Otorhinolaryngol 274:1–14
15. Böttger EC, Schacht J (2013) The mitochondrion: a perpetrator of acquired hearing loss. Hear Res 303:12–19
16. Wu X, Wang Y, Sun Y, Chen S, Zhang S, Shen L et al (2014) Reduced expression of connexin26 and its DNA promoter hypermethylation in the inner ear of mimetic aging rats induced by d-galactose. Biochem Biophys Res Commun 452:340–346
17. Gratton MA, Smyth BJ, Lam CF, Boettcher FA, Schmiedt RA (1997) Decline in the endocochlear potential corresponds to decreased Na,K-ATPase activity in the lateral wall of quiet-aged gerbils. Hear Res 108:9–16
18. Roth TN (2015) Aging of the auditory system. Handb Clin Neurol 129:357–373
19. Eckert MA, Cute SL, Vaden KI Jr, Kuchinsky SE, Dubno JR (2012) Auditory cortex signs of age-related hearing loss. J Assoc Res Otolaryngol 13:703–713
20. Schuknecht HF, (1964) Further observations on the pathology of presbycusis. Arch Otolaryngol Head Neck Surg 80(4):369–382
21. Schuknecht HF, Gacek MR (1993) Cochlear pathology in presbycusis. Ann Otol Rhinol Laryngol 102:1–16
22. Jr RDF (2000) Neural substrates for presbycusis: anatomy, chemistry, and neuroimaging of the central auditory system. J Acoust Soc Am 107:2798
23. Mills DM, Schmiedt RA (2004) Metabolic presbycusis: differential changes in auditory brainstem and otoacoustic emission responses with chronic furosemide application in the gerbil. J Assoc Res Otolaryngol 5:1–10
24. Schmiedt RA (2010) The physiology of cochlear presbycusis
25. Ohlemiller KK (2004) Age-related hearing loss: the status of Schuknecht's typology. Curr Opin Otolaryngol Head Neck Surg 12:439
26. Gates GA, Mills JH (2005) Presbycusis. Lancet 366:1111–1120

27. Doungkamol S, Paul M, Philip N, Maryanne G, Elena R, George R (2003) Prevalence and characteristics of tinnitus in older adults: the Blue Mountains Hearing Study. Int J Audiol 42:289
28. Song JJ, Ridder DD, Schlee W, Heyning PVD, Vanneste S (2013) "Distressed aging": the differences in brain activity between early- and late-onset tinnitus. Neurobiol Aging 34:1853–1863
29. Weij Kong LZ (2015) Binq Wang et. Presbycusis. People's medical publishing house
30. Liu CQ, Cheng XT, Zhu YH, Shen WD, Bian BW, Cao JY et al (2015) Clinical observation on hearing conditions of centenarians in northern district of China. Acta Otolaryngol 135:451–458
31. Wang RL, Zhang DM (2017) The comparison of clinical features and laboratory indexes between flat descending hearing loss and total hearing loss. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 31:1892–1895
32. Gates GA, Cooper JC (1991) Incidence of hearing decline in the elderly[J]. Acta Otolaryngol 111(2):240–248
33. Mom T (2007) Otoacoustic emissions in clinical and surgical practice. Ann Otolaryngol Chir Cervicofac 124:80–89
34. van Beeck Calkoen EA, Merkus P, Goverts ST, van de Kamp JM, Mulder MF, Sanchez Aliaga E et al (2018) Evaluation of the outcome of CT and MR imaging in pediatric patients with bilateral sensorineural hearing loss. Int J Pediatr Otorhinolaryngol 108:180–185
35. Verschuur CA, Dowell A, Syddall HE, Ntani G, Simmonds SJ, Baylis D et al (2012) Markers of inflammatory status are associated with hearing threshold in older people: findings from the Hertfordshire Ageing Study. Age Ageing 41:92–97
36. Alonso-Lujan LR, Gutierrez-Farfan I, Luna-Reyes FA, Chamlati-Aguirre LE, Durand Rivera A (2014) Audiometric evaluation short and medium term in cochlear implants. Rev Investig Clin 66:415–421
37. Musiek FE, Bornstein SP (1992) Contemporary aspects of diagnostic audiology. Am J Otolaryngol 13:23–33
38. Herdman AT, Stapells DK (2003) Auditory steady-state response thresholds of adults with sensorineural hearing impairments. Int J Audiol 42:237–248
39. Tunkel DE, Bauer CA, Sun GH, Rosenfeld RM, Chandrasekhar SS, Cunningham ER Jr et al (2014) Clinical practice guideline: tinnitus executive summary. Otolaryngol Head Neck Surg 151:533–541
40. Song JJ, An GS, Choi I, De Ridder D, Kim SY, Choi HS et al (2016) Objectification and differential diagnosis of vascular pulsatile tinnitus by transcanal sound recording and spectrotemporal analysis: a preliminary study. Otol Neurotol 37:613–620
41. Olsen WO, Rose DE, Hedgecock LD (2003) A brief history of audiology at Mayo Clinic. J Am Acad Audiol 14:173–180
42. Paul A, Marlin S, Parodi M, Rouillon I, Guerlain J, Pingault V et al (2017) Unilateral sensorineural hearing loss: medical context and etiology. Audiol Neurootol 22:83–88
43. Kurien M, Thomas K, Bhanu TS (1989) Hearing threshold in patients with diabetes mellitus. J Laryngol Otol 103:164–168
44. Sun AH, Li JY, Xiao SZ, Li ZJ, Wang TY (1990) Changes in the cochlear iron enzymes and adenosine triphosphatase in experimental iron deficiency. Ann Otol Rhinol Laryngol 99:988
45. Niu X, Canlon B (2002) Protective mechanisms of sound conditioning. Adv Otorhinolaryngol 59:96–105
46. Han C, Ding D, Lopez MC, Manohar S, Zhang Y, Kim MJ et al (2016) Effects of long-term exercise on age-related hearing loss in mice. J Neurosci 36:11308
47. Lotfi Y, Mehrkian S, Moossavi A, Faghih-Zadeh S (2009) Quality of life improvement in hearing-impaired elderly people after wearing a hearing aid. Arch Iran Med 12:365–370

Chapter 5
Diagnosis, Intervention, and Prevention of Genetic Hearing Loss

Tao Yang, Luo Guo, Longhao Wang, and Xiaoyu Yu

Abstract It is estimated that at least 50% of congenital or childhood hearing loss is attributable to genetic causes. In non-syndromic hearing loss, which accounts for 70% of genetic hearing loss, approximately 80% of cases are autosomal recessive, 15% autosomal dominant, and 1–2% mitochondrial or X-linked. In addition, 30% of genetic hearing loss is syndromic. The genetic causes of hearing loss are highly heterogeneous. So far, more than 140 deafness-related genes have been discovered. Studies on those genes tremendously increased our understanding of the inner ear functions at the molecular level. It also offers important information for the patients and allows personalized and accurate genetic counseling. In many cases, genetic diagnosis of hearing loss can help to avoid unnecessary and costly clinical testing, offer prognostic information, and guide future medical management. On the other hand, a variety of gene therapeutic approaches have been developed aiming to relieve or converse the hearing loss due to genetic causes. Prevention of genetic hearing loss is feasible through prepregnancy and prenatal genetic diagnosis and counseling.

Keywords Genetic hearing loss · Deafness genes · Genetic screening · Genetic diagnosis · Gene therapy

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5.1 Introduction of Deafness Genes

Causative genes associated with hearing loss can be classified based on their functions in the inner ear, which include, but are not limited to, gap junctions, tight junctions, cytoskeleton, transmembrane proteins, regulatory elements, and miRNAs.

5.1.1 The Gap Junctions

Gap junctions, or connexins, form intercellular connection between multitudes of animal cell types, which allow various molecules, ions, and nucleotides to pass through a regulated gate between cells [83]. The connexins are essential for the formation and maintenance of the unique ion composition of the endolymph [57].

GJB2 encodes the connexin-26 protein [35]. Mutations in *GJB2* may lead to autosomal recessive deafness DFNB1A and autosomal dominant deafness DFNA3A. In European countries, mutations in *GJB2* are responsible for 50% of autosomal recessive non-syndromic hearing impairment [32]. Mouse with conditional disruption of *Gjb2* is profoundly deaf, exhibiting severe hair cell loss and supporting cell degeneration. The apoptosis begins soon after the onset of hearing (postnatal day 14), suggesting that apoptosis could be triggered by the inner hair cell response to sound stimulation. The endocochlear potential and endolymphatic potassium concentration were significantly decreased in this mouse model. It was hypothesized that loss of connexin-26 compromises recycling of K^+ ion. The high concentration of K^+ in the extracellular space inhibits uptake of the glutamate, which leads to the apoptosis of the hair cells ultimately [10].

GJB3 and *GJB6*, encoding connexin-31 and connexin-30, respectively, are two other gap junction genes associated with genetic hearing loss. Mutations in *GJB3* may cause autosomal dominant deafness DFNA2B. The mechanism of hearing loss caused by *GJB3* mutations remains unclear. Mutations in *GJB6* may lead to autosomal recessive deafness DFNB1B and autosomal dominant deafness DFNA3B [66]. *Gjb6*-deficient mice exhibited a severe constitutive hearing impairment, but the cochlea and vestibular end organs developed normally. From the age of the hearing onset, these mice lacked the endocochlear potential, which plays a key role in the high sensitivity of the mammalian auditory organ. At P18, the cochlear sensory epithelium started to degenerate via cell apoptosis. These phenotypes suggest a critical role of *Gjb6* in generating the endocochlear potential and for survival of the auditory hair cells after the onset of hearing [86].

5.1.2 Tight Junction Proteins

Tight junctions play important roles in making a barrier between different components of the organ of Corti. They help to maintain the unique ionic composition essential for the generation and maintenance of the endocochlear potential (EP) [36]. Tight junction proteins, known as claudins, are critical for hearing [39].

CLDN14 encodes tight junction protein claudin-14. Mutations in *CLDN14* cause autosomal recessive non-syndromic hearing loss DFNB29 [39]. *CLDN14* is mainly expressed in the cochlea, liver, and kidney. Claudin-14 protein increases transepithelial resistance by decreasing cation permeability, particularly for potassium. In *Cldn14*-null mice, the absence of claudin 14 from tight junctions in the reticular lamina leads to rapid and massive death of outer hair cells, followed by slower degeneration of the inner hair cells [4].

5.1.3 The Cytoskeleton

Cytoskeleton is a complex network of interlinking filaments and tubules that extent from the nucleus to the plasma membrane. It consists of intermediate filaments, microtubules, and actin filaments [28]. A number of cytoskeletal genes are associated with hereditary deafness including *ACTG1* that encodes gamma-actin (DFNA20/26), *DIAPH1* that regulates the polymerization of actin filaments (DFNA1), *ESPN* that encodes actin-bundling protein espin (DFNB36), *RDX* that links the actin filaments to the membrane in stereocilia (DFNB24), and several unconventional myosin-encoding genes *MYO7A* (DFNA11, DFNB2, Usher syndrome 1B), *MYO6* (DFNA22 and DFNB37), and *MYO15A* (DFNB3).

ACTG1 encodes gamma-actin, which is abundantly expressed in inner ear hair cells. Mutations in *ACTG1* can cause autosomal dominant hearing loss (DFNA20/DFNA26) [89]. It may affect bunching, gelation, polymerization, or myosin movement in hair cells, obstructing the repair of the cochlear structure when it is damaged by noise or aging, thus causing progressive hearing loss [51].

DIAPH1 plays an important role in regulation of actin polymerization in hair cells of the inner ear. In mouse cochlea, *Diaph1* is expressed in inner pillar cell, the base of the outer hair cells, and outer pillar cells. It is also expressed in the neuronal structures in the spiral ganglion neurons and the cochlear nerve [55]. Mutations in *DIAPH1* cause autosomal dominant hearing loss (DFNA1) [45]. Mice with homozygous deletion of *Diaph1* developed unilateral dilatation of the ventricles without blockage of the cerebral aqueducts. The absence of *Diaph1* did not grossly alter the organization of actin filaments or tubulin, though auditory system was not particularly investigated in this study [17].

Both *MYO7A* and *MYO15A* encode unconventional myosin proteins. Variations in *MYO7A* lead to approximately 50% of Usher syndromes [48]. Mutation in the *MYO7A* gene can also cause autosomal recessive non-syndromic hearing impairment in humans. The shaker-1 and the headbanger mice which carry mutations in *Myo7A* have been studied. Shaker-1 mutants show vestibular dysfunction symptoms such as hyperactivity, head tossing, and circling, and the organ of Corti shows progressive degeneration [22]. In headbanger mice, outer hair cell stereocilia form O instead of V shapes, and giant stereocilia were observed among inner hair cells [69]. It was suggested that both the vestibular dysfunction and deafness were caused by a defective morphogenesis of the hair cell stereocilia.

Mutations in *MYO15A* cause autosomal recessive hearing impairment (DFNB3) in humans. MYO15A helps in elongation and the development of stereocilia and actin filament. Interaction of whirlin and MYO15A assists in the cohesion of stereocilia [3]. The mutation in the *MYO7A* gene was first identified in the families of Indonesia. The shaker-2 J mouse has a 14.7 kb deletion that removes the last six exons from the 3′-terminus of the *Myo15* transcript. It showed very short stereocilia in the cochlear and vestibular dysfunction [2]. It suggested that *Myo15* may be important for both the structure and function of these sensory epithelia in the inner ear.

5.1.4 Transmembrane Proteins

Transmembrane proteins act as gateways to permit the transport of specific substances across the biological membrane. They often undergo significant conformational changes to enable a substance move through the membrane. Various genes encode transmembrane proteins. Mutations in these genes including *SLC26A4*, *KCNQ1*, and *KCNQ4* cause hereditary hearing impairment.

SLC26A4 encodes an anion transporter known as pendrin. Pendrin works as a transporter of anion ($Cl-$, $I-$, and HCO_3-) in the cell membranes [84]. It is mostly expressed in the inner ear, thyroid, and kidney [5]. Dysfunction of pendrin results in Pendred syndrome (PDS) and non-syndromic (DFNB4) hearing impairment associated with enlarged vestibular aqueduct (EVA) [7]. *SLC26A4* knockout mice are completely deaf and show signs of vestibular dysfunction. The inner ears appear to develop normally until embryonic day 15, after which time severe endolymphatic dilatation occurs, reminiscent of what is seen radiologically in deaf individuals with *SLC26A4* mutations [15, 18].

KCNQ is a small family of potassium channels that were known to be associated with different human diseases such as deafness and cardiac arrhythmia [71]. *KCNQ* proteins are classified as voltage-gated channels that depend on the membrane potential and can be activated upon depolarization of the cell membrane. The pivotal role of potassium in the inner ear fluids and its dynamics are emphasized by the fact that two members of the *KCNQ* family, *KCNQ1* and *KCNQ4*, are essential for normal hearing [58, 67]. Mutations in *KCNQ1* can lead to long QT syndrome and

Jervell and Lange-Nielsen cardioauditory syndrome [56, 92]. Disruption of *KCNQ1* in mice leads to deafness with severe morphological abnormalities of the inner ear [70]. Histologic analysis of the inner ear structures of these mice showed morphologic anomalies because of drastic reduction in the volume of endolymph [70]. Mutations in *KCNQ4* can lead to DFNA2 [38]; KCNQ4 is expressed in the sensory outer hair cell [34]. It dictates the efflux of potassium outside the cell in order to bring the cell back to the excitatory state. Disruption of the KCNQ4 channel in mice mimics the human hearing phenotype and indicates that the progressive HL is due to degeneration of the outer hair cell. In addition, comprehensive electrophysiological measurements confirmed the hypothesis that the constant potassium overloading of the outer hair cells leads to cell death [33].

5.1.5 Transcription Factors

Transcription factors (TFs) are proteins that play important role in cell proliferation and differentiation and death [54]. Each TF contains at least one DNA-binding domain. They work by attaching to a specific sequence of DNA. Examples of TFs associated with hearing loss include *POU3F4*, *POU4F3*, *EYA4*, and *GRHL2*.

POU3F4 encodes a member of the POU-domain transcription factor family and is responsible for DFX2 (deafness, X-linked 2) [12]. *Pou3f4*-deficient mice showed profound deafness. There was a dramatic reduction in endocochlear potential, but the morphology of the inner ear appeared normal. Electron microscopy showed that the mesenchymal in cochlear spiral ligament, which plays a vital role in potassium ion homeostasis, was replaced by fibrocytes [49]. *POU4F3*, another member of the POU-domain transcription factor family, is vital for the maintenance of outer hair cells [95]. Deficiency of *POU4F3* leads to reduced expression of *Gfi1*. Outer hair cell degeneration was observed in *Gfil*-deficient mice which appeared comparable to what was observed in *Pou4f3* mutants. Therefore, the hair cell-specific transcription factor *Gfi1* may be the direct downstream target gene of *Pou4f3* [26].

EYA4 is responsible for DFNA10. It was first discovered in an American and a Belgian family with autosomal dominant non-syndromic hearing loss [93], *EYA4* is important for continued function of the mature organ of Corti. *Eya4*-null mice developed otitis media with effusion. Anatomy studies of *Eya4*-null mice showed abnormal middle ear cavity and the eustachian tube [13].

GRHL2 is widely expressed in human tissues such as the prostate, thymus, kidney, lung, salivary gland, mammary gland, and digestive tract. Mutations in *GRHL2* can lead to autosomal dominant form of non-syndromic sensorineural hearing loss DFNA 28 and ectodermal dysplasia/short stature syndrome [61, 62]. *grhl2bT086* mutant zebrafish showed enlarged otocysts, smaller or eliminated otoliths, malformed semicircular canals, insensitiveness to sound stimulation, and imbalanced swimming motion. The expression of claudin b (*cldnb*) and *epcam* is abolished or dramatically reduced, and apical junctional complexes are abnormal in otic epithelial cells of mutant embryos [24].

5.1.6 microRNAs

A microRNA is a small (~25-nucleotide) noncoding RNA molecule. It functions in RNA silencing and posttranscriptional regulation. MicroRNA works through binding to the 3'UTR region of the target gene and then reducing the gene expression by translational suppression and mRNA destabilization. miR-96 was the first miRNA shown associated with hearing impairment in human [47]. In a ENU-induced mouse mutant with a single base change in the seed region of miR-96, both heterozygotes and homozygotes mutant mice showed irregular hair bundles and ectopic stereocilia. Heterozygous mice showed progressive hearing loss similar to the phenotype in humans, while homozygotes have no cochlear responses [47]. A zebrafish model was used to understand the role of miR-96, miR-182, and miR-183 cluster in inner ear development. Overexpression of miR-96 and miR-182 in embryos exhibited body deformities and ectopic hair cells. Knockdown of each miRNAs showed a decrease number of hair cell. Overexpression of miR182 in miR-96 knockdown embryo exhibited a rescue effect. These results suggest that miRNAs play an important role in hair cell formation and development [42].

5.2 Genetic Screening and Diagnosis of Hearing Loss

5.2.1 Genetic Diagnosis

Most cases of hearing loss have a genetic etiology and are highly heterogeneous. Currently, more than 100 genes were identified associated with hearing loss in human (http://www.hereditaryhearingloss.org), and the number of causative genes continues to increase. Identifying the genetic etiology of hearing loss is important for many reasons. It can offer important genetic information for the children and allows personalized and accurate genetic counseling. Genetic diagnosis could also help to avoid unnecessary and costly testing, offer prognostic information, and guide future medical management. For instance, it helps to choose appropriate management options (e.g., hearing aids or cochlear implantation), identify syndromic hearing loss that needs early monitoring and intervention, and secure preventable risk factors for future hearing deterioration (e.g., aminoglycoside use or head trauma) [37]. The importance of an etiological diagnosis is underlined by the 2014 ACMG guidelines for diagnosis of hearing loss, in which it has been recommended that genetic testing should be included in the workup of patients with NSHL [1]. The universal newborn hearing screening program (UNHS) has resulted in an increased demand for genetic diagnosis of hearing loss, and the recent progress in understanding the genes associated with the function of hearing has made it possible to detect the molecular basis of hearing loss through DNA testing. In the following section, we'll discuss the advances in the genetic testing technology, as well as the currently available genetic assays for hereditary hearing loss and their advantages and limitations.

5.2.2 *Sanger Sequencing*

Sanger sequencing has been the predominant method in the genome sequencing field for over 40 years since Sanger first introduced his "plus and minus" method for DNA sequencing in 1975 [75, 76]. This method has an extremely high sensitivity and specificity and remains the gold standard for DNA sequencing accuracy. Over the years, the cost of Sanger sequencing has steadily reduced due to incremental improvements in methodologies, throughput, and instrumentation, so that laboratories are able to gradually add content to their tests. The current generation of automated Sanger sequencing machines was used to sequence the first human genome, which can read about two million bases per day.

For human hearing loss, due to the great number and the large size of deafness-associated genes, it will be time-consuming and expensive to test all possible candidate genes using routine Sanger sequencing. However, when the number of genes responsible for the subtype of hearing loss are limited, diagnosis of genetic hearing loss using Sanger sequencing can be effective. Examples include mutation screening of *OTOF* in cases with prelingual, profound NSHL accompanied by auditory neuropathy [73]. If hearing loss is found to be progressive, late onset combined with vestibular abnormalities, mutations in *COCH* may be suspected [31]. In cases of X-linked hearing loss associated with a defect in the bony labyrinth, POU3F4 should be tested for mutations [91]. Mutation screening of mitochondrial gene m.1555A > G is warranted if the hearing loss is progressive with history of aminoglycoside exposure and family history of mitochondrial inheritance [9]. Some genes cause hearing loss with a distinct audioprofile. For example, mitochondrial deafness gene *WFS1* mutations cause moderate mid-frequency hearing loss and *TECTA* low-frequency hearing loss [41, 63]. Moreover, in patients with genetically heterogeneous hearing loss phenotypes, Sanger sequencing can also be valuable when a single gene is responsible for a significant percentage of cases. For instance, mutations in *GJB2* are the most frequent cause of non-syndromic hearing loss in most populations worldwide, accounting for up to 50% of autosomal recessive NSHL cases [32]. In all individuals identified with non-syndromic hearing loss, it is now a standard practice to perform molecular testing for *GJB2* mutations. Mutations in *SLC26A4* are the second most frequent cause of autosomal recessive non-syndromic hearing loss [68], and it should be considered for mutation screening if hearing loss is progressive and is associated with enlarged vestibular aqueduct (EVA) or a goiter. In China, 33% patients can be diagnosed by screening only three genes *GJB2*, *SLC26A4*, and mtDNA 1555A > G in the Han, Hui, and Tibetan ethnicities [16]. However, most identified deaf genes cause hearing loss without any recognizable audioprofile. Because of this, until recently most patients with non-syndromic hearing loss, or with a syndromic hearing loss that is heterogeneous (for instance, Usher syndrome), cannot have a genetic diagnosis.

5.2.3 Next-Generation Sequencing

In the past 10 years, the advent of next-generation sequencing (NGS) has provided an extremely powerful tool to query the genetic landscape. Next-generation sequencing is capable of sequencing very large numbers of DNA fragments simultaneously in the same reaction, generating massive amounts of data within an extremely short period of time. The most comprehensive NGS technique is the whole genome sequencing (WGS), which sequences an individual's entire genome and is able to identify variants in both exonic and noncoding regions. The whole exome sequencing (WES) sequences only the protein-coding regions within a genome, which is believed to contain about 85% of disease-causing mutations [64]. The targeted gene panel (TGP) is the most focused NGS approach, which sequences only a specific cohort of genes. Each method has its own pros and cons (Table 5.1). For human deafness, gene panel approach could be used to sequence all known deafness genes, in which case only 0.014% of the entire genome is sequenced. Alternatively, if the patient has a mutation in a gene that hasn't been associated with sensorineural hearing loss yet, the whole exome sequencing could be considered. Since the exome contains approximately 2% of the entire genome, the costs and analytical complexity are relatively low [77]. Both of these two approaches depend on target enrichment, meaning that the region of interest must be captured to form sequencing libraries. In comparison, WGS skips the capture step and the entire genome is sequenced. As a result, it is much more bioinformatically complex and costly. Nevertheless, the

Table 5.1 Comparison of targeted panels, whole exome sequencing, and whole genome sequencing

	Targeted panel	WES	WGS
Target	<200 genes	~2% of genome	Entire genome
Cost	Low	Moderate	High
Variants detected	Depends on the panel size	~20,000	~4000,000
Advantages	Low cost	Identifies novel genetic causes of hearing loss in coding regions	Identifies novel genetic causes of hearing loss both in coding and noncoding regions
	Customizable		
	Easier to interpret	Low cost	
			Detects structural variants
			Most uniform depth of sequencing
Limitations	Requires constant updates as new deafness genes are discovered	Sequencing depth affected by poor/incomplete exome capture	High cost
			Largest volume of data and the most complex analysis
	Variants limited to the preselected gene panel	Cannot detect noncoding or structural variants	
	Cannot detect structural variants		

whole genome can be uniformly covered, and variants in noncoding regions can also be detected. NGS is bioinformatically challenging because of its high throughput and the generation of large amounts of data. In spite of this, the application of NGS in identifying genes for sensorineural hearing loss has been successful, resulting in an increase in diagnostic rate to around 40% [81]. A large study using comprehensive genetic testing by Sloan-Heggen reported a diagnostic rate of 39% in 1119 sequentially accrued and unrelated patients [80]. OtoSCOPE v4 (66 genes) and v5 (89 genes) were used in their study, and variants in 49 different genes were identified as deafness-causing in 440 patients in their cohort. Several smaller studies have reported similar diagnostic rates [78], which make comprehensive genetic testing the best diagnostic test in the evaluation of hearing loss. As new causes of hearing loss are discovered, the diagnostic rates using NGS will continue to improve. Choosing the most comprehensive genetic test will improve the chances of a genetic diagnosis so that better and more cost-effective patient care could be provided (Fig. 5.1).

5.2.4 Genetic Screening

Hearing loss is the most common sensory defect in children. It's estimated that 1.86 per 1000 children has permanent sensorineural hearing loss of 35 dB or more at birth. The prevalence of sensorineural hearing loss is reported to continue to increase during childhood, and by the time of 5 years of age, the rate reaches to about 2.7 per 1000 children [52]. Early identification and management of hearing loss are critical to improve the language, communication, and cognitive development of children with hearing loss [99]. The average age at which hearing loss is confirmed has dropped from 24–30 months to 2–3 months with the launch of universal newborn hearing screening (UNHS) program [25]. However, current UNHS program has its limitations. Firstly, most screening programs target hearing loss of 35 dB or more,

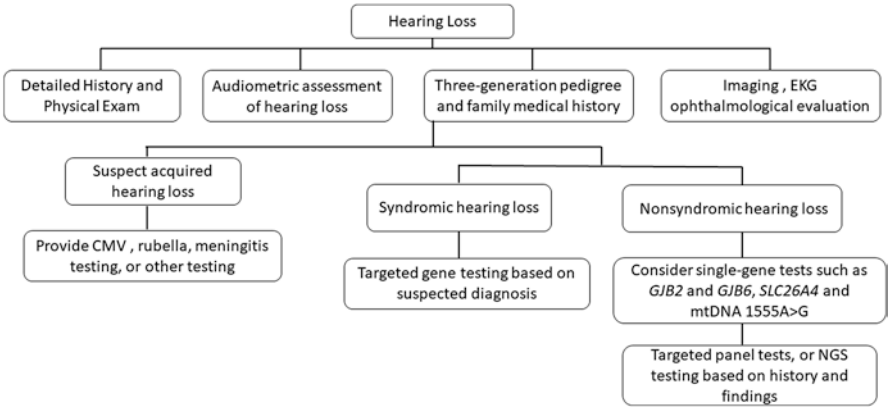

Fig 5.1 Genetic diagnosis of hearing loss

which are not able to detect newborns with slight or mild sensorineural hearing loss [30]. Secondly, children with late-onset or progressive sensorineural hearing loss after birth cannot be identified as these newborns have normal hearing at birth [100]. Finally, most current screening programs do not focus on the etiology, which makes it difficult to interpret the results of early intervention.

It is estimated that genetic causes are responsible for over two-thirds of prelingual cases of sensorineural hearing loss. Hence, genetic screening of newborns to identify the risk of future hearing loss from a genetic cause might be beneficial because it would allow family and child to consider use of parallel communication strategies before hearing deteriorates. Genetic screening could also help to identify newborns with mild hearing loss that cannot be detected with current newborn screening programs. In addition, genetic screening for specific mutations such as mitochondrial m.1555A > G could possibly minimize hearing loss in children by protecting them from avoidable risk factors such as the usage of aminoglycoside antibiotics [43].

There have been several studies demonstrating that newborn genetic screening might augment the current newborn hearing screening program by detecting children with mild sensorineural hearing loss and those at risk for late-onset hearing loss. Wu et al. [96] reported their results from a preliminary genetic screening study in 1017 newborns. The screen targeted four deafness-associated mutations, c.235delC and p. V37I (c.109G.A) of *GJB2*, c.919-2A > G of *SLC26A4*, and mitochondrial m.1555A > G that are common in the Taiwanese population. They found out nine babies that passed the hearing screen at birth had positive genetic screen results that suggested potential for hearing loss when analyzing the results of the newborn genetic screen along with the newborn hearing screen. Three months later the audiological follow-up in these babies identified two with mild hearing losses and one with slight hearing loss. Their results suggested that newborn genetic screening might play a potential role in identifying those newborns at risk of developing hearing loss at a later stage, so that they could be monitored with repeat audiological testing. Several other studies subsequently validated the feasibility and prognostic value of newborn genetic screening as well [98, 101, 102].

Although evidence has shown that newborn genetic screening for deafness genes is valuable in identifying infants with slight, mild, late-onset, or progressive sensorineural hearing loss, the integration of genetic screening into UNHS might pose new challenges. The implementation of newborn genetic screening for deafness genes might lead to new ethical issues such as risks of discrimination or stigmatization (misuse by insurers or employers) and undue anxiety of the family [14]. In addition, it might be difficult to make a precise interpretation of newborn genetic screen results due to the genetic heterogeneity of hereditary hearing loss. Besides, it is not possible to make prediction of the precise genetic risk for future hearing loss using screening tests that target only a selection of deafness genes.

Despite this, as more genes involved in hearing loss are discovered and more is known about the clinical significance of the identified mutations, 1 day it will be totally justified to adopt genetic screening to predict the risk of future hearing loss in infants. For now, it's suggested that newborn genetic screening for specific muta-

tions like mitochondrial m.1555A > G mutation be adopted. This mutation has a population prevalence of 1.9‰ [6] and can cause permanent profound hearing loss in carriers taking standard therapeutic doses of aminoglycoside antibiotics with a penetrance close to 100%. Knowledge of the carrier status of m.1555A > G would help to avoid aminoglycoside antibiotics usage for infection control for these children and potentially prevent unnecessary hearing loss.

5.3 Intervention and Prevention of Genetic Hearing Loss

5.3.1 Gene Therapy

Sensorineural hearing loss (SNHL) is the most common form of hearing loss and usually occurs due to loss of functional sensory hair cells in the cochlea, which accounts for approximately 90% of all human hearing loss cases [82]. For patients with severe to complete SNHL, cochlear implants can provide the pitch and time cues required for speech perception by electrically stimulating the spiral ganglion neurons (SGN). These recipients integrate the auditory information provided by the acoustic input of their residual hair cells and the electrical stimulation of the SGN and show improved speech perception. However, the signals produced by the cochlear implant are very different from the signals produced by the cochlear hair cells, requiring extended rehabilitation therapy to maximize the benefits of the implant [53, 72]. In addition, many patients are reluctant to accept implants because the procedure will permanently leave the signal receiver device on the surface of the head. Hence there is a strong need for development of biological treatments for restoration of auditory function [21]. Gene therapies have the potential to maintain or restore hearing with more natural sound perception because their theoretical frequency resolution is much higher than cochlear implants. Recent researches have showed partial hearing recovery in some specific forms of genetic hearing loss.

5.3.1.1 Design of Gene Therapy for HL

The design of gene therapy depends on the pathogenic mechanism of the mutation. There are two main strategies for gene therapy of genetic HL: replacement or augmentation by exogenous expression of wildtype genes and blocking or eradication of the mutant alleles. Bi-allelic recessive mutations and loss-of-function dominant mutations can be generally treated by the first strategy, whereas gain-of-function mutations require the second strategy [19].

Chang et al. reported a successful cochlear gene replacement therapy on the Kcnq1 null mutation in the marginal cells [8]. AAV1 expressing Kcnq1 was injected P0-P2 into to the endolymphatic spaces, which induced Kcnq1 expression in about 70% cochlear marginal cells where the native Kcnq1 is exclusively expressed. Examination of cochlear morphology showed that the collapse of the Reissner's

membrane and degeneration of HCs and SGNs are corrected. Auditory brainstem responses showed significant hearing preservation in the injected ears, ranging from 20 dB improvement to complete correction of the deafness phenotype. In a mouse model of Usher syndrome, mice with mutant WHRN gene were treated with AAV8-whirlin, resulting in rescue of both hearing and balance [29].

An antisense oligonucleotide (ASO) was used to correct defective pre-mRNA splicing of transcripts from the USH1C gene with the c.216G > A mutation [40]. Treatment of neonatal mice with a single systemic dose of ASO partially corrects Ush1c c.216G > A splicing, increases protein expression, improves stereocilia organization in the cochlea, and rescues cochlear hair cells, vestibular function, and low-frequency hearing in mice. These effects were sustained for several months, providing evidence that congenital deafness can be effectively overcome by treatment early in the development to correct gene expression and demonstrating the therapeutic potential of ASOs in the treatment of deafness [40]. Using an Ush1c c.216G > A knock-in mouse model to study the Usher type 1C disease, recently, Pan et al. [60] used the same Ush1c c.216G > A knock-in mouse model to test whether cochlear gene therapy could be used to target hair cells to correct the deafness phenotype [60]. Using a synthesized adeno-associated virus (AAV), they achieved auditory thresholds improvement of 60–70 dB compared to untreated ears when recombinant viral vectors were injected at P0-P1 through round window membrane into the scala tympani. The treatment effect lasted at least 6 months, as demonstrated by hair cell survival in the cochlea. The same treatment at P10-P12 showed no hearing recovery.

A recent study using a CRISPR-Cas9 genome-editing approach showed that hearing thresholds were improved in a mouse model of dominant deafness caused by a mutation in Tmc1, Beethoven (Bth) mice [20]. Injection into the neonatal cochlea of Tmc1Bth/+ mutant mice substantially reduced progressive hearing loss. These results demonstrated the applicability of cochlear gene therapy for recessive and dominant mutations.

5.3.1.2 Vectors

Previous studies have shown that herpes simplex virus type I, vaccinia virus, lentivirus, retrovirus, adenovirus, and adeno-associated virus (AAV) can be used as vectors for transfection of inner ear cells. Expression of lentiviral transfection is restricted only to cochlear cells lining the paralymphatic space and may be randomly integrated into the host's genome. Adenovirus is widely used in the transfection of cochlea and vestibular cells, transfection efficiency of inner hair cells is over 90%, and outer hair cells are more than 50% [44]. However, a major drawback of adenovirus as a gene therapy vector is that integration of the viral gene into the host cell can cause an immune response, produce cytotoxicity, and even kill cells [27]. A few features of AAV are that it is a good carrier for gene therapy: first, AAV is a harmless parvovirus; second, AAV has a wide range of hosts that can be transfected

into most cell types, including postmitotic cells; third, AAV has been used in clinical trials, and the results show that it has no significant effect on cell growth, morphology, and differentiation [65]. Therefore, most inner ear gene therapy studies use adeno-associated virus as a vector.

At present, there are at least a dozen serotypes of AAV and different subtypes of AAV target different cell types in cochlear cell transfection. A recent research investigated the specificity of 12 different serotypes of AAV (AAV1, 2, 5, 6, 6.2, 7, 8, 9, rh8, rh10, rh39, rh43) for transfection of various cells in living neonatal mice [79]. It does not affect hearing after surgery and provides an ideal transfection pathway for gene therapy of hereditary deafness. Recently, a novel synthetic adeno-associated virus (AAV), Anc80L65, was used, and this viral vector was able to transduce >90% of outer HCs [60]. Another independent study that carried the injection through the posterior semicircular canal has demonstrated excellent transduction efficiency in both inner and outer hair cells at the adult stage [85].

5.3.2 Genetic Implications for Cochlear Implantation

Cochlear implant (CI) is a surgically implanted device that bypasses the normal auditory pathway and interacts with the cochlear nerve through the electrode array. The primary treatments for hearing loss at present are hearing aids and CI. For patients with severe-to-complete hearing loss, CI is the only effective treatment option. However, hearing outcomes after CI may vary for genetic HL. Many researches have shown that patients with GJB2 or SLC26A4 mutations have favorable outcomes than others [11, 23, 46, 97]. Other genes that have been associated with good CI outcomes are TECTA [50], MYH9 [59], CDH23 [88], TMPRSS3 [94], MYO6 [90], OTOF [74, 87], COCH [87], and MYO15A [50]. A recent study showed that patients with gene mutations in SGNs have worse postoperative speech perception testing outcomes than those with variants mainly affect cochlea. Because CI directly stimulates the cochlear nerve, the electrical signal of CI cannot be normally transmitted to auditory center when the mutations affect the function of cochlear nerve.

5.3.3 Prevention of Genetic Hearing Loss

The World Health Organization divides prevention into three levels: primary prevention to avoid adverse health conditions, secondary prevention to carry out early detection and timely treatment, and tertiary prevention to reduce the impact of established conditions and possible recovery function.

The primary prevention of genetic HL mainly refers genetic counseling at prepregnancy or early pregnancy stage, through genetic screening and diagnosis of

high-risk population, to fundamentally reduce the incidence of children with congenital deafness. People with high risk of having HL children include HL patients and their relatives and couples who have already had deaf children. However, due to the high frequencies of specific HL mutation, it is also necessary to screen common HL genes in normal-hearing populations to identify carriers of recessive gene mutations. For carriers of mutations in the HL genes, further genetic testing of their spouses is required. If both couples have hereditary HL genotype, it is recommended to carry out genetic counseling, fertility guidance, and prenatal diagnosis. Prenatal diagnosis of genetic HL can give parents the opportunity to prepare psychologically, economically, and medically for the health and educational needs of the affected newborn.

References

1. Alford RL, Arnos KS, Fox M, Lin JW, Palmer CG, Pandya A, Rehm HL, Robin NH, Scott DA, Yoshinaga-Itano C (2014) American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss. Genet Med 16(4):347–355
2. Anderson DW, Probst FJ, Belyantseva IA, Fridell RA, Beyer L, Martin DM, Wu D, Kachar B, Friedman TB, Raphael Y, Camper SA (2000) The motor and tail regions of myosin XV are critical for normal structure and function of auditory and vestibular hair cells. Hum Mol Genet 9(12):1729–1738
3. Belyantseva IA, Boger ET, Naz S, Frolenkov GI, Sellers JR, Ahmed ZM, Griffith AJ, Friedman TB (2005) Myosin-XVa is required for tip localization of whirlin and differential elongation of hair-cell stereocilia. Nat Cell Biol 7(2):148–156
4. Ben-Yosef T, Belyantseva IA, Saunders TL, Hughes ED, Kawamoto K, Van Itallie CM, Beyer LA, Halsey K, Gardner DJ, Wilcox ER, Rasmussen J, Anderson JM, Dolan DF, Forge A, Raphael Y, Camper SA, Friedman TB (2003) Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration. Hum Mol Genet 12(16):2049–2061
5. Bidart JM, Mian C, Lazar V, Russo D, Filetti S, Caillou B, Schlumberger M (2000) Expression of pendrin and the Pendred syndrome (PDS) gene in human thyroid tissues. J Clin Endocrinol Metab 85(5):2028–2033
6. Bitnerglindzicz M, Pembrey M, Duncan A, Heron J, Ring SM, Hall A, Rahman S (2009) Prevalence of mitochondrial 1555A->G mutation in European children. N Engl J Med 360(6):640–642
7. Campbell C, Cucci RA, Prasad S, Green GE, Edeal JB, Galer CE, Karniski LP, Sheffield VC, Smith RJ (2001) Pendred syndrome, DFNB4, and PDS/SLC26A4 identification of eight novel mutations and possible genotype-phenotype correlations. Hum Mutat 17(5):403–411
8. Chang Q, Wang J, Li Q, Kim Y, Zhou B, Wang Y, Li H, Lin X (2015) Virally mediated Kcnq1 gene replacement therapy in the immature scala media restores hearing in a mouse model of human Jervell and Lange-Nielsen deafness syndrome. EMBO Mol Med 7(8):1077–1086
9. Chennupati SK, Levi J, Loftus P, Jornlin C, Morlet T, O'Reilly RC (2011) Hearing loss in children with mitochondrial disorders. Int J Pediatr Otorhinolaryngol 75(12):1519–1524
10. Cohen-Salmon M, Ott T, Michel V, Hardelin JP, Perfettini I, Eybalin M, Wu T, Marcus DC, Wangemann P, Willecke K, Petit C (2002) Targeted ablation of connexin26 in the inner ear epithelial gap junction network causes hearing impairment and cell death. Curr Biol 12(13):1106–1111

11. Davcheva-Chakar M, Sukarova-Stefanovska E, Ivanovska V, Lazarevska V, Filipche I, Zafirovska B (2014) Speech perception outcomes after cochlear implantation in children with GJB2/DFNB1 associated deafness. Balkan Med J 31(1):60–63
12. de Kok YJ, van der Maarel SM, Bitner-Glindzicz M, Huber I, Monaco AP, Malcolm S, Pembrey ME, Ropers HH, Cremers FP (1995) Association between X-linked mixed deafness and mutations in the POU domain gene POU3F4. Science 267(5198):685–688
13. Depreux FF, Darrow K, Conner DA, Eavey RD, Liberman MC, Seidman CE, Seidman JG (2008) Eya4-deficient mice are a model for heritable otitis media. J Clin Invest 118(2):651–658
14. Dhondt JL, Cornejo V, Hoffmann GF, Pollitt R (2010) Expanded newborn screening: social and ethical issues. J Inherit Metab Dis 33(2):211–217
15. Dror AA, Politi Y, Shahin H, Lenz DR, Dossena S, Nofziger C, Fuchs H, Hrabe de Angelis M, Paulmichl M, Weiner S, Avraham KB (2010) Calcium oxalate stone formation in the inner ear as a result of an Slc26a4 mutation. J Biol Chem 285(28):21724–21735
16. Duan SH, Zhu YM, Wang YL, Guo YF (2015) Common molecular etiology of nonsyndromic hearing loss in 484 patients of 3 ethnicities in northwest China. Acta Otolaryngol 135(6):586–591
17. Ercan-Sencicek AG, Jambi S, Franjic D, Nishimura S, Li M, El-Fishawy P, Morgan TM, Sanders SJ, Bilguvar K, Suri M, Johnson MH, Gupta AR, Yuksel Z, Mane S, Grigorenko E, Picciotto M, Alberts AS, Gunel M, Sestan N, State MW (2015) Homozygous loss of DIAPH1 is a novel cause of microcephaly in humans. Eur J Hum Genet 23(2):165–172
18. Everett LA, Belyantseva IA, Noben-Trauth K, Cantos R, Chen A, Thakkar SI, Hoogstraten-Miller SL, Kachar B, Wu DK, Green ED (2001) Targeted disruption of mouse Pds provides insight about the inner-ear defects encountered in Pendred syndrome. Hum Mol Genet 10(2):153–161
19. Gao L, Bryan BA (2017) Finding pathways to national-scale land-sector sustainability. Nature 544(7649):217–221
20. Gao X, Tao Y, Lamas V, Huang M, Yeh WH, Pan B, Hu YJ, Hu JH, Thompson DB, Shu Y, Li Y, Wang H, Yang S, Xu Q, Polley DB, Liberman MC, Kong WJ, Holt JR, Chen ZY, Liu DR (2018) Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. Nature 553(7687):217–221
21. Geleoc GS, Holt JR (2014) Sound strategies for hearing restoration. Science 344(6184):1241062
22. Gibson F, Walsh J, Mburu P, Varela A, Brown KA, Antonio M, Beisel KW, Steel KP, Brown SD (1995) A type VII myosin encoded by the mouse deafness gene shaker-1. Nature 374(6517):62–64
23. Green GE, Scott DA, McDonald JM, Teagle HFB, Tomblin BJ, Spencer LJ, Woodworth GG, Knutson JF, Gantz BJ, Sheffield VC, Smith RJH (2002) Performance of cochlear implant recipients with GJB2-related deafness. Am J Med Genet 109(3):167–170
24. Han Y, Mu Y, Li X, Xu P, Tong J, Liu Z, Ma T, Zeng G, Yang S, Du J, Meng A (2011) Grhl2 deficiency impairs otic development and hearing ability in a zebrafish model of the progressive dominant hearing loss DFNA28. Hum Mol Genet 20(16):3213–3226
25. Harrison M, Roush J, Wallace J (2003) Trends in age of identification and intervention in infants with hearing loss. Ear Hear 24(1):89–95
26. Hertzano R, Montcouquiol M, Rashi-Elkeles S, Elkon R, Yucel R, Frankel WN, Rechavi G, Moroy T, Friedman TB, Kelley MW, Avraham KB (2004) Transcription profiling of inner ears from Pou4f3(ddl/ddl) identifies Gfi1 as a target of the Pou4f3 deafness gene. Hum Mol Genet 13(18):2143–2153
27. Holt JR, Johns DC, Wang S, Chen ZY, Dunn RJ, Marban E, Corey DP (1999) Functional expression of exogenous proteins in mammalian sensory hair cells infected with adenoviral vectors. J Neurophysiol 81(4):1881–1888
28. Hoyt MA, Hyman AA, Bahler M (1997) Motor proteins of the eukaryotic cytoskeleton. Proc Natl Acad Sci U S A 94(24):12747–12748

29. Isgrig K, Shteamer JW, Belyantseva IA, Drummond MC, Fitzgerald TS, Vijayakumar S, Jones SM, Griffith AJ, Friedman TB, Cunningham LL, Chien WW (2017) Gene therapy restores balance and auditory functions in a mouse model of Usher syndrome. Mol Ther 25(3):780–791
30. Johnson JL, White KR, Widen JE, Gravel JS, James M, Kennalley T, Maxon AB, Spivak L, Sullivan-Mahoney M, Vohr BR (2005) A multicenter evaluation of how many infants with permanent hearing loss pass a two-stage otoacoustic emissions/automated auditory brainstem response newborn hearing screening protocol. Pediatrics 116(3):663–672
31. Kemperman MH, De Leenheer EM, Huygen PL, Van DG, Morton CC, Robertson NG, Cremers FP, Kremer H, Cremers CW (2005) Audiometric, vestibular, and genetic aspects of a DFNA9 family with a G88E COCH mutation. Otol Neurotol 26(5):926–933
32. Kenneson A, Van Naarden Braun K, Boyle C (2002) GJB2 (connexin 26) variants and nonsyndromic sensorineural hearing loss: a HuGE review. Genet Med 4(4):258–274
33. Kharkovets T, Dedek K, Maier H, Schweizer M, Khimich D, Nouvian R, Vardanyan V, Leuwer R, Moser T, Jentsch TJ (2006) Mice with altered KCNQ4 K+ channels implicate sensory outer hair cells in human progressive deafness. EMBO J 25(3):642–652
34. Kharkovets T, Hardelin JP, Safieddine S, Schweizer M, El-Amraoui A, Petit C, Jentsch TJ (2000) KCNQ4, a K+ channel mutated in a form of dominant deafness, is expressed in the inner ear and the central auditory pathway. Proc Natl Acad Sci U S A 97(8):4333–4338
35. Kiang DT, Jin N, Tu ZJ, Lin HH (1997) Upstream genomic sequence of the human connexin26 gene. Gene 199(1–2):165–171
36. Kitajiri SI, Furuse M, Morita K, Saishin-Kiuchi Y, Kido H, Ito J, Tsukita S (2004) Expression patterns of claudins, tight junction adhesion molecules, in the inner ear. Hear Res 187(1–2):25–34
37. Korver AM, Smith RJ, Van CG, Schleiss MR, Bitner-Glindzicz MA, Lustig LR, Usami SI, Boudewyns AN (2017) Congenital hearing loss. Nat Rev Dis Primers 71(10):467
38. Kubisch C, Schroeder BC, Friedrich T, Lutjohann B, El-Amraoui A, Marlin S, Petit C, Jentsch TJ (1999) KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. Cell 96(3):437–446
39. Lee K, Ansar M, Andrade PB, Khan B, Santos-Cortez RL, Ahmad W, Leal SM (2012) Novel CLDN14 mutations in Pakistani families with autosomal recessive non-syndromic hearing loss. Am J Med Genet A 158A(2):315–321
40. Lentz JJ, Jodelka FM, Hinrich AJ, McCaffrey KE, Farris HE, Spalitta MJ, Bazan NG, Duelli DM, Rigo F, Hastings ML (2013) Rescue of hearing and vestibular function by antisense oligonucleotides in a mouse model of human deafness. Nat Med 19(3):345–350
41. Lesperance MM, San Agustin TB, Leal SM (2003) Mutations in the Wolfram syndrome type 1 gene (WFS1) define a clinical entity of dominant low-frequency sensorineural hearing loss. Arch Otolaryngol Head Neck Surg 129(4):411
42. Li H, Kloosterman W, Fekete DM (2010) MicroRNA-183 family members regulate sensorineural fates in the inner ear. J Neurosci 30(9):3254–3263
43. Linden PL, Bitnerglindzicz M, Lench N, Steel KP, Langford C, Dawson SJ, Davis A, Simpson S, Packer C (2013) The future role of genetic screening to detect newborns at risk of childhood-onset hearing loss. Int Audiol 52(2):124–133
44. Luebke AE, Steiger JD, Hodges BL, Amalfitano A (2001) A modified adenovirus can transfect cochlear hair cells in vivo without compromising cochlear function. Gene Ther 8(10):789–794
45. Lynch ED, Lee MK, Morrow JE, Welcsh PL, Leon PE, King MC (1997) Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the Drosophila gene diaphanous. Science 278(5341):1315–1318
46. Matsushiro N, Doi K, Fuse Y, Nagai K, Yamamoto K, Iwaki T, Kawashima T, Sawada A, Hibino H, Kubo T (2002) Successful cochlear implantation in prelingual profound deaf-

ness resulting from the common 233delC mutation of the GJB2 gene in the Japanese. Laryngoscope 112(2):255–261
47. Mencia A, Modamio-Hoybjor S, Redshaw N, Morin M, Mayo-Merino F, Olavarrieta L, Aguirre LA, del Castillo I, Steel KP, Dalmay T, Moreno F, Moreno-Pelayo MA (2009) Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. Nat Genet 41(5):609–613
48. Millan JM, Aller E, Jaijo T, Blanco-Kelly F, Gimenez-Pardo A, Ayuso C (2011) An update on the genetics of usher syndrome. J Ophthalmol 2011:417217
49. Minowa O, Ikeda K, Sugitani Y, Oshima T, Nakai S, Katori Y, Suzuki M, Furukawa M, Kawase T, Zheng Y, Ogura M, Asada Y, Watanabe K, Yamanaka H, Gotoh S, Nishi-Takeshima M, Sugimoto T, Kikuchi T, Takasaka T, Noda T (1999) Altered cochlear fibrocytes in a mouse model of DFN3 nonsyndromic deafness. Science 285(5432):1408–1411
50. Miyagawa M, Nishio S-Y, Ikeda T, Fukushima K, Usami S-I (2013) Massively parallel DNA sequencing successfully identifies new causative mutations in deafness genes in patients with cochlear implantation and EAS. PLoS One 8(10):e75793
51. Morin M, Bryan KE, Mayo-Merino F, Goodyear R, Mencia A, Modamio-Hoybjor S, del Castillo I, Cabalka JM, Richardson G, Moreno F, Rubenstein PA, Moreno-Pelayo MA (2009) In vivo and in vitro effects of two novel gamma-actin (ACTG1) mutations that cause DFNA20/26 hearing impairment. Hum Mol Genet 18(16):3075–3089
52. Morton CC, Nance WE (2006) Newborn hearing screening—a silent revolution. N Engl J Med 354(20):2151–2164
53. Mueller U, Barr-Gillespie PG (2015) New treatment options for hearing loss. Nat Rev Drug Discov 14(5):346–U384
54. Nagashima R, Sugiyama C, Yoneyama M, Ogita K (2005) Transcriptional factors in the cochlea within the inner ear. J Pharmacol Sci 99(4):301–306
55. Neuhaus C, Lang-Roth R, Zimmermann U, Heller R, Eisenberger T, Weikert M, Markus S, Knipper M, Bolz HJ (2017) Extension of the clinical and molecular phenotype of DIAPH1-associated autosomal dominant hearing loss (DFNA1). Clin Genet 91(6):892–901
56. Neyroud N, Tesson F, Denjoy I, Leibovici M, Donger C, Barhanin J, Faure S, Gary F, Coumel P, Petit C, Schwartz K, Guicheney P (1997) A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. Nat Genet 15(2):186–189
57. Nickel R, Forge A (2008) Gap junctions and connexins in the inner ear: their roles in homeostasis and deafness. Curr Opin Otolaryngol Head Neck Surg 16(5):452–457
58. Nie L (2008) KCNQ4 mutations associated with nonsyndromic progressive sensorineural hearing loss. Curr Opin Otolaryngol Head Neck Surg 16(5):441–444
59. Nishiyama N, Kawano A, Kawaguchi S, Shirai K, Suzuki M (2013) Cochlear implantation in a patient with Epstein syndrome. Auris Nasus Larynx 40(4):409–412
60. Pan B, Askew C, Galvin A, Heman-Ackah S, Asai Y, Indzhykulian AA, Jodelka FM, Hastings ML, Lentz JJ, Vandenberghe LH, Holt JR, Geleoc GS (2017) Gene therapy restores auditory and vestibular function in a mouse model of Usher syndrome type 1c. Nat Biotechnol 35(3):264–272
61. Peters LM, Anderson DW, Griffith AJ, Grundfast KM, San Agustin TB, Madeo AC, Friedman TB, Morell RJ (2002) Mutation of a transcription factor, TFCP2L3, causes progressive autosomal dominant hearing loss, DFNA28. Hum Mol Genet 11(23):2877–2885
62. Petrof G, Nanda A, Howden J, Takeichi T, McMillan JR, Aristodemou S, Ozoemena L, Liu L, South AP, Pourreyron C, Dafou D, Proudfoot LE, Al-Ajmi H, Akiyama M, McLean WH, Simpson MA, Parsons M, McGrath JA (2014) Mutations in GRHL2 result in an autosomal-recessive ectodermal Dysplasia syndrome. Am J Hum Genet 95(3):308–314
63. Pfister M, Thiele H, Camp GV, Fransen E, Apaydin F, Aydin Ö, Leistenschneider P, Devoto M, Zenner HP, Blin N (2004) A genotype-phenotype correlation with gender-effect for hearing impairment caused by TECTA mutations. Cell Physiol Biochem 14(4–6):369–376

64. Rabbani B, Mahdieh N, Hosomichi K, Nakaoka H, Inoue I (2012) Next-generation sequencing: impact of exome sequencing in characterizing Mendelian disorders. J Hum Genet 57(10):621–632
65. Rabinowitz JE, Samulski RJ (2000) Building a better vector: the manipulation of AAV virions. Virology 278(2):301–308
66. Rabionet R, Zelante L, Lopez-Bigas N, D'Agruma L, Melchionda S, Restagno G, Arbones ML, Gasparini P, Estivill X (2000) Molecular basis of childhood deafness resulting from mutations in the GJB2 (connexin 26) gene. Hum Genet 106(1):40–44
67. Reardon W, Lewis N, Hughes HE (1993) Consanguinity, cardiac arrest, hearing impairment, and ECG abnormalities: counselling pitfalls in the Romano-Ward syndrome. J Med Genet 30(4):325–327
68. Reardon W, Mahoney CFO, Trembath R, Jan H, Phelps PD (2000) Enlarged vestibular aqueduct: a radiological marker of Pendred syndrome, and mutation of the PDS gene. QJM 93(2):99–104
69. Rhodes CR, Hertzano R, Fuchs H, Bell RE, de Angelis MH, Steel KP, Avraham KB (2004) A Myo7a mutation cosegregates with stereocilia defects and low-frequency hearing impairment. Mamm Genome 15(9):686–697
70. Rivas A, Francis HW (2005) Inner ear abnormalities in a Kcnq1 (Kvlqt1) knockout mouse: a model of Jervell and Lange-Nielsen syndrome. Otol Neurotol 26(3):415–424
71. Robbins J (2001) KCNQ potassium channels: physiology, pathophysiology, and pharmacology. Pharmacol Ther 90(1):1–19
72. Roche JP, Hansen MR (2015) On the horizon cochlear implant technology. Otolaryngol Clin N Am 48(6):1097–1116
73. Rodriguez-Ballesteros M, Reynoso R, Olarte M, Villamar M, Morera C, Santarelli R, Arslan E, Meda C, Curet C, Volter C, Sainz-Quevedo M, Castorina P, Ambrosetti U, Berrettini S, Frei K, Tedin S, Smith J, Cruz Tapia M, Cavalle L, Gelvez N, Primignani P, Gomez-Rosas E, Martin M, Moreno-Pelayo MA, Tamayo M, Moreno-Barral J, Moreno F, del Castillo I (2008) A multicenter study on the prevalence and spectrum of mutations in the otoferlin gene (OTOF) in subjects with nonsyndromic hearing impairment and auditory neuropathy. Hum Mutat 29(6):823–831
74. Rouillon I, Marcolla A, Roux I, Marlin S, Feldmann D, Couderc R, Jonard L, Petit C, Denoyelle F, Garabedian EN, Loundon N (2006) Results of cochlear implantation in two children with mutations in the OTOF gene. Int J Pediatr Otorhinolaryngol 70(4):689–696
75. Sanger F (1975) The Croonian Lecture, 1975: nucleotide sequences in DNA. Proc R Soc Lond B Biol Sci 191(1104):317–333
76. Sanger F, Coulson AR (1975) A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol 94(3):441–448
77. Shearer AE, Deluca AP, Hildebrand MS, Taylor KR, Scherer S, Scheetz TE, Smith RJ (2010) Comprehensive genetic testing for hereditary hearing loss using massively parallel sequencing. Proc Natl Acad Sci U S A 107(49):21104–21109
78. Shearer AE, Smith RJH (2015) Massively parallel sequencing for genetic diagnosis of hearing loss: the new standard of care. Otolaryngol Head Neck Surg 153(2):175–182
79. Shu Y, Tao Y, Wang Z, Tang Y, Li H, Dai P, Gao G, Chen ZY (2016) Identification of adeno-associated viral vectors (AAV) that target neonatal and adult mammalian inner ear cell subtypes. Hum Gene Ther
80. Sloanheggen CM, Bierer AO, Shearer AE, Kolbe DL, Nishimura CJ, Frees KL, Ephraim SS, Shibata SB, Booth KT, Campbell CA (2016) Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. Hum Genet 135(4):441–450
81. Sloan-Heggen CM, Smith RJ (2016) Navigating genetic diagnostics in patients with hearing loss. Curr Opin Pediatr 28(6):705
82. Smith RJH, Bale JF, White KR (2005) Sensorineural hearing loss in children. Lancet 365(9462):879–890

83. Sohl G, Willecke K (2004) Gap junctions and the connexin protein family. Cardiovasc Res 62(2):228–232
84. Soleimani M, Greeley T, Petrovic S, Wang Z, Amlal H, Kopp P, Burnham CE (2001) Pendrin: an apical Cl-/OH-/HCO3- exchanger in the kidney cortex. Am J Physiol Renal Physiol 280(2):F356–F364
85. Suzuki J, Hashimoto K, Xiao R, Vandenberghe LH, Liberman MC (2017) Cochlear gene therapy with ancestral AAV in adult mice: complete transduction of inner hair cells without cochlear dysfunction. Sci Rep 7(1):45524
86. Teubner B, Michel V, Pesch J, Lautermann J, Cohen-Salmon M, Sohl G, Jahnke K, Winterhager E, Herberhold C, Hardelin JP, Petit C, Willecke K (2003) Connexin30 (Gjb6)-deficiency causes severe hearing impairment and lack of endocochlear potential. Hum Mol Genet 12(1):13–21
87. Tsukada K, Ichinose A, Miyagawa M, Mori K, Hattori M, Nishio S-Y, Naito Y, Kitajiri S-I, Usami S-I (2015) Detailed hearing and vestibular profiles in the patients with COCH mutations. Ann Otol Rhinol Laryngol 124:100S–110S
88. Usami S-I, Miyagawa M, Nishio S-Y, Moteki H, Takumi Y, Suzuki M, Kitano Y, Iwasaki S (2012) Patients with CDH23 mutations and the 1555A > G mitochondrial mutation are good candidates for electric acoustic stimulation (EAS). Acta Otolaryngol 132(4):377–384
89. van Wijk E, Krieger E, Kemperman MH, De Leenheer EM, Huygen PL, Cremers CW, Cremers FP, Kremer H (2003) A mutation in the gamma actin 1 (ACTG1) gene causes autosomal dominant hearing loss (DFNA20/26). J Med Genet 40(12):879–884
90. Volk AE, Lang-Roth R, Yigit G, Borck G, Nuernberg G, Rosenkranz S, Nuernberg P, Kubisch C, Beutner D (2013) A novel MYO6 splice site mutation causes autosomal dominant sensorineural hearing loss type DFNA22 with a favourable outcome after cochlear implantation. Audiol Neuro Otol 18(3):192–199
91. Vore AP, Chang EH, Hoppe JE, Butler MG, Forrester S, Schneider MC, Smith LL, Burke DW, Campbell CA, Smith RJ (2005) Deletion of and novel missense mutation in POU3F4 in 2 families segregating X-linked nonsyndromic deafness. Arch Otolaryngol Head Neck Surg 131(12):1057
92. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, Schwartz PJ, Toubin JA, Moss AJ, Atkinson DL, Landes GM, Connors TD, Keating MT (1996) Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat Genet 12(1):17–23
93. Wayne S, Robertson NG, DeClau F, Chen N, Verhoeven K, Prasad S, Tranebjarg L, Morton CC, Ryan AF, Van Camp G, Smith RJ (2001) Mutations in the transcriptional activator EYA4 cause late-onset deafness at the DFNA10 locus. Hum Mol Genet 10(3):195–200
94. Weegerink NJD, Schraders M, Oostrik J, Huygen PLM, Strom TM, Granneman S, Pennings RJE, Venselaar H, Hoefsloot LH, Elting M, Cremers CWRJ, Admiraal RJC, Kremer H, Kunst HPM (2011) Genotype-phenotype correlation in DFNB8/10 families with TMPRSS3 mutations. J Assoc Res Otolaryngol 12(6):753–766
95. Weiss S, Gottfried I, Mayrose I, Khare SL, Xiang M, Dawson SJ, Avraham KB (2003) The DFNA15 deafness mutation affects POU4F3 protein stability, localization, and transcriptional activity. Mol Cell Biol 23(22):7957–7964
96. Wu CC, Hung CC, Lin SY, Hsieh WS, Tsao PN, Lee CN, Su YN, Hsu CJ (2011) Newborn genetic screening for hearing impairment: a preliminary study at a tertiary center. PLoS One 6(7):e22314
97. Wu C-C, Lee Y-C, Chen P-J, Hsu C-J (2008) Predominance of genetic diagnosis and imaging results as predictors in determining the speech perception performance outcome after cochlear implantation in children. Arch Pediatr Adolesc Med 162(3):269–276
98. Yao GD, Shou-Xia LI, Chen DL, Feng HQ, Zhao SB, Liu YJ, Guo LL, Yang ZM, Zhang XF, Sun CX (2014) Combination of hearing screening and genetic screening for deafness-susceptibility genes in newborns. Exp Ther Med 7(1):218–222

99. Yoshinagaitano C, Sedey AL, Coulter DK, Mehl AL (1998) Language of early- and later-identified children with hearing loss. Pediatrics 102(5):1161–1171
100. Young NM, Reilly BK, Burke L (2016) Limitations of universal newborn hearing screening in early identification of pediatric cochlear implant candidates. Arch Otolaryngol Head Neck Surg 137(3):230
101. Zhang J, Wang P, Han B, Ding Y, Pan L, Zou J, Liu H, Pang X, Liu E, Wang H (2013) Newborn hearing concurrent genetic screening for hearing impairment-a clinical practice in 58,397 neonates in Tianjin, China. Int J Pediatr Otorhinolaryngol 77(12):1929–1935
102. Zhang Z, Ding W, Liu X, Xu B, Wan D, Nan S, Guo Y (2012) Auditory screening concurrent deafness predisposing genes screening in 10,043 neonates in Gansu province, China. Int J Pediatr Otorhinolaryngol 76(7):984–988

Chapter 6
Protection of Spiral Ganglion Neurons and Prevention of Auditory Neuropathy

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Abstract In the auditory system, the primary sensory neurons, spiral ganglion neurons (SGNs), transmit complex acoustic information from hair cells to the second-order sensory neurons in the cochlear nucleus for sound processing, thus building the initial bridge between the physical world of sound and the perception of that sound. Cochlear SGN loss causes irreversible hearing impairment because this type of neural cell cannot regenerate. A better understanding of the molecular mechanisms of formation, structure, degeneration, and protection of SGNs will help to design potential therapeutic strategies for preservation and replacement of them in the cochlear implant recipient. In this review, we described and summarized the following about SGNs: (1) their cell biology and their peripheral and central connections, (2) mechanisms of their neuronal damage and their protection, and (3) the neural and synaptic mechanism of auditory neuropathy and current options for hearing rehabilitation from auditory neuropathy. The updates of the research progress and the significant issues on these topics were discussed.

Keywords Spiral ganglion neuron · Synapse · Neuronal damage · Auditory neuropathy · Cochlea implantation

6.1 Introduction

In mammals, the function of the auditory system relies on two neurosensory tissues: one is the organ of Corti, which is consist of sensory hair cells (HCs) and supporting cells, and the other is the sensory spiral ganglion neurons (SGNs), which project radial fibers to innervate the organ of Corti and also project auditory nerve tonotopically to the auditory brainstem. The bipolar SGNs delaminate from the growing

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cochlear duct around embryonic day (E) 10 and migrate to Rosenthal's canal. Postnatally, SGNs elaborate projections to HCs by postnatal day (P) P0 and refine them during the first few postnatal weeks [14, 23, 24], and the cochlea HCs are finally innervated by SGNs with distinct synaptic organization. This unique organization establishes a point-to-point communication between the cochlea HCs and the cochlear nucleus [53]. Early studies have been focused on the questions in relation to the survival, neurite sprouting, neurite growth, pathfinding, and synaptogenesis of SGN [10, 53, 93], while later progress has begun to disclose the biological mechanisms underlying the regulation of neural survival, growth, and function of SGNs. Recently, however, the work in auditory neuron research has taken on a new significance, which is aimed at slowing the death of SGNs and stimulating the neurite regeneration [11, 48], for the purpose of improving auditory prostheses and/or reengineering damaged cochleas. In this paper, we reviewed firstly the molecular basis of sensory neuron formation, including migration of SGNs and initial fiber growth to the organ of Corti, followed by the new data collected on the role of neurotrophins (NTs) in pathfinding, neuronal survival, and maintenance of connections.

6.2 The Spiral Ganglion: Connecting the Peripheral and Central Auditory Systems

In the course of sound transmission, the HCs convert mechanical energy into bioelectricity, and the auditory nerve generates nerve impulses and transmits to the intracranial auditory center along the synapses, and then the auditory center excites to produce subjective feelings. The SGN is the first-order neuron on the auditory conduction pathway. In the inner ear, the somata of SGNs are resided in the Rosenthal's canal, which is formed by latticework of bone spirals around in parallel with the coiled labyrinth. Each cell body of SGNs gives rise to a peripheral process that extends toward the organ of Corti and a central process that collects together to form the auditory nerve emitting into the brain, thus building a bridge between perception of the sound and the physical world of sound.

Human SGNs are divided into two types, type I and type II; each type has its own characteristics. Their key differences are the basis of somatic size, relative abundance, cytologic traits, and characteristics of the central and peripheral processes [78, 84]. Type I SGNs are large cell bodies and bipolar neuritis. The cytoplasm of them is characterized by the plentiful ribosomes, cisternae of endoplasmic reticulum, and Golgi bodies [64, 79]. Type I neurons are mainly associated with the inner hair cells (IHCs) of Corti by means of synapses. Despite SGNs coupled with IHCs in one-to-one pattern, each IHC is innervated by 5–30 type I SGNs monosynaptically that transmit sound information to the cochlea nucleus. Type I SGNs account for 90–95% of the total, and considering the significantly greater number of their fibers, it is assumed that they and IHCs transduce the majority of all auditory input into the brain [10]. Classic work by Liberman et al. in cat further subdivided type I

SGNs into two types based on where they terminate on IHCs [40]. However, the latest researches demonstrated that mouse type I SGNs comprising three subtypes which express unique combinations of calcium (Ca^{2+}) binding proteins, ion channel regulators, guidance molecules, and transcription factors [76, 83]. Like hair cells, type I SGNs are organized tonotopically, and thus differing in sensitivity to sound, the most sensitive frequency of each SGN is coincided with its relative position along the cochlear partition. Besides, type I SGNs also has variable spontaneous rates (SRs) that are inversely correlated with their threshold to sound and dynamic range [39, 68]. Based on the relation between threshold and SR in cats, Liberman et al. have classified SGNs into three classes: high-SR (> 18 spikes/s), medium-SR (0.5–18 spikes/s), and low-SR (< 0.5 spikes/s) fibers. In rodents, single IHCs appear to be innervated by SGN fibers with different SRs [42, 94]; such diversity enables the wide dynamic range of sound intensities encoded in the cochlea and helps maintain hearing in noisy environments [41, 90].

Compared with the axons of type I SGN, type II spiral ganglion axons are shorter [8], the body is small, and there is more variability in their shape either as bipolar or psuedounipolar [29]. The cytoplasm of type II SGNs is highly filamentous and lacks the usual organelles. Morphologically, type II SGNs actually resemble pain-sensing sensory fibers from the dorsal root ganglion [38, 53], they emit central projections to the granule cell layer in the cochlear nucleus and peripheral projections to the outer hair cells (OHCs) by way of synapses, and each OHC is innervated by 2–5 type II SGNs [24, 45, 100]. Type II SGNs only account for 5–10% of the total. The axons of both type I SGNs and type II SGNs ascend into the cochlear nucleus and bifurcate (Fig. 6.1). The bifurcation creates an ascending branch that projects through the anteroventral cochlear nucleus, and a descending branch that passes through the posteroventral cochlear nucleus to terminate in the dorsal cochlear nucleus.

Although we are just starting to understand how different SGN subtypes are produced during development, recent studies including physiological and molecular researches have recognized significant changes in SGNs that correlate with longitudinal position accounting for the known differences in SR, and more importantly, the elucidation of the range, nature, and origins of SGN diversity complemented the etiology of hearing loss and may be implicated in its treatment [1].

6.3 Mechanisms of Cochlear Neuronal Damage

The survival of cochlear neurons depends at least partially on the support of neurotrophic factors provided by the HCs. Loss of SGNs are frequently observed secondary to HC loss. Recent evidence from ototoxic and age-related sensory neural hearing loss (SNHL) has suggested that SGN damage or loss can also occur in the absence of HC damage [89]. One reason of this is considered to be neurotropic viruses, but the evidence is difficult to obtain. However, an established mechanism of noise-induced cochlear neuronal damage is the excessive release of the

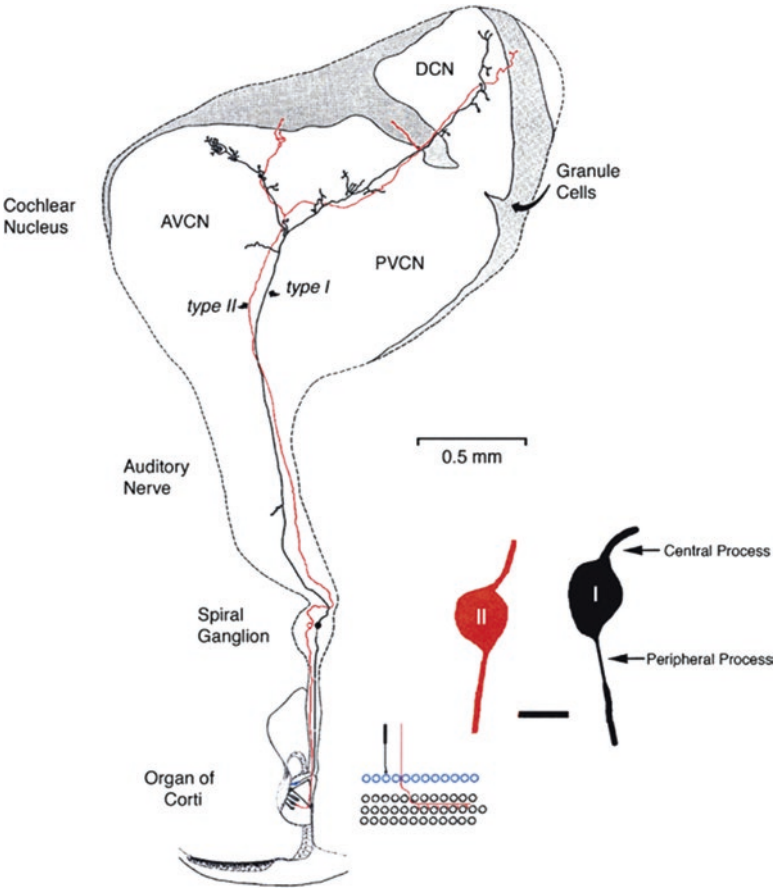

Fig. 6.1 Schematic drawing of SGNs and their central and peripheral terminations. The representative type I SGN (black) innervates a single IHC and projects topographically into the cochlear nucleus. The representative type II SGN (red) has a similar central projection mode but with additional terminations in the granule cell domain. Abbreviations: *AVCN* anteroventral cochlear nucleus, *DCN* dorsal cochlear nucleus, *PVCN* posteroventral cochlear nucleus. (Adapted from [8])

excitatory neurotransmitter glutamate from the inner HC [19, 57, 86]. When the cochlear glutamate cycle is impaired, the glutamate receptors on the postsynaptic membrane will be overactivated after glutamate accumulation. When the AMPA receptors are overactivated, it causes a large influx of Na^+, and then Cl^- and water molecules passively flow in, leading to edema and even death of the afferent nerve fibers of the cochlea. In addition, the overactivation of NMDA receptors leads to Ca^{2+} overload in the afferent nerves of the cochlea, which in turn produces intracellular osmotic imbalance and cytotoxic damage, causing postsynaptic nerve fibers swelling, even degeneration and apoptosis [58, 88]. Steinbach et al. reported that the damage or loss of cochlear neurons caused by the toxicity of glutamate is

mainly related to the glutamate-induced apoptosis [81]. This process has been applied by administration of exogenous glutamate receptor antagonist to block potential generation and excitotoxicity in the guinea pig cochlea [66]. Harris et al. reported a unique case in which cisplatin-associated hearing loss occurred after some years of successful cochlear implant use, and the patient subsequently lost the benefit from the device following cisplatin therapy [21]. This case study shows that structures such as spiral ganglion may be affected by cisplatin ototoxicity and that impairment is not limited to OHCs. Given the experience of SNHL and cochlear implant (CI) placement, it can be speculated that the changes demonstrated in this patient's hearing are highly unlikely to be related to any further damage to the OHCs resulting from cisplatin. Conversely, changes in hearing are most likely to directly represent the damage of SGNs.

Loss of many afferent IHC synapses and degeneration of type I SGNs have been implicated as contributors to noise-induced hearing loss [30]. Indeed, certain noise exposure can lead to a temporary threshold shift but a persistent substantial decrease of compound action potential (CAP) amplitudes in SGNs. This form of impairment is thought to reduce the ability to process and analyze auditory inputs [59]. Cellular mechanisms of noise-induced SGN degeneration are less clearly known. However, several studies have shown that damage and loss of synapses after noise might reflect the impairment of neurotrophic signals in Corti organs and synaptic loss could be reduced by the expression of virus-mediated neurotrophic factors in the mouse cochlea. For example, Wan et al. found that NT-3 could regulate the ribbon synapse density and induce the regeneration of synapse after acoustic trauma in the cochlea [87]. Interestingly, it has also been reported that the reduced activity and numbers of afferent synapses on IHCs, as well as the reduction of noise-induced loss of the remaining synapses could occur after conditional deletion of BDNF in HCs and SGNs [102]. These data indicate that neurotrophic support plays a critical role in maintaining the function and survival of afferent synapse in cochlea.

Aging in mice could also result in the loss of cochlea synapses, which is accelerated if animals were exposed to "non-damaging" noise before [31, 71, 80]. Currently, the effects of excitatory toxicity on synaptic damage in hearing loss have only been studied in animal models. Hence, we do not yet know the relationship between excitotoxic synapse loss and noise-induced and age-related hearing loss in human with certainty.

The use of therapeutic ototoxic drugs, such as aminoglycoside antibiotics, ouabain, and cisplatin, could cause loss of SGNs, leading to permanent SNHL in mammals. Cochlear neurons can produce reactive oxygen species (ROS) and undergo apoptosis after drug ototoxicity [15, 27, 63]. The oxidative imbalance initiates an oxidative stress response that causes oxidative damage to the SGNs, resulting in decreased hearing. Activation of the c-Jun *N*-terminal kinase signaling pathway is also implicated in SGNs apoptosis in response to oxidative stress [27]. In addition, the apoptotic pathway can be abnormally activated following the damage to cochlea, which causes normal functioning SGNs to die. The Bcl-2, caspase, and Apaf-1/ced-4 families constitute the central apoptotic machinery in neurons and many other cell types. Apoptosis can occur through the caspase-mediated sequential actions,

which are initiated by their associated intrinsic and extrinsic pathways [97]. There are also caspase-independent processes that lead to apoptosis, mediated by other factors including receptor-interacting serine/threonine-protein kinase 1 or AIF [43].

In recent years, researches on the genetics of SNHL have made rapid progress. To date, it has been identified that more than 64 genes and 125 loci are linked to the hearing impairment in various degrees [5, 13]. Some of these genes, such as SLC17A8, PJVK, and DIAPH3, are shown to play an important role in the regulation of synaptic transmission and neuronal survival and death. Primary SGN degeneration is more likely to occur after deficiency of these genes. In addition, the animal studies with gene defects have also revealed some transcription factors, for example, nuclear factor κB and forkhead box O3, that play vital roles in regulating the IHC synapse and maintaining the survival of SGNs and normal function of the auditory nerve [17, 36].

6.4 Repair and Protection of Spiral Ganglion Neuron

It has been reported that the progressive degeneration of SGNs following SNHL could reduce the effectiveness of hearing aid devices, including CI [20]. To prevent or reverse SGN degeneration may carry critical implications for CIs to patients with hearing loss and improve the restorement of auditory function.

The loss of neural activity and neurotrophic support after HC damage is one factor in the degradation of SGNs [4, 33]. Therefore, it is not surprising that using electrical stimulation (ES) to induce the neural activity and application of exogenous NT to these neurons were attempted to rescue SGNs. Neurotrophins, such as brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), and neurotrophin-3 (NT3), are known to play a vital role in the development of nervous system, the maintenance of normal physiological function, and the repair of nerve damage [65]. Exogenous NTs have been shown to rescue SGNs from degeneration, but the effects are limited by the source of NTs [2, 3, 91]. NT gene therapy, which transfected the cells in cochlea with genes that enable them to produce NTs, is one potential strategy to provide a long-term NTs support for SGNs. Previous studies have reported that injection of viral vectors for expression of NT genes into the scala tympani compartment or scala media compartment of the cochlea leads to the protection of SGNs from deafness-induced degeneration [9, 51, 75, 92]. However, the effectiveness of NT gene therapy may depend on the activity of the targeted cochlea cells for viral transfection [92]. It has been shown that the use of chronic ES to cochlear after HC loss could reduce the irreversible damage of SGNs [47]. Significantly, studies have demonstrated enhanced SGN survival in deafened cochleae treated with both exogenous NTs and ES [28, 73]. In addition, Shepherd et al. reported that chronic ES could prevent the rapid loss of SGNs once exogenous NTs were exhausted [74].

Over the past decades, a variety of molecules have been experimentally applied to protect SGNs. For example, Lallemend et al. reported that substance P, an unde-

capeptide belonging to a class of neuropeptides, could protect SGNs from apoptosis by an inhibition of caspase activation [34]. CEP-1347, a derivative of the indolocarbazole K252a, is an inhibitor of JNK activation that has been shown to rescue neuron from degeneration [55]. It has also been reported that PKC activators could promote the survival and neurite outgrowth of SGNs through PI3K/Akt and MEK/ERK pathways [35]. Recently, a number of small-molecule tyrosine receptor kinase B receptor agonists, such as 7,8-dihydroxyflavone and 7,8,3-trihydroxyflavone, have been developed and shown to protect SGN from degeneration with high potency [26, 85, 98]. Moreover, Liu et al. reported an interesting finding that Wnt signaling could activate TP53-induced glycolysis and apoptosis regulator, inhibit oxidative stress and apoptosis of SGN, and protect SGNs from cisplatin-induced damage. This study might provide a new therapeutic target for the repair and protection of SGNs [44].

In addition, stem cell replacement therapy is an important candidate for the treatment of auditory neurological disorders. Currently, some researchers focus on a viable method of developing stem cell transplantation to restore the neural elements that have degenerated or dead due to hearing loss and ultimately achieve the purpose of treating neurological deafness. As far as is known, some kinds of stem cells, such as bone marrow-derived mesenchymal stem cells [72, 82], embryonic stem cells (ESCs) [12], neural stem cells (NSCs) [22], induced pluripotent stem cells [25], and inner ear stem cells [37], have been studied in this regard and proved to protect and repair the damaged SGNs in varying degrees. However, the use of NSCs and ESCs is subject to various ethical and logistical constraints. Recently, adult peripheral tissues are shown to provide a source of stem and progenitor cells that are more easily studied and alternative. It has been reported that adipose tissue-derived stem cells may be induced into neural stem cell-like cells and functional neural cells in vitro [101]. Though there are still many problems that need to be resolved in stem cell replacement therapy, with a short survival time for the grafts transplanted into the inner ear and unestablished functional connection between differentiated neurons and host cochlear neurons, these all studies still have taken us one step closer to the effective translation of basic SGN neuroprotective research into clinical practice.

6.5 Neural and Synaptic Mechanisms of Auditory Neuropathy

Auditory neuropathy (AN) was first named in 1996 to define a group of individuals with auditory symptoms. Although these individuals retain the function of sensory transduction and the amplification of OHCs, they are commonly accompanied by hearing damage caused by the abnormal neural coding of sound stimulation. Today the most common denomination is auditory neuropathy/dyssynchrony (AN/AD) [49, 77]. Amount of studies about the neural and synaptic mechanisms of AN have

been conducted over the last two decades. Recently, the disease mechanisms that include loss of IHCs or IHC synapses, impaired synaptic transmission to SGNs, and disrupted propagation of auditory information along the auditory nerve have been elucidated. Actually, auditory synaptopathy and auditory neuropathy have been named to define the disease that derived separately from synaptic and neural defects.

The synaptic ribbon is an electron-intensive specialization that binds dozens of synaptic vesicles together. It is highly specialized, achieving a tireless afferent conduction with a frequency of a few hundred Hertz and a time accuracy of milliseconds [46, 50] (Fig. 6.2). As the first identified major unconventional synaptic protein that regulating exocytosis at the IHC synapse, the multi-C2-domain protein otoferlin is employed by HCs and operates in the active zone of the mature IHC ribbon synapses [54]. Besides, the IHC synapse vesicular glutamate uptake is mediated by VGluT3 [70], and Ca^{2+} signaling involves the CaV1.3 L-type Ca^{2+} channel [56]. As a result, Gene defects encoding the otoferlin, VGluT3, and the Ca^{2+} channel complex cause human auditory synaptopathy. In addition, mutations in optic atrophy 1 among individuals with syndromic dominant optic atrophy result in the neuropathic hearing impairments, and mutations of MPZ and DFNB59 gene can also cause genetic ANs.

In vivo extracellular recordings of SGN action potentials in response to sound among different model animals have provided important insight for the role of SGNs in sound encoding [16]. One or few spikes triggering are fired upon SGNs even in response to sustained currents [67]. SGNs are electrically "tight" cells and show large excitatory postsynaptic currents, which can achieve high-frequency transmission [18]. As a result, loss of IHC transmitter release, spike generation, or spike propagation damage accompanied by SGNs or SGNs transmission and

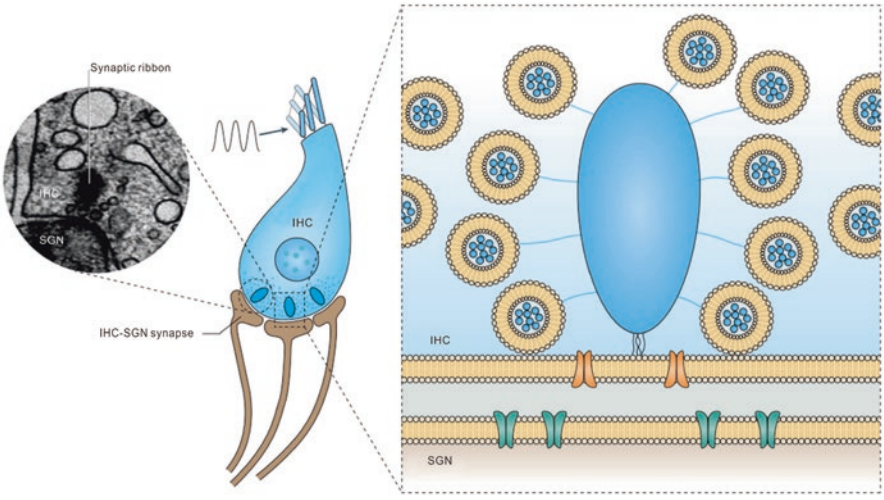

Fig. 6.2 The afferent ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) [49]. The ribbon synapses are highly specialized, achieving a tireless afferent conduction with a frequency of a few hundred Hertz and a time accuracy of milliseconds

changes in synaptic function of SGNs all influence the transmission of sound signals from cochlear to the brain. The following loss of time accuracy (or synchronization) and inaccurate neurological performance of the auditory signal are considered to be key disease mechanisms of neuropathy.

Disorders of auditory nerve function can occur as postsynaptic mechanisms of AN, which include axonal neuropathies, auditory ganglion cell disorders and myelin disorders, hypoplasia of auditory nerve, and auditory nerve conduction disorders [61]. Among these defects, demyelination disorders can be accompanied by axonal damage [95], and the loss of nerve fibers and ganglion cells happens concomitant with a reduction in the amplitude of synchronous input. Besides, the number, size, and position of auditory nerve terminals relative to the HCs vary systematically along the basilar membrane. Pathology affecting the dendritic nerve terminals results in a certain pattern of objective measures similar to ribbon synapse disorders [69].

6.6 Current Options for Hearing Rehabilitation from Auditory Neuropathy

With regard to the intervention effect of AN patients, the mainstream opinion is that AN patients can benefit from hearing aids and CI, but the expected benefit is less than that of sensorineural hearing loss patients, and the effect of drug therapy for AN is lack of definite evidence.

The current literature confirms that although the traditional hearing aid has a certain effect on hearing improvement in children with AN, it cannot solve the obstacle of speech recognition, and the individual variation of therapeutic effect is relatively great [60]. Some researchers believe that the traditional sound amplifier can only provide noisy and distorted signals for AN patients [7], which does not give AN patients effective hearing rehabilitation; conversely, this inappropriate high-gain sound amplification can cause the patient's secondary noise induced hearing loss [32]. According to the pathological mechanism and audiological characteristics of AN patients, researchers have proposed new algorithms or new techniques to adapt to the hearing aids rehabilitation of AN patients [99], but the results are in the primary stage. Narne et al. have studied the time domain envelope enhancement in improving the speech recognition ability of AN patients [52]; the results show that the technology is helpful to improve speech recognition in some AN patients. The FM system is also believed to play a role in improving rehabilitation for some AN patients. This kind of AN patients still have certain speech recognition ability in quiet environment, but it is difficult to identify speech in noisy condition. For such patients, if the background noise is interfered by the FM system, the residual speech recognition ability can be fully utilized [62].

Cochlear implantation is the only definitely testified effective treatment for AN patients. Theoretically, the mechanism of CI to improve hearing and speech recog-

nition in AN patients is that if the lesion site is in IHC or synapse, CI can bypass this part of the afferent pathway and directly stimulate the auditory neuron cell body or axon; if the lesion is in the trunk of the nerve, a better synchronous electrical signal can be produced by CI electrical stimulation, and the problem of auditory nerve synchronization is improved, which can be confirmed by EABR. On the other hand, if the lesion mechanism is the neuron deletion, then the effect of CI is not ideal. In fact, the main problem now is that there is no definite detection index to determine the lesion site before the operation, so that the effect of CI is still not clear. Preoperative imaging examinations such as auditory canal neuroimaging in MRI [6] and EABR may play a role in suggesting the lesion location of the AN patients and predicting the effect of implantation.

In addition to hearing aids and CI, the current drug treatment AN is still in the exploratory stage; the effect is not yet reported. Studies have shown that AN may be closely related to nerve demyelination; therefore, the validation of anti-demyelinating drugs has also become a possible solution [96].

In summary, the current understanding and intervention methods for the pathogenesis of AN are still at an early stage. In the future, it is necessary to further study the location and mechanism of AN, and explore the clinical feasible location diagnosis methods (such as gene diagnosis, electrophysiological diagnosis, psychophysical diagnosis, etc.), which is of great importance to the formulation of auditory intervention strategies and the prediction of auditory speech rehabilitation effect in patients with AN.

6.7 Conclusion

In spite of the research progress of SGNs, analyzing and understanding the biological mechanisms in SGNs are slow. The study is limited because of several reasons such as the anatomy inaccessible of SGNs within the bony Rosenthal's canal, the limited SGN numbers as about 10,000 in the mouse and about 30–40,000 in the human, the necessary labor-intensive histologic and anatomical procedures for SGN analysis, etc. Although mechanisms of the neuronal damage and protection have been explored in the past decades, the survival and neurite regeneration mechanisms of SGN have not been clearly elucidated. In order to promote the study of SGNs, alternative and complementary experimental methods have to be established to reduce the large numbers of animals in doing and analyzing potential mechanisms of SGN survival and regeneration. Furthermore, it is also especially important to implement broader discoveries on SGNs such as large-scale screens of genes and chemicals contributing to finding candidate mechanisms which may be manipulable with pharmaceutical, genetic, or other interventions. As biochemical, molecular, and imaging approaches become more advanced, the limitations mentioned above about the SGN research will no longer restrict findings of SGN and will lend insight into the etiology and mechanisms responsible for the SGN survival, damage, and regeneration, which may, in turn, offer novel effective therapeutic targets to SNHL.

References

1. Adamson CL, Reid MA, Davi RL (2002) Opposite actions of brain-derived neurotrophic factor and neurotrophin-3 on firing features and ion channel composition of murine spiral ganglion neurons. J Neurosci 22(4):1385–1396
2. Agterberg M, Versnel HG, Jc SG, Albers F, Klis S (2008) Morphological changes in spiral ganglion cells after intracochlear application of brain-derived neurotrophic factor in deafened guinea pigs. Hear Res 244(2):25–34
3. Agterberg MJ, Versnel H, Dijk LM, Groot JC, Klis SF (2009) Enhanced survival of spiral ganglion cells after cessation of treatment with brain-derived neurotrophic factor in deafened Guinea Pigs. Jaro J Assoc Res Otolaryngol 10(3):355–367
4. Alam SA, Robinson BK, Huang J, Green SH (2007) Prosurvival and proapoptotic intracellular signaling in rat spiral ganglion neurons in vivo after the loss of hair cells. J Comp Neurol 503(6):832–852. https://doi.org/10.1002/cne.21430
5. Angeli S, Lin X, Liu XZ (2012) Genetics of hearing and deafness. Anat Rec (Hoboken) 295(11):1812–1829. https://doi.org/10.1002/ar.22579
6. Bardley J, Beale T, Graham J, Bell M (2008) Variable long-term outcomes from cochlear implantation in children with hypoplastic auditory nerve. Cochlea Implants Int 9:34–35
7. Berlin CI (1999) Auditory neuropathy:using OAEs and ABRs from screening to management. Semin Hear 20:307–308
8. Brown MC, Berglund AM, Kiang NY, Ryugo DK (1988) Central trajectories of type II spiral ganglion neurons. J Comp Neurol 278(4):581–590
9. Chikar JA, Colesa DJ, Swiderski DL, Polo AD, Raphael Y, Pfingst BE (2008) Over-expression of BDNF by adenovirus with concurrent electrical stimulation improves cochlear implant thresholds and survival of auditory neurons. Hear Res 245(1):24–34
10. Coate TM, Kelley MW (2013) Making connections in the inner ear: recent insights into the development of spiral ganglion neurons and their connectivity with sensory hair cells. Semin Cell Dev Biol 24(5):460–469
11. Conde de Felipe MM, Feijoo Redondo A, García-Sancho J, Schimmang T, Durán Alonso MB (2011) Cell- and gene-therapy approaches to inner ear repair. Histol Histopathol 26(7):923–940
12. Corrales CE, Pan L, Li H, Liberman MC, Heller S, Edge ASB (2006) Engraftment and differentiation of embryonic stem cell–derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of corti. Dev Neurobiol 66(13):1489–1500
13. Dror AA, Avraham KB (2010) Hearing impairment: a panoply of genes and functions. Neuron 68(2):293–308. https://doi.org/10.1016/j.neuron.2010.10.011
14. Echteler SM (1992) Developmental segregation in the afferent projections to mammalian auditory hair cells. Proc Natl Acad Sci U S A 89:6324–6327
15. Fu Y, Ding D, Wei L, Jiang H, Salvi R (2013) Ouabain-induced apoptosis in cochlear hair cells and spiral ganglion neurons in vitro. Biomed Res Int 2013:628064. https://doi.org/10.1155/2013/628064
16. Geisler CD (1998) From sound to synapse: physiology of the mammalian ear. Oxford University Press, New York
17. Gilels F, Paquette ST, Zhang J, Rahman I, White PM (2013) Mutation of Foxo3 causes adult onset auditory neuropathy and alters cochlear synapse architecture in mice. J Neurosci 33(47):18409–18424. https://doi.org/10.1523/jneurosci.2529-13
18. Glowatzki E, Fuchs PA (2002) Transmitter release at the hair cell ribbon synapse. Nat Neurosci 5:147–154
19. Hackney CM, Osen KK, Ottersen OP, Storm-Mathisen J, Manjaly G (1996) Immunocytochemical evidence that glutamate is a neurotransmitter in the cochlear nerve: a quantitative study in the guinea-pig anteroventral cochlear nucleus. Eur J Neurosci 8(1):79–91

20. Hardie NA, Shepherd RK (1999) Sensorineural hearing loss during development: morphological and physiological response of the cochlea and auditory brainstem. Hear Res 128(1–2):147–165
21. Harris MS, Gilbert JL, Lormore KA, Musunuru SA, Fritsch MH (2011) Cisplatin ototoxicity affecting cochlear implant benefit. Otol Neurotol 32(6):969–972. https://doi.org/10.1097/MAO.0b013e3182255893
22. He Y, Zhang PZ, Sun D, Mi WJ, Zhang XY, Cui Y et al (2014) Wnt1 from cochlear schwann cells enhances neuronal differentiation of transplanted neural stem cells in a rat spiral ganglion neuron degeneration model. Cell Transplant 23(6):747–760
23. Huang LC, Thorne PR, Housley GD, Montgomery JM (2007) Spatiotemporal definition of neurite outgrowth, refinement and retraction in the developing mouse cochlea. Development 134:2925–2933
24. Huang LC, Barclay M, Lee K, Peter S, Housley GD, Thorne PR et al (2012) Synaptic profiles during neurite extension, refinement and retraction in the developing cochlea. Neural Dev 7:38
25. Ishikawa M, Ohnishi H, Skerleva D, Sakamoto T, Yamamoto N, Hotta A et al (2017) Transplantation of neurons derived from human iPS cells cultured on collagen matrix into guinea-pig cochleae. J Tissue Eng Regen Med 11(6):1766–1778
26. Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, Liu Y et al (2010) A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. Proc Natl Acad Sci U S A 107(6):2687–2692
27. Jeong SW, Kim LS, Hur D, Bae WY, Kim JR, Lee JH (2010) Gentamicin-induced spiral ganglion cell death: apoptosis mediated by ROS and the JNK signaling pathway. Acta Otolaryngol 130(6):670–678. https://doi.org/10.3109/00016480903428200
28. Kanzaki S, Stöver T, Kawamoto K, Prieskorn DM, Altschuler RA, Miller JM et al (2002) Glial cell line-derived neurotrophic factor and chronic electrical stimulation prevent VIII cranial nerve degeneration following denervation. J Comp Neurol 454(3):350–360
29. Kiang NY, Rho JM, Northrop CC, Liberman MC, Ryugo DK (1982) Hair-cell innervation by spiral ganglion cells in adult cats. Science 217(4555):175–177
30. Kujawa SG, Liberman MC (2009) Adding insult to injury: cochlear nerve degeneration after "Temporary" noise-induced hearing loss. J Neurosc Off J Soc Neurosci 29(45):14077–14085
31. Kujawa SG, Liberman MC (2015) Synaptopathy in the noise-exposed and aging cochlea: primary neural degeneration in acquired sensorineural hearing loss. Hear Res 330(Pt B):191–199
32. Kundu P, Rout N (2010) The impact of high gain conventional hearing aid on OAEs in a case of auditory neuropathy/dys-synchrony. East J Med 15:15–16
33. Ladrech S, Guitton M, Saido T, Lenoir M (2004) Calpain activity in the amikacin-damaged rat cochlea. J Comp Neurol 477(2):149–160. https://doi.org/10.1002/cne.20252
34. Lallemend F, Lefebvre PP, Hans G, Rigo JM, Tr VDW, Moonen G et al (2003) Substance P protects spiral ganglion neurons from apoptosis via PKC-Ca2+-MAPK/ERK pathways. J Neurochem 87(2):508–521
35. Lallemend F, Hadjab S, Hans G, Moonen G, Lefebvre PP, Malgrange B (2005) Activation of protein kinase CbetaI constitutes a new neurotrophic pathway for deafferented spiral ganglion neurons. J Cell Sci 118(19):4511–4525
36. Lang H, Schulte BA, Zhou D, Smythe N, Spicer SS, Schmiedt RA (2006) Nuclear factor κB deficiency is associated with auditory nerve degeneration and increased noise-induced hearing loss. J Neurosci 26(13):3541–3550. https://doi.org/10.1523/jneurosci.2488-05.2006
37. Li H, Liu H, Heller S (2003) Pluripotent stem cells from the adult mouse inner ear. Nat Med 9(10):1293–1299
38. Li L, Rutlin M, Abraira VE, Cassidy C, Kus L, Gong S et al (2011) The functional organization of cutaneous low-threshold mechanosensory neurons. Cell 147(7):1615–1627
39. Liberman MC (1978) Auditory-nerve response from cats raised in a lownoise chamber. J Acoust Soc Am 63:442–455

40. Liberman MC (1982) Single-neuron labeling in the cat auditory nerve. Science 216(4551):1239–1241
41. Liberman MC (2017) Noise-induced and age-related hearing loss: new perspectives and potential therapies. F1000Res 6:927
42. Liberman LD, Wang H, Liberman MC (2011) Opposing gradients of ribbon size and AMPA receptor expression underlie sensitivity differences among cochlear-nerve/hair-cell synapses. J Neurosci 31:801–808
43. Liu W, Fan Z, Han Y, Zhang D, Li J, Wang H (2012) Intranuclear localization of apoptosis-inducing factor and endonuclease G involves in peroxynitrite-induced apoptosis of spiral ganglion neurons. Neurol Res 34(10):915–922. https://doi.org/10.1179/1743132812y.0000000098
44. Liu W, Xu X, Fan Z, Sun G, Han Y, Zhang D et al (2018) Wnt signaling activates TP53-induced glycolysis and apoptosis regulator and protects against cisplatin-induced spiral ganglion neuron damage in the mouse Cochlea. Antioxid Redox Signal. https://doi.org/10.1089/ars.2017.7288
45. Martinez-Monedero R, Liu C, Weisz C, Vyas P, Fuchs PA, Glowatzki E (2016) GluA2-containing AMPA receptors distinguish ribbon-associated from ribbonless afferent contacts on rat cochlear hair cells. eNeuro 3(2):11080–11085
46. Matthews G, Fuchs P (2010) The diverse roles of ribbon synapses in sensory neurotransmission. Nat Rev Neurosci 11:812–822
47. Miller JM, Miller AL, Yamagata T, Bredberg G, Altschuler RA (2002) Protection and regrowth of the auditory nerve after deafness: neurotrophins, antioxidants and depolarization are effective in vivo. Audiol Neurootol 7(3):175–179. https://doi.org/10.1159/000058306
48. Mohammadian F, Eatemadi A, Daraee H (2017) Application of stem cell for the regeneration of spiral ganglion neurons. Cell Mol Biol 63(1):6–12
49. Moser T, Starr A (2016) Auditory neuropathy–neural and synaptic mechanisms. Nat Rev Neurol 12:135–149
50. Moser T, Predoehl F, Starr A (2013) Review of hair cell synapse defects in sensorineural hearing impairment. Otol Neurotol 34:995–1004
51. Nakaizumi T, Kawamoto K, Minoda R, Raphael Y (2004) Adenovirus-mediated expression of brain-derived neurotrophic factor protects spiral ganglion neurons from ototoxic damage. Audiol Neurotol 9(3):135–143
52. Narne VK, Vanaja CS (2009) Perception of speech with envelope enhancement in individuals with auditory neuropathy and simulated loss of temporal modulation processing. Int J Audiol 48:700–701
53. Nayagam BA, Muniak MA, Ryugo DK (2011) The spiral ganglion: connecting the peripheral and central auditory systems. Hear Res 278(1–2):2–20
54. Pangrsic T, Lasarow L, Reuter K, Takago H, Schwander M, Riedel D et al (2010) Hearing requires otoferlin-dependent efficient replenishment of synaptic vesicles in hair cells. Nat Neurosci 13:869–876
55. Pirvola U, Xingqun L, Virkkala J, Saarma M, Murakata C, Camoratto AM et al (2000) Rescue of hearing, auditory hair cells, and neurons by CEP-1347/KT7515, an inhibitor of c-Jun N-terminal kinase activation. J Neurosci 20(1):43–50
56. Platzer J, Engel J, Schrott-Fischer A, Stephan K, Bova S, Chen H et al (2000) Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca2+ channels. Cell 102:89–97
57. Pouyatos B, Morel G, Lambert-Xolin AM, Maguin K, Campo P (2004) Consequences of noise- or styrene-induced cochlear damages on glutamate decarboxylase levels in the rat inferior colliculus. Hear Res 189(1–2):83–91
58. Pujol R, Puel JL (1999) Excitotoxicity, synaptic repair, and functional recovery in the mammalian cochlea: a review of recent findings. Ann N Y Acad Sci 884(1):249–254

59. Pujol R, Rebillard G, Puel JL, Lenoir M, Eybalin M, Recasens M (1990) Glutamate neurotoxicity in the cochlea: a possible consequence of ischaemic or anoxic conditions occurring in ageing. Acta Otolaryngol Suppl 476:32–36
60. Rance G, Barker EJ (2009) Speech and language outcomes in children with auditory neuropathy/dys-synchrony managed with either cochlear implants or hearing aids. Int J Audiol 48(6):313–320
61. Rance G, Starr A (2015) Pathophysiological mechanisms and functional hearing consequences of auditory neuropathy. Brain 138:3141–3158
62. Rance G, Corben LA, Du Bourg E, King A, Delatycki MB (2010) Successful treatment of auditory perceptual disorder in individuals with Friedreich ataxia. Neuroscience 171:552–553
63. Rathinam R, Ghosh S, Neumann WL, Jamesdaniel S (2015) Cisplatin-induced apoptosis in auditory, renal, and neuronal cells is associated with nitration and downregulation of LMO4. Cell Death Discovery 1:15052
64. Rosenbluth J (1962) The fine structure of acoustic ganglia in the rat. J Cell Biol 12:329–359
65. Rubel EW, Fritzsch B (2002) Auditory system development: primary auditory neurons and their targets. Annu Rev Neurosci 25(1):51–101
66. Ruel J, Bobbin RP, Vidal D, Pujol R, Puel JL (2000) The selective AMPA receptor antagonist GYKI 53784 blocks action potential generation and excitotoxicity in the guinea pig cochlea. Neuropharmacology 39(11):1959–1973
67. Rutherford MA, Chapochnikov NM, Moser T (2012) Spike encoding of neurotransmitter release timing by spiral ganglion neurons of the cochlea. J Neurosci 32:4773–4789
68. Sachs MB, Abbas PJ (1974) Rate versus level functions for auditory-nerve fibers in cats: tone-burst stimuli. J Acoust Soc Am 56:1835–1847
69. Santarelli R, Rossi R, Scimemi P, Cama E, Valentino ML, La Morgia C et al (2015) OPA1-related auditory neuropathy: site of lesion and outcome of cochlear implantation. Brain 138:563–576
70. Seal RP, Akil O, Yi E, Weber CM, Grant L, Yoo J et al (2008) Sensorineural deafness and seizures in mice lacking Vesicular Glutamate Transporter 3. Neuron 57:263–275
71. Sergeyenko Y, Lall K, Liberman MC, Kujawa SG (2013) Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. J Neurosci Off J Soc Neurosci 33(34):13686–13694
72. Sharif S, Nakagawa T, Ohno T, Matsumoto M, Kita T, Riazuddin S et al (2007) The potential use of bone marrow stromal cells for cochlear cell therapy. Neuroreport 18(4):351
73. Shepherd RK, Coco A, Epp SB, Crook JM (2005) Chronic depolarization enhances the trophic effects of brain-derived neurotrophic factor in rescuing auditory neurons following a sensorineural hearing loss. J Comp Neurol 486(2):145–158
74. Shepherd RK, Coco A, Epp SB (2008) Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss. Hear Res 242(1):100–109
75. Shibata SB, Cortez SR, Beyer LA, Wiler JA, Polo AD, Pfingst BE et al (2010) Transgenic BDNF induces nerve fiber regrowth into the auditory epithelium in deaf cochleae. Exp Neurol 223(2):464
76. Shrestha BR, Chia C, Wu L, Kujawa SG, Liberman MC, Goodrich LV (2018) Sensory neuron diversity in the inner ear is shaped by activity. Cell 174(5):1229–1246
77. Soares ID, Menezes PL, Carnauba AT, de Andrade KC, Lins OG (2016) Study of cochlear microphonic potentials in auditory neuropathy. Braz J Otorhinolaryngol 82:722–736
78. Spoendlin H (1971) Degeneration behaviour of the cochlear nerve. Arch Klin Exp Ohren Nasen Kehlkopfheilkd 200:275e291
79. Spoendlin H (1981) Differentiation of cochlear afferent neurons. Acta Otolaryngol 91(5–6):451–456
80. Stamataki S, Francis HW, Lehar M, May BJ, Ryugo DK (2006) Synaptic alterations at inner hair cells precede spiral ganglion cell loss in aging C57BL/6J mice. Hear Res 221(1):104–118
81. Steinbach S, Lutz J (2007) Glutamate induces apoptosis in cultured spiral ganglion explants. Biochem Biophys Res Commun 357(1):14–19

82. Sujeong J, Hyong-Ho C, Song-Hee K, Kyung-Hwa L, Yeoul JJ, Jong-Seong P et al (2015) Neural-induced human mesenchymal stem cells promote cochlear cell regeneration in deaf guinea pigs. Clin Exp Otorhinolaryngol 8(2):83–91
83. Sun S, Babola T, Pregernig G, So KS, Nguyen M, Su SM et al (2018) Hair cell mechanotransduction regulates spontaneous activity and spiral ganglion subtype specification in the auditory system. Cell 174(5):1247–1263
84. Thomsen E (1966) The ultrastructure of the spiral ganglion in the guinea pig. Acta Otolaryngol 63(Suppl. 224):442
85. Uluc K, Kendigelen P, Fidan E, Zhang L, Chanana V, Kintner D et al (2013) TrkB receptor agonist 7, 8 dihydroxyflavone triggers profound gender- dependent neuroprotection in mice after perinatal hypoxia and ischemia. CNS Neurol Disord Drug Targets 12(3):360–370
86. Verleye M, Steinschneider R, Fx GJ (2007) Moclobemide attenuates anoxia and glutamate-induced neuronal damage in vitro independently of interaction with glutamate receptor subtypes. Brain Res 1138(1):30–38
87. Wan G, Gómez-Casati ME, Gigliello AR, Liberman MC, Corfas G (2014) Neurotrophin 3 regulates ribbon synapse density in the cochlea and induces synapse regeneration after acoustic trauma. elife 3:e03564
88. Wang Y, Hirose K, Liberman MC (2002) Dynamics of noise-induced cellular injury and repair in the mouse Cochlea. J Assoc Res Otolaryngol Jaro 3(3):248–268
89. Wang J, Ding D, Salvi RJ (2003) Carboplatin-induced early cochlear lesion in chinchillas. Hear Res 181(1–2):65–72
90. Winter IM, Robertson D, Yates GK (1990) Diversity of characteristic frequency rate-intensity functions in guinea pig auditory nerve fibres. Hear Res 45:191–202
91. Wise AK, Richardson R, Hardman J, Clark G, O'Leary S (2005) Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3. J Comp Neurol 487(2):147–165
92. Wise AK, Hume CR, Flynn BO, Jeelall YS, Suhr CL, Sgro BE et al (2010) Effects of localized neurotrophin gene expression on spiral ganglion neuron resprouting in the deafened cochlea. Mol Ther J Am Soc Gene Ther 18(6):1111–1122
93. Wong AC, Ryan AF (2015) Mechanisms of sensorineural cell damage, death and survival in the cochlea. Front Aging Neurosci 21(7):58
94. Wu JS, Young ED, Glowatzki E (2016) Maturation of spontaneous firing properties after hearing onset in rat auditory nerve fibers: spontaneous rates, refractoriness, and interfiber correlations. J Neurosci 36:10584–10597
95. Wynne DP, Zeng FG, Bhatt S, Michalewski HJ, Dimitrijevic A, Starr A (2013) Loudness adaptation accompanying ribbon synapse and auditory nerve disorders. Brain 136:1626–1638
96. Xiao L, Xu H, Zhang Y, Wei Z, He J, Jiang W et al (2008) Quetiapine facilitates oligodendrocyte development and prevents mice from myelin breakdown and behavioral change. Mol Psychiatry 13:697–698
97. Yakovlev AG, Faden AI (2001) Caspase-dependent apoptotic pathways in CNS injury. Mol Neurobiol 24(1–3):131–144
98. Yu Q, Chang Q, Liu X, Wang Y, Li H, Gong S et al (2013) Protection of spiral ganglion neurons from degeneration using small-molecule TrkB receptor agonists. J Neurosci Off J Soc Neurosci 33(32):13042
99. Zeng FG, Liu S (2006) Speech perception in individuals with auditory neuropathy. Speech Lang Hear Res 49:367–368
100. Zhang KD, Coate TM (2017) Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear. Semin Cell Dev Biol 65:80–87
101. Zhang Y, Liu N, Tang Y, Yang E, Dong S, Huang M et al (2014) Efficient generation of neural stem cell-like cells from rat adipose derived stem cells after lentiviral transduction with green fluorescent protein. Mol Neurobiol 50(2):647–654
102. Zuccotti A, Kuhn S, Johnson SL, Franz C, Singer W, Hecker D et al (2012) Lack of brain-derived neurotrophic factor hampers inner hair cell synapse physiology, but protects against noise-induced hearing loss. J Neurosci 32(25):8545–8553

Chapter 7
Advances in Understanding, Diagnosis, and Treatment of Tinnitus

Dongmei Tang, Huawei Li, and Lin Chen

Abstract Tinnitus is one of the most common hearing disorders, with wide-ranging risk factors including age, hearing loss, noise exposure, inflammatory diseases or tumors of the ear, ototoxic drugs, head or cervical vertebra trauma, and psychological disorders (e.g., anxiety and depression). Tinnitus can be a lifelong disorder and will bring about annoyance, anxiety, depression, insomnia, hyperacusis, concentration difficulty, and, in some extreme cases, suicide. Not every tinnitus patient will require medical attention, and the majority often get accustomed to the phantom sound; however, about 20% of the sufferers will seek clinical intervention. As a matter of fact, evidence was rare for successful tinnitus treatment with a randomized clinical trial. With recent advances in neuroimaging approaches and development of novel tinnitus animal models, scientists have gained new insights into the neural basis of tinnitus. Current theories regarding mechanisms underlying tinnitus focus on abnormal activities in the central nervous system, such as elevated spontaneous neuronal firing rate and increased neuronal synchronization caused by the auditory deprivation, changes in the tonotopic map, auditory cortical reorganization, dysregulation of the limbic system, and the central auditory cortex. At the present, there is a lack of objective indicator of tinnitus, and the diagnosis battery for tinnitus mainly relies on subjective assessments and self-reports, such as case history, audiometric tests, detailed tinnitus inquiry, tinnitus matching, and neuropsychological assessment. While there is currently no golden standard treatment for tinnitus, counseling, psychotherapy, pharmacological approaches, masking devices, indi-

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vidualized sound stimulation, and cognitive behavioral therapy (CBT) are the most widely used strategies, and among these only CBT treatment has been shown to have a definite improvement effect on tinnitus in a large randomized controlled trial. In summary, this article reviews recent advances in understanding, diagnosis, and treatment of tinnitus.

Keywords Tinnitus · Hearing loss · Diagnosis · Treatment strategy

7.1 Introduction

Tinnitus has been a worldwide complaint and refers to the bothersome auditory perception in the absence of external acoustic or electric stimulus. There is no consolidated criterion for tinnitus definition for the purpose of research. The most common definition of tinnitus demonstrates that tinnitus must exceed a 5-min duration [1]. The other tinnitus questions include "Do you have permanent tinnitus all the time?" or "Do you have recurrent tinnitus once a month or more?"

Strictly speaking, tinnitus is a symptom of auditory system rather than a disease. It can be the concomitant symptom of many diseases, such as otitis media, Meniere's disease, presbycusis, impacted cerumen, or otosclerosis. In some other cases, tinnitus can be the first sign of other diseases, for instance, acoustic neuroma or sudden sensorineural hearing loss. Furthermore, some systematic diseases like arteriosclerosis, cervical spondylosis, and anemia can also cause tinnitus, and there is still some portion of tinnitus that is hardly attributed to some specific causes.

Being different from the verbal auditory hallucination, the sound of tinnitus conveys no meaning, while the former always conveys intact content with clear sound. Tinnitus is clinically heterogeneous that the sound characteristics, the underlying pathophysiology, and the influence factors can vary. Tinnitus perception can be localized unilaterally or bilaterally in the ears or within the head. Buzzing in the brain is often called tinnitus cerebri, included in the range of tinnitus definition. Tinnitus can be constant or intermittent, and it may occur suddenly or develop slowly. The common described sounds of tinnitus are chirping, buzzing, ringing, hissing, or whistling sounds. In many cases, more than one sound but several mixed forms of noise or music-liked sounds are perceived. The sound heard as a form of tinnitus can sometimes be pulsatile, which synchronized with the heartbeat or peripheral pulses.

The most common classification of tinnitus is subjective or objective tinnitus based on if it can be perceived by other people. Objective tinnitus is less common, often generated by biological activities in the body, for example, the sound produced by the blood turbulence of the middle ear, eustachian tube, and soft palate, pulse beats, and muscle contraction that is transmitted to the ear. Others can also detect the presence of objective tinnitus directly or with the aid of medical devices. Subjective tinnitus is more common, refers to tinnitus that lacks corresponding sound source, and is currently thought to be caused by disorders of the auditory nervous system.

For the choice of treatment approaches, the "Clinical Practice Guideline of Tinnitus" by American Academy of Otolaryngology—Head and Neck Surgery recommended that tinnitus should be classified as either primary or secondary. Primary tinnitus is idiopathic that may or may not be associated with sensorineural hearing loss (SNHL). Secondary tinnitus is associated with a specific underlying cause (other than SNHL) or an identifiable organic condition. The current cure for primary tinnitus is to provide symptomatic relief, while the management of secondary tinnitus is targeted firstly toward treatment of the specific underlying condition [2].

7.2 Epidemics

McCormack, A. et al. reviewed all adult population studies reporting the prevalence of tinnitus from January 1980 to July 2015 [1]. The prevalence of tinnitus according to papers is 5.1–42.7% around the world, and it increases with age and noise exposure. If the tinnitus diagnosis criterion is lasting for more than 5 min at a time, the self-reported prevalence varies widely from 11.9% to 30.3%. A cross-sectional analysis of largest sample adults (n = 75,764) who reported tinnitus in the preceding 12 months was identified in the United States. The estimated prevalence of tinnitus in the United States is approximately 1 in 10 adults. Higher rates of tinnitus were reported in those with occupational or recreational noise exposures [3].

As for the gender preference, it seemed higher tinnitus prevalence exited among males than females. In another large survey in Norway (n = 51,574), 21.3% of men and 16.2% of women reported perception of tinnitus, with 9.6% of men and 9.3% of women reporting low tinnitus intensity, 7.3% of men and 4.8% of women reporting intermediate tinnitus intensity, and 4.4% of men and 2.1% of women reporting high tinnitus intensity [4]. This study further verified the higher prevalence of tinnitus among males. The largest study sample for people aged over 14 years in New Zealand was 69,976. The overall prevalence for tinnitus was 6.0%, with respective 6.5% among males compared to 5.5% among females. Tinnitus prevalence increased with age, peaking at 13.5% for older adults aged over 65 years [5].

Similar prevalence to the adults in younger population was revealed. A questionnaire was completed by 3892 high school students in Belgium; the prevalence of temporary noise-induced tinnitus and permanent tinnitus in high school students was, respectively, 74.9% and 18.3%. An increasing prevalence of temporary tinnitus with age was present [6]. A study was conducted to learn the prevalence of tinnitus in US adolescents aged 12–19 years (n = 3520). Overall, tinnitus lasting 5 min or more in the preceding 12 months was reported by 7.5% of the whole population. The prevalence of chronic tinnitus (lasting for more than 3 months) was 4.7%, corresponding to about 1.6 million adolescents in the United States [7]. Coincidentally, 3047 participants aged 12–19 years in Korea were included in a study; the prevalence of tinnitus in the young population was 17.7%, although only 0.3% of subjects reported severe discomfort caused by tinnitus [8].

Prevalence in children is often overlooked and often difficult to estimate. However, results of available studies suggest that their tinnitus experience is as common as adults. A large sample study of 15,199 students aged from 7 to 12 years was taken in Poland. Overall 6.0% of them reported tinnitus lasting for 5 min or more. Prevalence of tinnitus in children was similar between sexes [9].

As for the localization of tinnitus, a dominant left ear preponderance of tinnitus sensation was established in several studies [10, 11]. A possible reason is that the left ear is more sensitive to many hearing damage risk factors such as noise and ototoxic drugs for its difference in central auditory system with right ear [12, 13]. In a recent study, although an increase of activity in the left auditory cortex versus the right auditory cortex was present, the left-sided hyperactivity in the auditory cortex also existed in a control group without tinnitus. This data showed that hemisphere asymmetries in tinnitus patients seemed to be a normal characteristic of the normal brain, which might be not specific in tinnitus [14].

7.3 Hearing Loss and Tinnitus

The prevalence of tinnitus is associated with many factors—otological infection like otitis media, acoustic pathway neoplasms, impacted cerumen, presbyacusis, sensorineural hearing loss, noise exposure, neurological disorders such as meningitis and migraine, head trauma or temporal bone fracture, some other systematic diseases like hypertension and diabetes, psychological disorder, and ototoxic medications [15]; among these hearing loss is the main risk factor [16, 17]. Tinnitus can be the precursor or concomitant symptom, occurring in 80% of sudden sensorineural hearing loss patients [18]. These suggest correlation existing between the generation of tinnitus and damaged hearing.

7.3.1 The Tonotopic Reorganization Model

The tonotopic reorganization model suggests that hearing loss results in a reorganization in disturbed tonotopic map in primary auditory cortex whereby the neurons with characteristic frequencies within the deprived region adopt the tuning properties of their less-affected neighbors [19]. The boundary of normal hearing region and hearing loss region is so-called edge frequency, which was believed to correspond to the frequency perceived by tinnitus patients in several studies [20–22] (Fig. 7.1). Konig et al. found a weak but still significant relationship between tinnitus pitch and audiometric edge, while Moore et al. claimed that a stronger and more significant correspondence existed between tinnitus pitch and the low edge frequency after the octave error training.

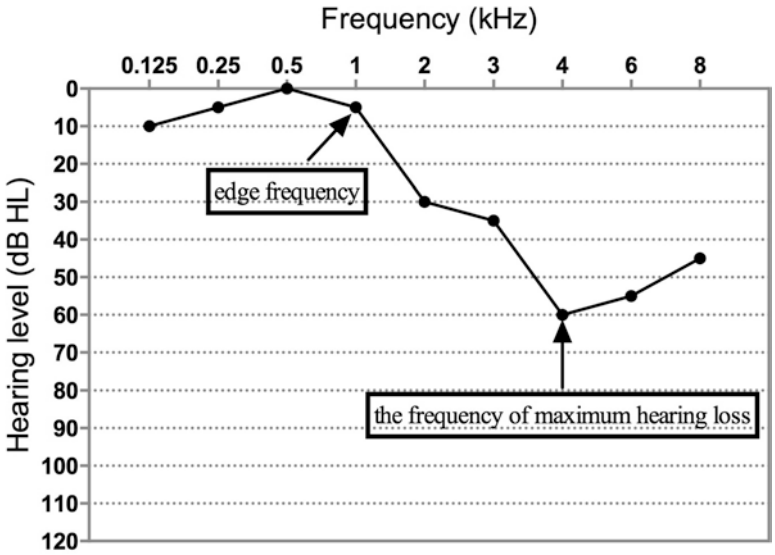

Fig. 7.1 An example of the edge frequency and the frequency of maximum hearing loss both labelled on the audiometric graph

7.3.2 The Neural Synchrony Model

Nevertheless, the neural synchrony model has different points with the hypothesis that tinnitus is generated by increased neuronal synchronization of the hearing impairment region [23, 24]. The theory suggests that tinnitus pitch generally falls at the area where hearing is impaired [25–27] and is more corresponding to the frequency of maximum hearing loss [28, 29] (Fig. 7.1). In a cohort of 195 patients, Pan and colleagues [30] could not find a clear relationship between tinnitus pitch and edge frequency both in normal and abnormal hearing, although they tried repeatedly in subgroups based on the audiogram types and tinnitus properties. Similarly, they failed to demonstrate a significant correlation between the frequency of maximum hearing loss and the pitch of tinnitus. Sereda et al. [27] also failed to detect an association of tinnitus pitch and edge frequency in the whole 67 subjects with chronic bilateral tinnitus. However, in a subgroup of 23 subjects with narrowband width tinnitus, a significant positive relationship existed between edge frequency and tinnitus pitch. Then, in a more recent study, they amended the prediction on the relationship of narrowband tinnitus pitch and edge frequency in an enlarged cohort of 129 patients. Surprisingly they didn't demonstrate that tinnitus pitch was corresponding to the audiometric edge in patients with narrowband width tinnitus. On the contrary, it fell within the hearing loss region, contrast to the reorganization theory [31].

To date, Schecklmann and colleagues [29] established the association of both the edge frequency and frequency of maximum hearing loss with tinnitus pitch in the largest scale of 286 subjects. They did multidimensional comparatives based on pairs of subgroups of unilateral vs. bilateral tinnitus, pure tone vs. noise-like tinnitus, low vs. high sloping audiograms, and right vs. left ears. They could not detect significant relationship between tinnitus pitch and edge frequency similar with the studies of Pan et al. and Sereda et al. The dominant tinnitus frequency was within the area of hearing loss in both left and right ears. Nevertheless, only in the right or the left ears, tinnitus pitch was correlated with maximum hearing loss, and the cause was owing to tinnitus laterality. However, they failed to interpret exact mechanisms.

7.3.3 The "Hidden Hearing Loss" Hypothesis

An abnormal hearing is not detected in all subjects with tinnitus; however, 20% of them do not have hearing loss [32]. Research on the presence of tinnitus in patients with normal hearing has been conducted to discover the "hidden hearing loss" undetectable by the traditional pure tone audiometry (0.125–8 kHz), using auditory brainstem responses (ABR), otoacoustic emissions (OAEs), and high-frequency audiometry. Weisz et al. argue that a tinnitus subject's possession of normal auditory hearing does not mean the subject lacks cochlear impairment [33]. A reduced neural output from the cochlea is accompanied by significantly reduced amplitude of the wave I potential by ABR, indicating deafferentation of high-threshold auditory nerve fibers [34]. Several studies have revealed, using distortion product otoacoustic emissions (DPOAEs) or transient evoked otoacoustic emissions (TEOAEs), that minor abnormalities exist in the outer hair cells, indicating that tinnitus can be generated by the abnormalities in the outer hair cells or by some undetectable hearing damage [35–37]. Nevertheless, the findings of research using OAEs in subjects with normal audiograms are ambiguous. Serra et al. also found a higher prevalence of alterations in DPOAEs and TEOAEs in patients with normal hearing. However, this feature is not always present in those who have the symptoms of tinnitus, suggesting that OHC dysfunction is not necessary for tinnitus development [38]. Moreover, a decrease in DPOAEs is seen in tinnitus patients with normal hearing [39]. A recent study demonstrated additional pathological high-frequency audiograms using the high-frequency audiometry (> 8 kHz), suggesting a potential causal role for the high-frequency hearing loss in tinnitus etiopathogenesis [40].

In conclusion, the sensation of tinnitus associated with hearing loss is usually localized toward the affected ear, and the matched pitch of tinnitus corresponds to frequencies at which hearing is impaired [25, 26]. Even in tinnitus patients with normal hearing whose hearing loss is beyond detectable, some cochlear dead regions or outer hair cell damage exists compared with controls [41], reconfirming the relevance of hearing impairment for tinnitus sensations.

7.4 Neural Basis of Tinnitus

Tinnitus is often triggered by hearing damage; however, cochlear ablation or auditory nerve section never eradicates the perception of tinnitus [42]. This indicates that the generation of tinnitus involves a central mechanism. Although the exact mechanism of subjective tinnitus generation is not clear, the central mechanism relevant to tinnitus was gradually uncovered with the development of neuroimaging techniques such as magneto- and electro-encephalography or functional magnetic resonance. EEG data have revealed that tinnitus is associated with increased γ-band activity in the auditory cortex [43]. The enhanced gamma activity may lie between deafferented (hearing loss) and normally afferented regions because of the loss of lateral inhibition of the deafferented region, which is termed as "edge effect." Functional MRI (fMRI) is available to study the brain networks of tinnitus. Under normal conditions, the limbic system may identify an irrelevant signal (e.g., noise) and inhibit the unwanted signal from reaching auditory cortex. However, under abnormal states, the limbic regions failed to recognize and cancel the noise signal, and that is the mechanism of chronic tinnitus generation [44, 45].

Current view is that tinnitus is considered to be a neuroplastic response to acoustic deprivation [19]. Hearing loss reduces the affected peripheral auditory nerve activity, leading to downregulation in inhibitory cortical processes, which induces elevated spontaneous firing rates in central auditory system, including primary auditory cortex (dorsal cochlear nucleus, DCN) [46, 47]. The increased spontaneous firing rates are also accompanied by an increase in the neural synchronization both spatially and temporally after noise-induced hearing loss. The frequency distribution of affected neuron is consistent with noise-damaged frequency domain [46].

Tinnitus and chronic pain share similar features in terms of physiology, assessment, and management, so does the mechanisms. Sensory deafferentation results in increased activation of the primary sensory cortexin the case of chronic tinnitus. Awareness of the stimulus arises when perceptual network is co-activated, and the percept is enhanced by activation of salience network. Tinnitus perception becomes associated to distress if a nonspecific distress network is activated, and once memory network becomes active, the persistence of tinnitus results [48].

7.5 Diagnosis

The diagnosis of tinnitus involves patient history taken, clinical examination, neuropsychological assessments, and audiometric and tinnitus tests. Most tinnitus is subjective, perceived only by the patient; thus, the diagnosis of tinnitus mainly relies on patients' self-report. Objective tinnitus, on the contrary, is rare and can also be heard by others, which enables it easier to recognize. In most cases, objective tinnitus is relevant to vascular abnormality, so its diagnosis often relies on radioactive imaging examination such as magnetic resonance imaging (MRI) or computed tomography (CT).

7.5.1 Subjective Report

Before diagnosis, the general patient history collection including age, gender, education level, and tinnitus-related family history makes great senses. Then, a specialized and detailed tinnitus inquisition is quite essential. The self-reported perceptive position, tinnitus duration, and sound properties of pitch and loudness, particularly whether it has a rhythmical or pulsatile component, are the main concerns. Besides, tinnitus-relevant factors such as noise exposure, sudden hearing loss, presbyacusis, traumatic deafness, tympanitis, ototoxic drug application, and so on should also be identified. The diseases originated from ears, and some tinnitus-relevant chronic systematic diseases such as hypertension, diabetes, coronary heart disease, and especially neurological disorders should also be figured out.

Several validated questionnaires are available to assess the severity of tinnitus and to evaluate the effects of tinnitus treatment, of which the tinnitus handicap inventory (THI), tinnitus questionnaire (TQ), and visual analogue scale (VAS) for tinnitus loudness are the most commonly used rating scales. TQ [49] with high internal consistency reliability and stability in different languages consists of 52 items to assess tinnitus-related psychological disorders involving five dimensions of tinnitus complaints: cognitive and emotional distress, auditory perceptual difficulties, intrusiveness, sleep disturbance, and somatic complaints. Subjects rate their conditions by circling one from the three response alternatives.

The THI is a 25-item self-report questionnaire, firstly introduced by Newman at 1996. THI scores on a three-label category scale (0, 1, 2 scores) and assesses the severity of tinnitus on three domains: functional, emotional, and catastrophic. The total score of global tinnitus distress and impact ranges from 0 to 100 points, and guidelines for classification of tinnitus severity constitute no handicap (0–16), mild handicap (18–36), moderate handicap (38–56), or severe handicap (58–100).

The hospital anxiety and depression scale (HADS), which contains 14 items, is often used for secondary outcome measure of tinnitus [50]. The tinnitus catastrophizing scale (TCS) is an adapted version of the pain catastrophizing scale and assesses catastrophic misinterpretations of the tinnitus sound with 13 items rated on a 5-point scale (0–4 scores, 0 is not at all, 4 is always) [51]. The fear of tinnitus questionnaire (FTQ) has 17 items of true or false scale to measure the fear related to tinnitus [52].

7.5.2 Objective Assessment

7.5.2.1 Audiometry Examination

An otoscopic screening is firstly performed to identify abnormalities from normal ear canals and eardrums. Overmuch or impacted cerumen in the external auditory canal often causes buzzing sound as well as hearing loss. The situation is frequently

confused with tinnitus, and the solution is clearing away the cerumen immediately. Some conditions like otorrhea and tympanic membrane perforation are helpful to understand the etiology of tinnitus.

Pure tone (0.125, 0.25, 0.5, 1, 2, 4, 8 kHz) and speech audiometry, tympanometry for the two ears are extremely important in guiding the pathogenesis and pathogenic sites. For those without hearing loss in the regular pure tone audiometry (PTA), the extended high-frequency PTA sometimes may have abnormal findings. Patients who have asymmetric tinnitus and hearing or with other associated neurological symptoms need further investigation, and generally the chosen tools are MRI or CT. Patients with heartbeat-synchronous pulsatile tinnitus need more detailed investigation by a complex algorithm that includes ultrasonography, CT, MRI, CT, and MR angiography.

7.5.2.2 Tinnitus Matching

No objective measure exists to detect subjective tinnitus; however, some psychoacoustic measures such as pitch, loudness, maskability, and residual inhibition match can show the characteristics of tinnitus indirectly. Pure tone or narrowband noise is the common form of sounds used for tinnitus pitch and loudness match (PM and LM). The three main tinnitus matching procedures are, respectively, the traditional audiometry method, two-alternative forced choice (2AFC) procedure, and computer-automated procedure by Henry JA et al. Audiometry method is conducted in audiological testing paradigm by using an audiometer to try step by step to find the closest frequency to tinnitus sound following the means of pure tone audiometry and then adjust the loudness level equivalent to tinnitus.

The 2 FAC method is to give pairs of pure tones once a time by asking the patients to distinguish the one closer to tinnitus sensation, and the test tone pairs for pitch-matching are always separated by a third octave frequency except for octave-confusion testing [53]. The computer-automated procedure can be simplified as follows: Firstly, test the pure tone thresholds from 125 Hz to 12 kHz by a third octave step in frequencies. Then, match the loudness level of tinnitus at each test frequency with the combination of computer-aided instructions and external adjustable feedback handle. Finally, finish the tinnitus pitch match by gradually comparing the stimulus sound with tinnitus perception at equal loudness curve acquired by tinnitus loudness-matching [54, 55].

The minimum masking level (MML) testing, according to Henry JA et.al [55], uses a 2–12 kHz broad band of noise as the sound stimulus. The noise threshold in each ear is separately tested at first. The noise is presented to each ear at the same sensation level (dB SL, i.e., level above threshold), respectively, to totally cover tinnitus. The average of the two MML value is the final MML result. As with MML testing, residual inhibition (RI) is tested binaurally at the intensity level of 10 dB above the MML value. Subjects are instructed to listen to the noise for 1 min and then respond immediately to the question “what percentage of the usual loudness does tinnitus loudness decrease to?”

7.6 Treatment

Although tinnitus is in high incidence, only the patients with bothersome and persistent (lasting 6 months or longer) tinnitus will ask for clinical intervention. The proportion is around 20% of the adults who experience tinnitus. In the past, tinnitus was thought to be caused by disorders of the ear. However, the treatment targeting cochlea did not yield curative effect, and there was rare randomized clinical trial evidence of successful tinnitus treatment. Due to the complicated etiology and unclear pathogenesis, the current treatments for tinnitus are diversified, including psychotherapy, medication, noise maskers and tinnitus retraining therapy, sound stimulation, and surgical treatment. Among them, physiotherapy like brain stimulation and laser treatment is often considered to be ineffective. Surgery is only suitable for tinnitus caused by some specific organic diseases. At present, there is no effective medication for tinnitus. Increasing sound stimulation strategies emerge, while psychotherapy has become a fundamental part of tinnitus treatment (Fig. 7.2).

7.6.1 Cognitive Behavioral Therapy

The term cognitive behavioral therapy (CBT) comes originally from the field of psychotherapy, aiming at recognition of maladaptive cognition (cognitive) and modification of negative thoughts with some effective behaviors (behavioral). CBT approaches include diverse elements such as psychological education, counseling, relaxation training, behavioral reactivation, and mindfulness-based practice. It is widely applied in improving anxiety disorders, depression, and insomnia and even used in disposing unhealthy lifestyles relevant diseases such as diabetes, obesity, and alcohol dependence. CBT treatment, to some degree, is generally considered as the current gold standard in psychotherapy [56].

CBT has been used to treat tinnitus since the 1980s. For decades, CBT approaches have been repeatedly shown to be effective in controlling tinnitus by decreasing tinnitus-induced annoyance, dysthesia, depression, anxiety, and insomnia. The clinical guideline for tinnitus announced by the American Academy of Otolaryngology-Head and Neck Surgery Foundation made a statement that clinicians should recommend CBT to patients with persistent, bothersome tinnitus [2].

Most studies of CBT for tinnitus consist 8–24 weekly sessions, each lasting 60–120 min. Cognitive behavioral therapy can be delivered face to face to individuals or groups, and the group size (usually 6–8 participants) can vary according to tinnitus severity. Such treatment theme involves psychoeducation, cognitive reconstruction of dysfunctional beliefs, exposure techniques, mindfulness-based elements, stress relief, and attention redirecting techniques by means of movement therapy, and applied relaxation. Cognitive behavioral therapy can also be performed remotely through internet, making it more acceptable and implementable, and sharing the overall equivalent effects as face-to-face CBT.

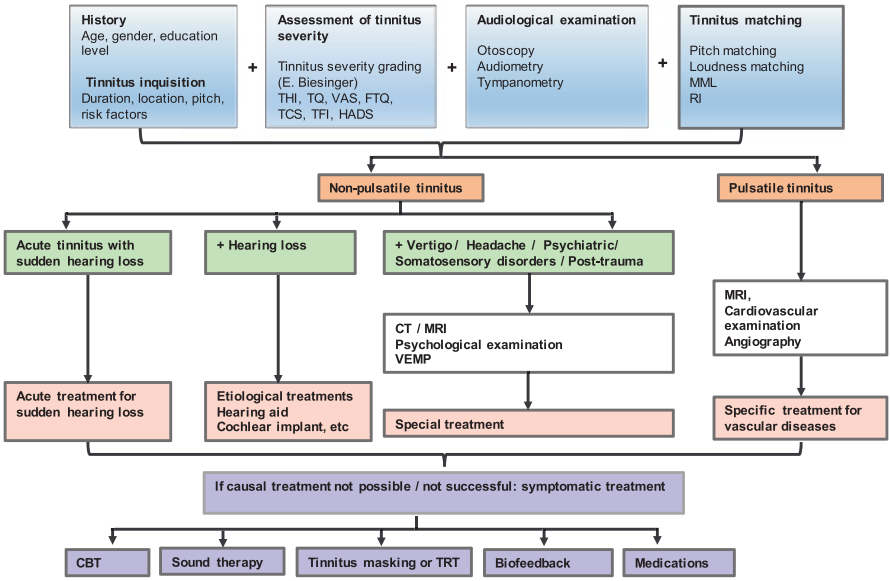

Fig. 7.2 A flow chart for diagnosis and treatment strategies for patients with tinnitus. Abbreviations: *THI* tinnitus handicap inventory, *TQ* tinnitus questionnaire, *VAS* visual analogue scale, *FTQ* the fear of tinnitus questionnaire, *TCS* the tinnitus catastrophizing scale, *TFI* tinnitus functional index, *HADS* the hospital anxiety and depression scale, *MML* minimum masking level, *RI* residual inhibition, *CBT* cognitive behavioral therapy, *TRT* tinnitus retraining therapy

The largest and most credible randomized controlled trial on CBT for tinnitus to date (n = 492) was undertaken in Netherlands. In this trial, specialized care of tinnitus for up to 12 weeks based on CBT group treatments was proven to improve the quality of life significantly when compared with the usual care control group [52]. Furthermore, another study concluded that the special stepped-care tinnitus treatment based on CBT was cost-effective as compared with usual care by cost analysis of quality adjusted life years (QALYs) as measured with the Health Utilities Index Mark III.

7.6.2 Masking Devices

Many patients with complaint of tinnitus have the experience that their tinnitus loudness can diminish or even vanish with the presence of appropriate background sounds. The common sense makes the basis of tinnitus masking (TM) more comprehensible, which makes tinnitus inaudible by complete masking through raising the intensity of noise. Though tinnitus masking has been one of the most commonly used means to cope with tinnitus, and indeed some temporary relief can be obtained,

the negative comments on TM have never stopped. The opponents argue that the positive effect if any is often short; however, in a long run, the repeated louder noises applied for tinnitus masking can inversely involve increased awareness of tinnitus or induce further hearing impairment (e.g., [57, 58]).

There are two types of masking therapy: complete masking and partial masking, the latter leading both tinnitus and the masking sound be heard. Many researchers preferred the concept "partial masking" rather than "complete masking" for preventing the underlying hearing damage. Further work showed that the low level white noise treatment could be used to achieve habituation of tinnitus by perception of both the tinnitus sound and the external noise, simultaneously. The partial masking method is the core of tinnitus retraining therapy (TRT), which is based on Jastreboff's neurophysiological model in 1990, often involved in habituation of tinnitus at the mixing point level (minimally appreciable) when tinnitus comes to be partially masked.

The model postulates that the annoyance and distress associated with tinnitus arises from abnormal subconscious non-auditory mechanisms, mediated primarily by the inappropriate limbic and autonomic nervous systems. Jastreboff and colleagues claimed that the acoustic enrichment, in combination with directive counseling sessions, would ameliorate tinnitus impairment by diminishing awareness of tinnitus. Another benefit of the sound enrichment reflects in the compensation for hearing loss. The acoustic enrichment can be implemented with white noise or natural sound generators or hearing aids.

Most published reports for the TRT therapeutic effect derive from retrospective and uncontrolled or non-randomized clinical trials. However, the controlled randomized trials for TRT treatment are scarce. In a prospective non-randomized clinical assay (n = 158), 82% of the patients in TRT group improved their tinnitus according to their self-evaluation. THI score was reduced from 48% to 32%, and VAS decreased from 6.6 to 5.3 after 1 year ($p < 0.05$) in a 12-month period [59]. In another multisite randomized controlled trial, the audiologists also concluded significant reduction of tinnitus severity over 18 months using TM, TRT, and tinnitus educational counseling (TED) approaches, respectively. Nevertheless, they failed to find any significant difference among the three conditions [60].

Bauer, C.A reported benefit from both TRT and general counseling treatment in adults with chronic tinnitus and normal to near-normal hearing in the speech frequencies [61]. Recently, Bauer, C.A again with colleagues, published a randomized controlled trial (n = 39) to compare TRT with standard of care treatment (SC) for subjects with chronic tinnitus and hearing loss. The similar conclusion was reached to their previous research on tinnitus patients without hearing loss. Both TRT and SC therapy significantly improved tinnitus severity and impact through the measure of THI and tinnitus functional index (TFI) scales. Furthermore, the lasting therapeutic benefit was evident until 18 months after the beginning of treatment in both groups, and the larger treatment effect was obtained in the TRT group [62].

7.6.3 Sound Therapy

There are four main strategies for individualized and specialized sound stimulation with music modulation according to tinnitus frequency. One approach is called tailor-made notched music therapy, established by Pantev C team, by removing the frequency band of one octave width centered at tinnitus frequency from the music energy spectrum. The underlying mechanism is supposed to reduce the activity of tinnitus-related brain region by enhancing lateral inhibition. After 12 months of regular listening, the notched music group (n = 8) showed significantly both reduced tinnitus loudness and reduced evoked activity in auditory cortex areas corresponding to the tinnitus frequency compared to the placebo controls (n = 8) [63].

The second strategy is noted as acoustic enrichment, including neuromonics and frequency discrimination training. The neuromonics tinnitus treatment aims to induce desensitization to tinnitus signal through the combination of structured counseling with acoustic stimulation that enriches the hearing-deprived region of auditory pathways by intermittent and momentary tinnitus suppression. In the three formal clinical trials, the approach was certified to be effective in improving both the tinnitus-related symptoms and quality of life [64]. The research of Davis PB et al. indicated that neuromonics group achieved more benefits in tinnitus alleviation over both the noise + counseling group and counseling-only group [65]. However, in another study, the researchers found equivalent efficacy for neuromonics treatment and tinnitus masking while masking therapy had preponderance in a cost-effectiveness analysis [66].

The proposal of frequency (or auditory) discrimination training was based on the tinnitus cortical reorganization mechanisms that tinnitus probably arises from the modified central auditory system following peripheral auditory deprivation (i.e., hearing loss). The curative effect relied on the hypothesis that the sound enrichment across the hearing loss frequencies would alleviate the neighboring over-activated region (corresponding to tinnitus pitch). Herraiz et al. reported a total 43% improvement rate of auditory discrimination therapy (ADT) group compared with the waiting list group; however, they failed to find significant THI reduction between the two groups [67]. In another study by Herraiz et al., the superiority of ADT to waiting list control group was further confirmed. Besides, they draw a conclusion that the training frequencies one octave below the tinnitus pitch had significantly better outcome than those similar or same to the tinnitus pitch when performing the ADT procedure [68]. However, the curative effect could not be replicated by others. Hoare et al. trained total 70 patients with three different frequencies: within the normal hearing region, within the hearing loss region, a high-pass harmonic complex tone spanning a region of hearing loss, and resulted in no significant differences between groups. Moreover, they failed to find significant improvement pre- and posttreatment when assessing the psycho-acoustical characteristics of tinnitus [69]. Furthermore, in a double-blind controlled study, music tailored to compensate for hearing loss was not beneficial in alleviating tinnitus, whereas overcompensating hearing loss worsened tinnitus [70].

A third approach is so-called acoustic coordinated reset (CR) neuromodulation in which individualized auditory stimuli are presented as short and different frequencies above and below the dominant tinnitus pitch as a new approach to induce desynchronization of the region that tinnitus-related topological synchrony increases in the auditory cortex [71]. In a prospective, placebo-controlled trial (n = 63), CR treatment turned out to be invasive and well tolerated and caused a significant reduction of tinnitus symptoms as measured by VAS and TQ scales. CR therapy also significantly reversed the tinnitus-related EEG alterations according to the EEG recordings [72]. In another crossover designed trial, 18 subjects treated respectively by acoustic CR neuromodulation and noisy CR-like stimulation acquired similar tinnitus relief both clinically and electro-physiologically as assessed with VAS and TQ scores and EEG data [73].

The fourth strategy is known as S-Tones, referred to the amplitude modulated tones, which are commercially applied on production of the Serenade device from SoundCure. Reavis et al. figured out that the high carrier frequency in the 6000–9000 Hz region with a 40 Hz rate 100% depth of amplitude modulation had the greatest likelihood of temporary suppression on tinnitus after testing 17 external stimuli in 20 subjects with chronic tinnitus. What was worth mentioning is that all the stimuli were presented in a low level just below tinnitus loudness [74].The possible mechanism speculated by authors is that 40 Hz amplitude modulation can generate a strong 40 Hz auditory steady-state response, thus enhancing the gamma rhythm to potentially disorganize thalamocortical dysrhythmia [75]. However, the actually underlying mechanisms remain to be further investigated. In another study (n = 56), listening to S-Tones at a carrier frequency corresponding to the tinnitus pitch (amplitude modulation rate of 40 Hz) was proven to be more effective in reducing tinnitus loudness than noise [76].

7.6.4 Biofeedback Therapy

Biofeedback is a self-regulation technique in which individuals learn to voluntarily control themselves to modify their involuntary body processes (e.g., muscle relaxation, heart rate variability, mental sedation) according to their body feedback. The information of physiological functions is picked up by specialized instruments that then convert physiological signals into meaningful visual and auditory cues to show on a screen. Patients get feedback inside their bodies from the screen. Numerous studies have revealed biofeedback training to be beneficial for tinnitus treatment, mostly in the aspects such as improvements in tinnitus annoyance, distress, and feelings of controllability [77–80]. It is currently believed that biofeedback therapy is a relaxation technique in relieving patient's nervous state and reducing tinnitus arousal, but not changing the loudness of tinnitus.

7.6.5 *Medications and Alternative Medicine*

Various medications have been used for obtaining relief from tinnitus mainly for the purpose of coping with tinnitus-relevant comorbidities such as insomnia, anxiety, or depression and promoting microcirculation. The commonly used medications include cortisone, vasodilators, diuretics, antidepressants, benzodiazepines, lidocaine, and neurotrophic drugs. The roles of some medications in tinnitus treatment are presented below.

The application of anticonvulsants in treating tinnitus is based on the assumption that tinnitus is related to central auditory hyperactivity. However, a Cochrane review about anticonvulsants in treating tinnitus was perorated to be ineffective and 18% of patients experienced side effects [81].

The common prescription for antidepressants is mainly because of their improvement to tinnitus comorbid depressive or anxiety disorders. The tricyclic antidepressants and the SSRIs (serotonin-specific reuptake inhibitors) are the common used drugs; some suggested that tinnitus patients with sleep disorders and depression should be considered for antidepressant therapy [82]. However, in recent four Cochrane reviews applying antidepressants, the positive effects have been mainly related to improvement of depression and anxiety rather than any character or intensity change of tinnitus. Because of the reported side effects and more dropped out ratio compared with the placebo controls, the trials failed to demonstrate a preponderance of benefit over harm [2].

Extracts of Ginkgo biloba leaves have been widely used for at least 5000 years in the traditional Chinese medicine. A Cochrane review showed that the evidence that Ginkgo biloba is effective was so limited, but the incidence of side effects was low [83]. Two Cochrane reviews [84, 85] indicated that tricyclic antidepressants didn't show any direct beneficial effect in the treatment of tinnitus.

Based on systematic reviews and RCTs, the 2014 Clinical Practice Guideline of Tinnitus draw a conclusion that medications such as antidepressants, anticonvulsants, anxiolytics, Ginkgo biloba, melatonin, zinc, or other dietary supplements were recommended against for the routine treatment of persistent and bothersome tinnitus. And, in fact, there is currently no drug approved by the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA) for the treatment of tinnitus [86].

7.7 Summary

This article provides a review of the known knowledge about tinnitus including tinnitus definition, prevalence, the relevance of hearing loss to tinnitus, diagnosis, and various treatment choices for tinnitus. The prevalence of tinnitus estimating is 10–15% of the adults around the world, and fortunately, only about 20% would

require clinical intervention. The diagnosis of tinnitus involves history taken, assessment of tinnitus severity, auditory examinations, and tinnitus matching. Considering various risk factors related to tinnitus, the secondary tinnitus should be identified firstly and its management targets at etiological treatment of the primary lesion. As for the primary tinnitus or if the causal treatment not possible or not successful, the symptomatic treatment including CBT, sound therapy, tinnitus masking devices, or TRT, biofeedback therapy, and medications come to be alternative approaches.

References

1. McCormack A, Edmondson-Jones M, Somerset S, Hall D (2016) A systematic review of the reporting of tinnitus prevalence and severity. Hear Res 337:70–79
2. Tunkel DE, Bauer CA, Sun GH, Rosenfeld RM, Chandrasekhar SS, Cunningham ER Jr, Archer SM, Blakley BW, Carter JM, Granieri EC, Henry JA, Hollingsworth D, Khan FA, Mitchell S, Monfared A, Newman CW, Omole FS, Phillips CD, Robinson SK, Taw MB, Tyler RS, Waguespack R, Whamond EJ (2014) Clinical practice guideline: tinnitus. Otolaryngol Head Neck Surg 151(2 Suppl):S1–S40
3. Bhatt JM, Lin HW, Bhattacharyya N (2016) Prevalence, severity, exposures, and treatment patterns of tinnitus in the United States. JAMA Otolaryngol Head Neck Surg 142(10):959–965
4. Krog NH, Engdahl B, Tambs K (2010) The association between tinnitus and mental health in a general population sample: results from the HUNT study. J Psychosom Res 69(3):289–298
5. Wu BP, Searchfield G, Exeter DJ, Lee A (2015) Tinnitus prevalence in New Zealand. N Z Med J 128(1423):24–34
6. Gilles A, Van Hal G, De Ridder D, Wouters K, Van de Heyning P (2013) Epidemiology of noise-induced tinnitus and the attitudes and beliefs towards noise and hearing protection in adolescents. PLoS One 8(7):e70297
7. Mahboubi H, Oliaei S, Kiumehr S, Dwabe S, Djalilian HR (2013) The prevalence and characteristics of tinnitus in the youth population of the United States. Laryngoscope 123(8):2001–2008
8. Park B, Choi HG, Lee HJ, An SY, Kim SW, Lee JS, Hong SK, Kim HJ (2014) Analysis of the prevalence of and risk factors for tinnitus in a young population. Otol Neurotol 35(7):1218–1222
9. Piotrowska A, Raj-Koziak D, Lorens A, Skarzynski H (2015) Tinnitus reported by children aged 7 and 12 years. Int J Pediatr Otorhinolaryngol 79(8):1346–1350
10. Baguley D, McFerran D, Hall D (2013) Tinnitus. Lancet 382(9904):1600–1607
11. Yenigun A, Dogan R, Aksoy F, Akyuz S, Dabak H (2014) Assessment of tinnitus with tinnitus severity index, tinnitus handicap inventory and distortion product otoacoustic emissions in patients with normal hearing and hearing loss. Kulak Burun Bogaz Ihtis Derg 24(1):11–16
12. Nageris BI, Raveh E, Zilberberg M, Attias J (2007) Asymmetry in noise-induced hearing loss: relevance of acoustic reflex and left or right handedness. Otol Neurotol 28(4):434–437
13. Schmidt CM, Knief A, Lagosch AK, Deuster D, am Zehnhoff-Dinnesen A (2008) Left-right asymmetry in hearing loss following cisplatin therapy in children--the left ear is slightly but significantly more affected. Ear Hear 29(6):830–837
14. Geven LI, de Kleine E, Willemsen AT, van Dijk P (2014) Asymmetry in primary auditory cortex activity in tinnitus patients and controls. Neuroscience 256:117–125
15. Cianfrone G, Pentangelo D, Cianfrone F, Mazzei F, Turchetta R, Orlando MP, Altissimi G (2011) Pharmacological drugs inducing ototoxicity, vestibular symptoms and tinnitus: a reasoned and updated guide. Eur Rev Med Pharmacol Sci 15(6):601–636
16. Lockwood AH, Salvi RJ, Burkard RF (2002) Tinnitus. N Engl J Med 347(12):904–910

17. Nondahl DM, Cruickshanks KJ, Huang GH, Klein BE, Klein R, Nieto FJ, Tweed TS (2011) Tinnitus and its risk factors in the beaver dam offspring study. Int J Audiol 50(5):313–320
18. Nosrati-Zarenoe R, Arlinger S, Hultcrantz E (2007) Idiopathic sudden sensorineural hearing loss: results drawn from the Swedish national database. Acta Otolaryngol 127(11):1168–1175
19. Eggermont JJ, Roberts LE (2004) The neuroscience of tinnitus. Trends Neurosci 27(11):676–682
20. Konig O, Schaette R, Kempter R, Gross M (2006) Course of hearing loss and occurrence of tinnitus. Hear Res 221(1–2):59–64
21. Moore BC, Vinay, Sandhya (2010) The relationship between tinnitus pitch and the edge frequency of the audiogram in individuals with hearing impairment and tonal tinnitus. Hear Res 261(1–2):51–56
22. Schaette R, Kempter R (2009) Predicting tinnitus pitch from patients' audiograms with a computational model for the development of neuronal hyperactivity. J Neurophysiol 101(6):3042–3052
23. Norena AJ (2011) An integrative model of tinnitus based on a central gain controlling neural sensitivity. Neurosci Biobehav Rev 35(5):1089–1109
24. Schaette R, Kempter R (2006) Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model. Eur J Neurosci 23(11):3124–3138
25. Henry JA, Meikle MB (1999) Pulsed versus continuous tones for evaluating the loudness of tinnitus. J Am Acad Audiol 10(5):261–272
26. Norena A, Micheyl C, Chery-Croze S, Collet L (2002) Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. Audiol Neurootol 7(6):358–369
27. Sereda M, Hall DA, Bosnyak DJ, Edmondson-Jones M, Roberts LE, Adjamian P, Palmer AR (2011) Re-examining the relationship between audiometric profile and tinnitus pitch. Int J Audiol 50(5):303–312
28. Ochi K, Ohashi T, Kenmochi M (2003) Hearing impairment and tinnitus pitch in patients with unilateral tinnitus: comparison of sudden hearing loss and chronic tinnitus. Laryngoscope 113(3):427–431
29. Schecklmann M, Vielsmeier V, Steffens T, Landgrebe M, Langguth B, Kleinjung T (2012) Relationship between audiometric slope and tinnitus pitch in tinnitus patients: insights into the mechanisms of tinnitus generation. PLoS One 7(4):e34878
30. Pan T, Tyler RS, Ji HH, Coelho C, Gehringer AK, Gogel SA (2009) The relationship between tinnitus pitch and the audiogram. Int J Audiol 48(5):277–294
31. Sereda M, Edmondson-Jones M, Hall DA (2015) Relationship between tinnitus pitch and edge of hearing loss in individuals with a narrow tinnitus bandwidth. Int J Audiol 54(4):249–256
32. Jastreboff PJ, Jastreboff MM (2003) Tinnitus retraining therapy for patients with tinnitus and decreased sound tolerance. Otolaryngol Clin N Am 36(2):321–336
33. Weisz N, Hartmann T, Dohrmann K, Schlee W, Norena A (2006) High-frequency tinnitus without hearing loss does not mean absence of deafferentation. Hear Res 222(1–2):108–114
34. Schaette R, McAlpine D (2011) Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. J Neurosci 31(38):13452–13457
35. Granjeiro RC, Kehrle HM, Bezerra RL, Almeida VF, Sampaio AL, Oliveira CA (2008) Transient and distortion product evoked oto-acoustic emissions in normal hearing patients with and without tinnitus. Otolaryngol Head Neck Surg 138(4):502–506
36. Paglialonga A, Del Bo L, Ravazzani P, Tognola G (2010) Quantitative analysis of cochlear active mechanisms in tinnitus subjects with normal hearing sensitivity: multiparametric recording of evoked otoacoustic emissions and contralateral suppression. Auris Nasus Larynx 37(3):291–298
37. Paglialonga A, Fiocchi S, Del Bo L, Ravazzani P, Tognola G (2011) Quantitative analysis of cochlear active mechanisms in tinnitus subjects with normal hearing sensitivity: time-frequency analysis of transient evoked otoacoustic emissions and contralateral suppression. Auris Nasus Larynx 38(1):33–40

38. Serra L, Novanta G, Sampaio AL, Oliveira CA, Granjeiro R, Braga SC (2015) The study of otoacoustic emissions and the suppression of otoacoustic emissions in subjects with tinnitus and normal hearing: an insight to tinnitus etiology. Int Arch Otorhinolaryngol 19(2):171–175
39. Shiomi Y, Tsuji J, Naito Y, Fujiki N, Yamamoto N (1997) Characteristics of DPOAE audiogram in tinnitus patients. Hear Res 108(1–2):83–88
40. Vielsmeier V, Lehner A, Strutz J, Steffens T, Kreuzer PM, Schecklmann M, Landgrebe M, Langguth B, Kleinjung T (2015) The relevance of the high frequency audiometry in tinnitus patients with Normal hearing in conventional pure-tone audiometry. Biomed Res Int 2015:302515
41. Job A, Raynal M, Kossowski M (2007) Susceptibility to tinnitus revealed at 2 kHz range by bilateral lower DPOAEs in normal hearing subjects with noise exposure. Audiol Neurootol 12(3):137–144
42. Jackson P (1985) A comparison of the effects of eighth nerve section with lidocaine on tinnitus. J Laryngol Otol 99(7):663–666
43. Weisz N, Muller S, Schlee W, Dohrmann K, Hartmann T, Elbert T (2007) The neural code of auditory phantom perception. J Neurosci 27(6):1479–1484
44. Leaver AM, Renier L, Chevillet MA, Morgan S, Kim HJ, Rauschecker JP (2011) Dysregulation of limbic and auditory networks in tinnitus. Neuron 69(1):33–43
45. Rauschecker JP, Leaver AM, Muhlau M (2010) Tuning out the noise: limbic-auditory interactions in tinnitus. Neuron 66(6):819–826
46. Norena AJ, Eggermont JJ (2003) Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. Hear Res 183(1–2):137–153
47. Seki S, Eggermont JJ (2003) Changes in spontaneous firing rate and neural synchrony in cat primary auditory cortex after localized tone-induced hearing loss. Hear Res 180(1–2):28–38
48. De Ridder D, Elgoyhen AB, Romo R, Langguth B (2011) Phantom percepts: tinnitus and pain as persisting aversive memory networks. Proc Natl Acad Sci U S A 108(20):8075–8080
49. Hallam RS, Jakes SC, Hinchcliffe R (1988) Cognitive variables in tinnitus annoyance. Br J Clin Psychol 27(Pt 3):213–222
50. Yang Y, Ding R, Hu D, Zhang F, Sheng L (2014) Reliability and validity of a Chinese version of the HADS for screening depression and anxiety in psycho-cardiological outpatients. Compr Psychiatry 55(1):215–220
51. Cima RF, Crombez G, Vlaeyen JW (2011) Catastrophizing and fear of tinnitus predict quality of life in patients with chronic tinnitus. Ear Hear 32(5):634–641
52. Cima RF, Maes IH, Joore MA, Scheyen DJ, El Refaie A, Baguley DM, Anteunis LJ, van Breukelen GJ, Vlaeyen JW (2012) Specialised treatment based on cognitive behaviour therapy versus usual care for tinnitus: a randomised controlled trial. Lancet 379(9830):1951–1959
53. Henry JA, Flick CL, Gilbert A, Ellingson RM, Fausti SA (2004) Comparison of manual and computer-automated procedures for tinnitus pitch-matching. J Rehabil Res Dev 41(2):121–138
54. Henry JA, Fausti SA, Flick CL, Helt WJ, Ellingson RM (2000) Computer-automated clinical technique for tinnitus quantification. Am J Audiol 9(1):36–49
55. Henry JA, Roberts LE, Ellingson RM, Thielman EJ (2013) Computer-automated tinnitus assessment: noise-band matching, maskability, and residual inhibition. J Am Acad Audiol 24(6):486–504
56. David D, Cristea I, Hofmann SG (2018) Why cognitive behavioral therapy is the current gold standard of psychotherapy. Front Psych 9:4
57. Penner MJ (1983) The annoyance of tinnitus and the noise required to mask it. J Speech Hear Res 26(1):73–76
58. Penner MJ, Brauth S, Hood L (1981) The temporal course of the masking of tinnitus as a basis for inferring its origin. J Speech Hear Res 24(2):257–261
59. Herraiz C, Hernandez FJ, Plaza G, de los Santos G (2005) Long-term clinical trial of tinnitus retraining therapy. Otolaryngol Head Neck Surg 133(5):774–779

60. Henry JA, Stewart BJ, Griest S, Kaelin C, Zaugg TL, Carlson K (2016) Multisite randomized controlled trial to compare two methods of tinnitus intervention to two control conditions. Ear Hear 37(6):e346–e359
61. Bauer CA, Brozoski TJ (2011) Effect of tinnitus retraining therapy on the loudness and annoyance of tinnitus: a controlled trial. Ear Hear 32(2):145–155
62. Bauer CA, Berry JL, Brozoski TJ (2017) The effect of tinnitus retraining therapy on chronic tinnitus: a controlled trial. Laryngoscope Investig Otolaryngol 2(4):166–177
63. Okamoto H, Stracke H, Stoll W, Pantev C (2010) Listening to tailor-made notched music reduces tinnitus loudness and tinnitus-related auditory cortex activity. Proc Natl Acad Sci U S A 107(3):1207–1210
64. Davis PB, Paki B, Hanley PJ (2007) Neuromonics tinnitus treatment: third clinical trial. Ear Hear 28(2):242–259
65. Davis PB, Wilde RA, Steed LG, Hanley PJ (2008) Treatment of tinnitus with a customized acoustic neural stimulus: a controlled clinical study. Ear Nose Throat J 87(6):330–339
66. Newman CW, Sandridge SA (2012) A comparison of benefit and economic value between two sound therapy tinnitus management options. J Am Acad Audiol 23(2):126–138
67. Herraiz C, Diges I, Cobo P, Plaza G, Aparicio JM (2006) Auditory discrimination therapy (ADT) for tinnitus managment: preliminary results. Acta Otolaryngol 126(Suppl 556):80–83
68. Herraiz C, Diges I, Cobo P, Aparicio JM, Toledano A (2010) Auditory discrimination training for tinnitus treatment: the effect of different paradigms. Eur Arch Otorhinolaryngol 267(7):1067–1074
69. Hoare DJ, Kowalkowski VL, Hall DA (2012) Effects of frequency discrimination training on tinnitus: results from two randomised controlled trials. J Assoc Res Otolaryngol 13(4):543–559
70. Vanneste S, van Dongen M, De Vree B, Hiseni S, van der Velden E, Strydis C, Joos K, Norena A, Serdijn W, De Ridder D (2013) Does enriched acoustic environment in humans abolish chronic tinnitus clinically and electrophysiologically? A double blind placebo controlled study. Hear Res 296:141–148
71. Silchenko AN, Adamchic I, Hauptmann C, Tass PA (2013) Impact of acoustic coordinated reset neuromodulation on effective connectivity in a neural network of phantom sound. NeuroImage 77:133–147
72. Tass PA, Adamchic I, Freund HJ, von Stackelberg T, Hauptmann C (2012) Counteracting tinnitus by acoustic coordinated reset neuromodulation. Restor Neurol Neurosci 30(2):137–159
73. Adamchic I, Toth T, Hauptmann C, Walger M, Langguth B, Klingmann I, Tass PA (2017) Acute effects and after-effects of acoustic coordinated reset neuromodulation in patients with chronic subjective tinnitus. Neuroimage Clin 15:541–558
74. Reavis KM, Rothholtz VS, Tang Q, Carroll JA, Djalilian H, Zeng FG (2012) Temporary suppression of tinnitus by modulated sounds. J Assoc Res Otolaryngol 13(4):561–571
75. Llinas RR, Ribary U, Jeanmonod D, Kronberg E, Mitra PP (1999) Thalamocortical dysrhythmia: a neurological and neuropsychiatric syndrome characterized by magnetoencephalography. Proc Natl Acad Sci U S A 96(26):15222–15227
76. Tyler R, Stocking C, Secor C, Slattery WH 3rd (2014) Amplitude modulated S-tones can be superior to noise for tinnitus reduction. Am J Audiol 23(3):303–308
77. Erlandsson SI, Rubinstein B, Carlsson SG (1991) Tinnitus: evaluation of biofeedback and stomatognathic treatment. Br J Audiol 25(3):151–161
78. Heinecke K, Weise C, Rief W (2009) Psychophysiological effects of biofeedback treatment in tinnitus sufferers. Br J Clin Psychol 48.(Pt 3:223–239
79. Landis B, Landis E (1992) Is biofeedback effective for chronic tinnitus? An intensive study with seven subjects. Am J Otolaryngol 13(6):349–356
80. Weise C, Heinecke K, Rief W (2008) Biofeedback-based behavioral treatment for chronic tinnitus: results of a randomized controlled trial. J Consult Clin Psychol 76(6):1046–1057
81. Hoekstra CE, Rynja SP, van Zanten GA, Rovers MM (2011) Anticonvulsants for tinnitus. Cochrane Database Syst Rev 7:CD007960

82. Henry JA, Dennis KC, Schechter MA (2005) General review of tinnitus: prevalence, mechanisms, effects, and management. J Speech Lang Hear Res 48(5):1204–1235
83. Hilton M, Stuart E (2004) Ginkgo biloba for tinnitus. Cochrane Database Syst Rev 2:CD003852
84. Baldo P, Doree C, Molin P, McFerran D, Cecco S (2012) Antidepressants for patients with tinnitus. Cochrane Database Syst Rev 9:CD003853
85. Robinson S (2007) Antidepressants for treatment of tinnitus. Prog Brain Res 166:263–271
86. Langguth B, Elgoyhen AB (2012) Current pharmacological treatments for tinnitus. Expert Opin Pharmacother 13(17):2495–2509

Chapter 8
Cochlear Implantation and Rehabilitation

Fei Chen, Wenli Ni, Wenyan Li, and Huawei Li

Abstract Cochlear implant (CI) is currently the only medical treatment available to partially restore hearing to patients with profound-to-severe hearing loss. CI is fundamentally distinct from hearing aid (HA) use, as implants are surgically placed under the skin behind the ear where they bypass the normal sound-conducting mechanism, convert sound signals into electrical stimulation, and directly stimulate the residual auditory nerves. In recent years, CI has evolved into one of the most profound advances in modern medicine and provided hearing to more than 320,000 deaf patients. According to the time of onset, deafness is classified as prelingual and postlingual deafness, and the indications of cochlear implants vary slightly. The medical evaluation must be made before surgery, including the medical history, objective and subjective audiometry, imaging of the ear, as well as the genetic diagnostic. Here we reviewed the surgical approaches for cochlear implants as well as the complications.

Keywords Cochlear implantation · Speech processing · Speech perception · Indication · Surgery approach · Outcome expectation

8.1 Background of Cochlear Implants

The first account of electrical stimulation of the auditory system was provided by Alessandro Volta. He placed two metal probes in his own ears and connected each pole of a battery to the metal probes. His experience of a "crackling and boiling" sensation was likely the first demonstration that electrical stimulation can induce

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auditory sensation [1]. In 1957, André Djourno and Charles Eyriès conducted the first experiment to directly stimulate the human auditory nerve with electricity [2]. Two patients with complete deafness who underwent surgery reported clear auditory perception. In 1961, Drs. William House and John Doyle implanted gold-insulated electrodes into the scalae tympanorum of two patients who were deaf [3]. These patients reported that they could detect changes in pitch from an electrical stimulus. In 1964, Blair Simmons and his colleagues at Stanford University implanted an electrode into the vestibule and directly onto the modiolus of the cochlea in one patient [4]. The patient could detect changes in duration and tonality.

Implant technology advanced markedly from the 1970s to 1990s. From 1972 to the mid-1980s, the number of implantation surgeries increased to more than 1000. The first single-channel cochlear implant (CI) was implanted in 1972, and an early single-channel device used during the mid-1980s notably enhanced speech perception [5]. However, the latter device did not achieve the level of normal neural activity that was produced by acoustic stimulation of the inner hair cells in a functional cochlea. In 1975, Bilger and his colleagues at University of Pittsburgh were commissioned by the National Institutes of Health to evaluate speech performance in the world's first group of single-electrode CI recipients [6]. The research report showed that, although the single-electrode CI could provide useful information to identify common environmental sound, it could not provide open-set speech recognition. In 1984, the 3M/House single-electrode CI was approved by the Food and Drug Administration for commercial use. The first multichannel CI system was developed that same year by the Cochlear Corporation. Large-scale clinical trials concluded that the multi-electrode device did indeed provide superior sound perception compared to the single-electrode device [7]. Introduction of the multi-electrode device led to the eventual disuse of the single-electrode device commercially. Since that time, many researchers have sought to improve the design and speech processing strategies used in the CI system. In the 1990s, major improvements in speech processing technology and miniaturization allowed CI to gain a foothold for use in mainstream medicine, where it has been recognized as substantially improving patients' quality of life.

Presently, CI systems are mainly manufactured by three corporations: Med-EL Corporation (Austria), Cochlear Corporation (Australia), and Advanced Bionics Corporation (United States). Multi-electrode CIs are currently also being developed by other companies, including Advanced Cochlear Systems (United States), Nurobiosys Corporation (Korea), and Nurotron Biotechnology (China). During the last 10 years, developments in the field of microelectronics and advances in signal processing techniques have led to significant improvements in CI systems. Although significant progress has been made, CIs present further challenges. Speech recognition in a quiet environment has plateaued. In evaluating new CI products and patient use, researchers should focus on improving speech recognition in noise, music perception, and tonal language understanding, all of which are challenging tasks for current implant users.

As of December 2012, over 324,200 patients with hearing loss have received CIs in the world. In the United States, approximately 58,000 devices have been implanted in adults and 38,000 in children. From 30 June 2012 to 30 June 2013, approximately 50,000 CIs were sold throughout the world. Nearly 30,000 of these 50,000 CIs were received by children. An estimated 134 million children are born each year, and this figure is predicted to remain stable over the next few years. However, approximately one to three newborns in a thousand have hearing loss, which can only be treated with CIs. Hence every year over 134,000 CIs are needed to provide for children with profound-to-severe sensorineural hearing loss. If patients consider using bilateral implantation, the number would continue to increase.

8.2 Design of Cochlear Implants

Cochlear implants bypass the normal auditory mechanism, which picks up the sound and transforms the acoustic pressure wave into the movements of the hair cells in the cochlea, and electrically stimulate the remaining auditory neurons directly, resulting in restoring partial hearing. Several available CI systems have been developed over the past years. Almost all of them consist of the following essential components in common [8, 9]: (1) one or more microphones that sense and receive the sound from the environment, (2) a signal processor that extracts features in the sound of microphone output and converts the features into a set of electrical signals, (3) a transmission system that sends power and transmits the electrical signals across the skin to the implanted array of electrodes, and (4) an electrode or an electrode array embedded in the cochlea. Figure 8.1 shows the typical architecture of a modern cochlear implant.

Most importantly, signal processing strategies used for deriving electrical stimuli from the speech signal may lead to the performance gap between CI listeners and normal-hearing people [8, 9]. Single-channel implants first implanted in human subjects in the early 1970s were capable of conveying temporal envelope information and limited spectral information. Multichannel implants providing electrical stimulation at multiple sites were introduced in the 1980s so that different auditory nerve fibers can be stimulated at different places. The CI signal processor is responsible for transforming the acoustic cues into electrical signals delivered to the appropriate electrodes.

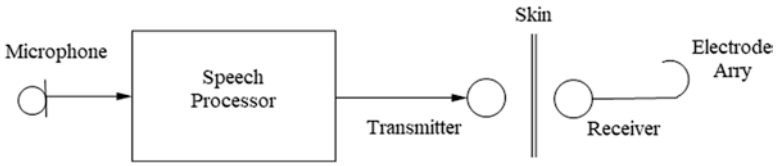

Fig. 8.1 The typical architecture of a modern cochlear implant [8]

Most CI speech processing strategies discard the fine structure and encode the coarse features only. Spectral features, such as the fundamental frequency (F0) and the second formant (F2), were extracted by the first-generation multi-electrode Nucleus 22 device [10]. In the advanced version of the implant, the addition of the first formant (F1) that emphasized low-frequency information improved the speech understanding but did not yield significant improvement on consonant-recognition scores [11]. A further improvement was multi-peaks (MPEAK) strategy that extracted high-frequency information using three additional band-pass filters (2000–2800 Hz, 2800–4000 Hz, 4000–6000 Hz) [12]. However, these three strategies had a limitation of dependence on the formant-extraction algorithms and would be subject to the situations where the speech signal was exposed to noise.

In the late 1980s and early 1990s, researches showed that the temporal envelopes obtained by filtering the sound into different frequency bands could support a high level of speech recognition [e.g., 15–16]. In the early 1980s, the compressed analog (CA) strategy was originally employed in the Ineraid device manufactured by Symbion, Inc., Utah [13]. In the CA strategy, the signal was first compressed with an automatic gain control; passed through 4 band-pass filters with center frequencies at 0.5, 1, 2, and 3.4 kHz; and then delivered simultaneously to corresponding electrodes. A potential problem associated with simultaneous simulation strategies was current interaction, which could distort speech spectrum information and therefore degrade speech understanding. The continuous interleaved sampling (CIS) approach utilized trains of non-simultaneous and interleaved pulses, addressing the channel interaction issue [14]. The original stimulus was pre-emphasized and then was divided into four analysis frequency bands within the whole frequency range of 100–6000 Hz. The temporal envelopes in each sub-band were used to amplitude modulate biphasic pulse trains, whose carriers interleaved with pulsatile carriers from other sub-bands. The CIS strategies have been successfully used in three commercially cochlear corporations, although they reduced some of the temporal information delivered to the cochlea and were lack of the temporal fine structure. The "HiResolution" (HiRes) strategy, a close version of CIS, used relatively high cutoff frequencies for the envelope detectors to analyze the acoustic signal and delivered relatively high rates of stimulation [15].

Unlike previous strategies, the spectral peak (SPEAK) strategy estimated the outputs of a bank of 20 filters and continuously selected those with the largest amplitude at each cycle rather than extracting any features from the speech waveform [16]. In a strategy called N-of-M, "N" bands with the largest envelope amplitude were selected, and "M" was corresponding to the total number of available channels [17, 18]. The Advanced Combination Encoder (ACE) strategy was extremely similar to the N-of-M strategy, with differences in some implementation details [17, 18]. The range of peak selection and the rate in the ACE strategy were both higher than those in the SPEAK strategy. The SPEAK and ACE strategies were the same as the CIS strategy when N = M. The parameter "N" was fixed in the N-of-M and ACE strategies, but that parameter depending upon the signal level and spectral composition condition could vary from frame to frame in the SPEAK strategy.

Generally, the above strategies demonstrated a good speech perception in a quiet environment, moderate perception ability in noise, and a poor capacity for music appreciation due to insufficient fine structure information [9]. At present, increasing attention has been devoted to representing temporal fine structure cues in cochlear implants [19, 20]. The current electrode manufacturing technology and the placement of these electrodes in the cochlea limit an increase of the physical electrode number and are unable to encode the fine structure. Existing solutions mainly include several advanced versions of the CIS, e.g., the fine structure processing strategy (FSP) [19] and HiRes with Fidelity 120 option (HiRes 120) [20]. FSP uses the filters with bell-shaped frequency response to provide fine spectral information. FSP also utilizes the simulations at the one to three most apical electrodes at a variable rate corresponding to the fine structure of the signal from the specific filter band to provide fine temporal information. The purpose of the FSP is to better enable users to perceive pitch variations which is useful to improve speech recognition especially in noise. The HiRes 120 strategy implements a current steering technique which has the capability of creating virtual spectral channels up to 120 channels to substantially enhance the spatial resolution of simulation and to increase the transmission of fine structure information [39, 40]. HiRes 120 also makes full use of steeper analysis filter slopes than HiRes, contributing to higher pitch-ranking capacity [20].

A hearing aid (HA) amplifies sound through the use of a microphone, an amplifier, a loudspeaker (receiver), and a battery. HAs are worn in the ear, in the ear canal, or behind the ear, and users can increase the volume of sound from external sources. HA amplifies sound waves in certain frequencies and then projects those sound toward the inner ear, where they can be processed by the auditory nerve. CI differs from HA use in that it additionally makes use of an external sound processor and internal components. For patients with absent or reduced cochlear hair cell function, HA is insufficient to compensate for hearing loss. To treat profound-to-severe sensorineural hearing loss, CI restores hearing loss by bypassing the normal sound-conducting mechanism of the damaged cochlea. The implant generates a signal that is sent to the auditory nerve and subsequently to the brain, where it is recognized as sound. Because CI directly stimulates the auditory nerve, hearing quality differs with a CI compared to normal hearing or hearing with a HA. CI users report that voices sound more robotic in quality. With HA use, sound quality is more similar to normal hearing, as the cochlea may not be as severely damaged.

8.3 Indications

Cochlear implants are now universally considered to be the standard medical treatment of severe to profound sensorineural hearing loss both in children and adults. The possibility of hearing rehabilitation must be confirmed before surgery. As the development of surgical techniques and auditory prostheses, the candidacy criteria also evolved.

The selection criteria for patients depends on the FDA approved clinical trial and criteria recommended by 2013 Hainan conference. According to the time of onset, deafness is classified as prelingual and postlingual deafness, and the criteria vary slightly.

1. Pre-lingual deafness
   (a) 12 months to 6 years of age; children implantation before 6 months is not recommended in China. For children implanted at or after age of 6, we recommend improved auditory detection abilities, improvements in speech perception, history of hearing aid use, and training.
   (b) Severely or profound sensorineural hearing loss (PTA thresholds>90 dB HL).
   (c) At least 3–6 months of hearing aid use with little or no benefit.
   (d) No surgery contraindication.
   (e) Realistic expectations by the patient and family members.
   (f) Willingness to comply with follow-up procedures.
2. Postlingual deafness
   (a) Severely or profoundly sensorineural hearing loss of all age, with no benefit from hearing aid use
   (b) No surgery contraindication
   (c) Realistic expectations by the patient and family members
   (d) Willingness to comply with follow-up procedures
3. Surgery contraindication

The basic condition for successful application of cochlear implants is a functional hearing nerve, intact central auditory pathways, and an anatomically developed cochlear for insertion of the electrode carrier. So, severely cochlear malformation, lack of auditory nerve, and acute suppurative otitis are absolute contraindication for cochlear implantation. Patients with uncontrolled epilepsy, severe intellectual disability, mental disease, or behavior disorder are not recommended for cochlear implantation, because they cannot comply with follow-up trainings.

4. Specific issues
   (a) Auditory neuropathy/dys-synchrony(AN/AD)

Auditory neuropathy/dys-synchrony has been specified as a hearing disorder in which inner hair cell ribbon synapses, auditory nerve dendrites, or auditory nerve axons are dysfunctional [21]. AN/AD occurs in about 10% of patients with a dys-synchronous ABR, and they may not benefit much with hearing aids because amplification only provides increased sound intensity but cannot improve neural synchrony. With careful evaluation, cochlear implants may be an optional treatment for both children and adults with AN/AD, and improvement of speech perceptions has been observed. However, cochlear implantation is still contraindicated in which neural function is significantly compromised or the auditory nerve is absent [22].

(b) Chronic suppurative otitis media

Cochlear implantation was initially viewed as contraindicated with CSOM because of the potential risk of infection. Some retrospective studies have shown that the prevalence and severity of otitis media do not increase following implantations [23, 24]. Surgeons can choose a two-stage or a single-stage approach accordingly. For patients with a dry ear, myringoplasty can be performed followed by cochlear implantation in 3–6 months. For patients with cholesteatoma, cochlear implantation is performed after the radical mastoidectomy and obliteration 3–6 months later. Patients with a stable cavity can receive a single-stage approach, which includes cavity obliteration and implantation.

(c) Inner ear malformation

Inner ear malformation is found on CT about 20–40% of patients with congenital sensorineural hearing loss, and it was regarded as a contraindication to surgery at the beginning of the cochlear implant. As the development of cochlear implant technique and the HRCT, in the present day, cochlear implantation surgery in inner ear malformation is accepted as a standard surgical approach. The identification of inner ear malformation before surgery is important, which can result in the outcome of the surgery.

8.4 Preoperative Evaluation

The possibility of hearing rehabilitation must be confirmed, including a developed cochlear and the connection to the functional hearing nerve. Meanwhile, healthy condition must be evaluated, especially for elderly patients and children, to make sure they are tolerated for the surgery. The medical evaluation must be made before surgery, including:

1. Patients' history

Surgeons should be detailed about patients' medical history. The otologic history includes age of onset, the progression, and the reason of hearing loss; the history of tinnitus and vertigo; risk factor exposure (such as noise, ototoxicity, infection); history of infectious diseases; history of vestibular dysfunction; the usage of hearing aids; prenatal and postnatal history, specifically drug use, alcohol intake, and tobacco use; and perinatal risk factor (such as low birth weight, prematurity, hyperbilirubinemia, birth hypoxia). Family history of hearing loss is also important. Physical and psychological condition should also be taken into account.

2. Otologic evaluation includes auricle, external ear meatus, and tympanic membrane.

3. Audiologic test

Function of hearing nerve and the central hearing pathway is tested before surgery, as well as speech recognition abilities. Pure tone threshold (PTA) and speech recognition are subjective tests; for children before 6 years old, behavioral audiometry and play audiometry are measured instead of PTA. Objective functional tests include otoacoustic emission (OAE) and cochlear microphonic (CM), which can be used to measure test cochlear function, while the auditory brainstem responses (ABR) are an objective measure for the function of peripheral and brainstem auditory system.

Objective and subjective audiometries are necessary for prelingually deaf patients. The following values are referral criteria for prelingually deaf patients:

Subjective audiometry: mean hearing threshold of behavioral audiometry without hearing aid >80 dBHL, threshold above 2 kHz with hearing aid >50dBHL.

Objective audiometry: threshold of ABR >90 dBnHL, ASSR of both ear (above 2 kHz) >90dBnHL, OAE of both ear is negative.

The postlingually deaf patients, who dispose of an acoustic memory, benefit highly from cochlear implantation. The referral criteria for postlingually deaf patients are mean hearing threshold of both ear without hearing aid >80dBHL and speech recognition of better ear with hearing aid <70%.

4. Imaging

Many patients with congenital hearing loss will be found to have an associated cochlear malformation. Imaging is important to diagnose if there is an inner ear malformation and its type. The inner ear malformation is associated with a malformed cochlear structure, abnormal distribution of spiral ganglion neuros, and cochlear nerve deficiency, which can result in less favorable post-implantation speech perception ability. It can also result in narrow facial recess, facial nerve anomaly, and defect in the IAC, which increase surgical risk including facial nerve injury and CSF gusher. The surgeons make decisions of treatment, surgical approach, and electrode according to the information from imaging.

High-resolution temporal bone computed tomography (HRCT) of axial and coronal sections should be scanned before surgery for all cochlear implant candidates. HRCT provides bony details of temporal bone, which is useful for the identification and classification of inner ear malformation. Facial nerve abnormalities and IAC width can also be evaluated through HRCT. Meanwhile, surgeons get valuable information such as size of mastoid, facial recess, and position of segments of the bony facial canal.

MRI is helpful to evaluate the nerves in the IAC and cochlear fluids. The absence of cochlear nerve is a contraindication to cochlear implantation. It can be absent in inner ear malformation, especially in patients with a common cavity abnormality.

5. Genetic diagnostics

At least 60% of pediatric hearing loss is genetic, and they are classified into non-syndromic and syndromic types. It is estimated that about 70% of all hereditary hearing losses are non-syndromic [25]. The diagnostic of hereditary hearing loss requires a multidisciplinary approach, including physical examination, genetic test-

ing, and counseling. Genetic syndromic hearing loss is a small proportion of all profound hearing impairment, such as Usher syndrome and Pendred syndrome, which is characterized by not only hearing loss but also malformations of oculus, branchial arch, or pharyngeal. It is necessary for all adult and children with profound hearing loss to have a complete physical examination, including skull, craniofface, ocular, ear, neck, oral cavity, and oropharynx. The ears should be examined for preauricular pits, abnormalities of the external ear canal and middle ear. The neck should be inspected for branchial anomalies. Meanwhile, tests of gait and balance should be taken into account to assess vestibular function.

8.5 Surgical Approaches

On 1984, the first single-channel CI was approved by the FDA for implantation in adult patients with profound postlingual deafness [26]. Over the last decades, CI design and surgical techniques have evolved dramatically.

1. Mastoidectomy with posterior tympanotomy approach (MPTA), also named facial recess approach

It is introduced by Dr. House and has been the most common used approach in cochlear implantation surgery. In this approach, a mastoidectomy is performed, then facial recess opened, and the round window niche and the promontory visualized [27]. Placement of the electrode array is accomplished through a cochleostomy or through the round window membrane. This approach provides better visualization and exposure of round window niche, which is helpful for electrode array insertion and retaining [28]. However, there are risks of facial and chorda tympani nerve paralysis in MPTA, and for patients with inner ear malformation, the cochlear anatomy is unfavorable through the facial recess area. Meanwhile, the approach resulted in the communication of tympanic cavity and external auditory canal, which raises the possibilities of postoperative infection and cholesteatoma formation.

2. Suprameatal approach (SMA)

In 1999, Kronenberg introduced a technique that avoids a mastoidectomy altogether and introduces the electrode into the middle ear via a suprameatal route. The SMA approach involves exposing the middle ear through the external auditory canal and inserting electrodes into the cochlea through a suprameatal tunnel bypassing the mastoid cavity [29]. There is lower risk for facial and chorda tympani nerve paralysis using SMA approach, and there is shorter duration of the surgery compared with MPTA technique. However, the possibility of observing the interior of the cochlea is compromised, which deters ability to observe the course of the electrode array along the basal turn after insertion. The SMA approach includes in increased risk of electrode stretched during insertion because of the difficult superior insertion of the electrode. Another disadvantage of the SMA technique is the difficult insertion for

round window and inferior cochleostomy. Additionally, this approach is difficult for low-lying dura patients [30].

3. Middle cranial fossa approach (MCF)

Colleti firstly published papers referring to cochlear implantation through the middle fossa in 1998. Middle fossa approach has widened the selection criteria of cochlear implantation surgery, such as patients with CSOM, ossified cochlea, and inner ear dysplasia, and has been proposed as a valuable alternative approach. Successful cochlear implantation via MCF has resulted in satisfactory hearing; however, this approach is difficult, and the duration of the surgery is long. Meanwhile, MCF approach provides the risk of intracranial complication and more mental pressure for patients. This approach should be used in special cases only [31].

4. Other non-mastoidectomy approach

Alternative approaches have been described in cochlear implantation surgery of inner ear malformation patients, such as transcanal approach in common cavity and hypoplastic cochlear. This cochlear implantation technique uses an endaural approach for access to the cochleostomy, which requires special tools to drill a direct tunnel and insert the electrode safely [32, 33].

5. Cochleostomy or round window insertion

In the CI surgery, it was believed that the electrode insertion resulted in intracochlear trauma, which irreversibly destroying the residual hearing. In order to preserve residual low-frequency hearing, many investigators have sought the least traumatic way to insert the electrode array and protect the delicate intracochlear structures.

(a) Cochleostomy technique was proposed by Lenhardt in 1993, which was performed by drilling the promontory anterior/inferior to the round window membrane. The design of the cochleostomy is determined the electrode. The cochleostomy should be made as small as possible to insert the electrode. This traditional cochleostomy approach has been used by many surgeons. However, in this approach, the inner ear might exposed to acoustic trauma while drilling, and once the endosteum is exposed, bone dust and blood might enter into the cochleostomy [34, 35].
(b) Round window approach involves only a minimal incision of the round window membrane, which avoids the potential acoustic trauma and postoperative vertigo. Round window approach in combination with a standard electrode has resulted in preservation in residual low-frequency hearing and the benefit of EAS in children [36]. However, several studies showed that there was no significant difference in the residual hearing preservation rates in patients using the two approaches [37, 38].

6. Cochlear malformation

Inner ear malformations used to be a contraindication to cochlear implantation. With the development of cochlear implant and the technique of surgery, more and more patients who received cochlear implantation were reported. Nowadays, cochlear implantation is a standard approach for inner ear malformation.

The outcome of cochlear implantation is manifold and depends on the anatomical situations. Detailed evaluation must be done before surgery, and the choice of electrode should be taken into consideration according to the malformation of inner ear. The classifications of malformation that are accepted and used most widely are those of Jackler, Sennaroglu and Saatci, and Sennaroglu. Based on Sennaroglu and Saatci, the classification and electrode choice of cochlear implantation are as followed. Based on the reviews of cochlear implantation in inner ear malformation by Sennaroglu and Lenarz [39–41], we summarized the following Table 8.1.

One of the problems for cochlear implantation in inner ear malformation patients is the facial nerve paralysis, because certain abnormalities may cause anomalies in the location of the facial canal. It is important for surgeons to possess good knowledge of the anatomy and excellent surgery skills. Intraoperative facial nerve moni-

Table 8.1 Malformations of inner ear and cochlear implantation

Type of malformation	Characteristics	Electrode choice
Aplasia		
Labyrinthine aplasia	Absent of cochlea, vestibule, vestibular aqueduct, and cochlear aqueduct	ABI
Cochlear aplasia	Absence of the cochlea	ABI
Common cavity	Cochlea and vestibule are represented by a single chamber	CI with ring electrodes
Incomplete partition		
IP type I	Absence of the entire modiolus and interscalar septa	CI, standard electrode
IP type II	Only basal part of the modiolus	CI, Standard electrode
IP type III	Present of interscalar septa, absence of modiolus	CI with pre-shaped electrode for securing the intracochlear electrode position
	Observed in X-linked deafness	
Hypoplasia		
Type I	Bud-like cochlea	CI with short electrode
	No modiolus or interscalar septa	
Type II	Cystic hypoplastic cochlea	CI with short electrode
	Smaller cochlea, no modiolus and interscalar septa	
Type III	Cochlea with less than two turns	CI with short electrode
Others		
Large vestibular aqueduct syndrome	Enlarged vestibular aqueduct	CI, standard electrode
Narrow IAC	IAC smaller than 2.5, with/without hypoplastic cochlear nerve	CI, in case of failure: ABI

toring is helpful in cases with inner ear malformations in cochlear implantation surgeries.

Most cochlear implantation in malformed inner ear can be done using the classical MPTA technique. However, the surgery may be impossible because of the complex malformations, such as abnormal location of the facial nerve and severe hypoplastic cochlea with underdeveloped promontory, and the surgeons must be ready to modify the surgical approach.

8.6 Surgical Complications

Cochlear implantation is a safe hearing rehabilitation surgical technique associated with a low complication rate. Since the application of this surgery, many complications have been reported, which are classified as the major and minor complications. Major complications, which require surgical revision or hospitalization treatment, include electrode failure, mastoiditis, permanent facial paralysis, and CSF otorrhea. Minor complications are those only requiring conservative management or minimal surgery, such as imbalance, temporary facial nerve palsy, and dysgeusia. These complications are associated with either the surgical technique or the foreign body implantation. Many cases of major complications such as facial paralysis were considered avoidable by strict operative and postoperative procedures, while some cases such as flap infection may not be able to be avoided completely. It is reported that the global complication rate is 5% of major complication and 14.9% of minor complications [42].

8.7 Outcome Expectations

The outcomes are assessed by means of standardized test according to different age. In the context of children, the Child Development after Cochlear Implantation (CDaCI) study developed a hierarchical battery of speech perception measures for tracking skills in pediatric implantees. This hierarchical test battery includes the Infant-Toddler Meaningful Auditory Integration Scale (IT-MAIS), Meaningful Auditory Integration Scale (MAIS), the Early Speech Perception (ESP) test, and the Pediatric Speech Intelligibility (PSI) test [43].

1. Prelingually deaf patients

Usually, early implanted children (1st year of life) achieve very good speech development scores compared with those of normally hearing children in quiet environments. In noise, poorer scores are observed. Compared to normally hearing people, deficits remain even in cases of early implantation [41].

A number of pre-implant factors influence early auditory and speech perception development in children with CIs. Earlier implantation is associated with higher

performance; preoperative residual hearing and consistency of hearing aid use also influence performance on speech perception skills [44, 45].

Research shows that speech perception, speech production, and language and reading skills improve over time after CI [46], and the first 6 months after CI activation is critical for early auditory development, leading to a measurable development in speech perception [47]. Some post-implant factors contribute to the performance level, including length of cochlea implant use, rehabilitative training, communication mode, and family support [48].

2. Postlingually deaf patients

The majority of postlingually deafened adult demonstrate significant pre- to postoperative implant. There are some factors that affect performance for postlingually deaf patients, including amount of residual hearing, duration of profound hearing loss, age of implant, cognitive abilities, and so on [49]. Although older age was associated with poorer recognition of words and negatively impact CI outcomes, elderly patients benefit from cochlear implantation during long-term use. Research showed that speech perception in geriatric patients continues to improve for up to 5 years following surgery. Cochlear implantation has been shown to significantly improve health-related quality of life and cognitive function [50].

Post-implant factors that contribute to performance levels include length of cochlear implant use, comfortable levels used for device programming, lifestyle, and social interaction.

3. Bilateral cochlea implantation

In the last few years, bilateral implantation has become more common, because studies demonstrated clear benefits in multiple aspects. Bilateral CIs provide binaural summation, which yields a benefit of approximately 2 dB for listeners with normal hearing [51]. Study suggested that a bilateral CI recipient would bring a 14% improvement in speech recognition in noise compared with a unilateral CI recipient [52]. Specifically, bilateral CI improved the ability to localize sound in not only quiet environment [53] but also in noise [54]. On the other hand, the application of bilateral CI is argued because of cost benefit and the increased risk of complication. However, bilateral CI is used increasingly, and several studies have recommended it as the standard treatment option for adults and children with bilateral profound sensorineural deafness [55, 56].

References

1. Volta (1800) On the electricity excited by mere contact of conducting substances of different kinds. Royal Soc Philos Trans 90:403–431
2. Djourno A, Eyries C (1957) Auditory prosthesis by means of a distant electrical stimulation of the sensory nerve with the use of an indwelt coiling. Presse Med 65(63):1417–1417
3. House WF, Urban J (1973) Long term results of electrode implantation and electronic stimulation of the cochlea in man. Ann Otol Rhinol Laryngol 82(4):504–517

4. Simmons FB, Epley JM, Lummis RC, Guttman N, Frishkopf LS, Harmon LD, Zwicker E (1965) Auditory nerve: electrical stimulation in man. Science 148:104–106
5. Fretz RJ, Fravel RP (1985) Design and function: a physical and electrical description of the 3M House cochlear implant system. Ear Hear 6(3):14S–19S
6. Bilger RC (1977) Psychoacoustic evaluation of present prostheses. Ann Otol Rhinol Laryngol Suppl 86:92–104
7. Gantz B, Tyler RS, Abbas P, Tye-Murray N, Knutson JF, Mccabe BF, Lansing C, Brown CJ, Woodworth G, Hinrichs J, Kuk F (1988) Evaluation of five different cochlear implant designs: audiologic assessment and predictors of performance. Laryngoscope 98(10):1100–1106
8. Loizou PC (1999) Introduction to cochlear implants. IEEE Eng Med Biol Mag 18(1):32–42
9. Loizou PC (1999) Signal-processing techniques for cochlear implants. IEEE Eng Med Biol Mag 18(3):34–46
10. Seligman PM, Patrick JF, Tong YC, Clark GM, Dowell RC, Crosby PA (1984) A signal processor for a multiple-electrode hearing prosthesis. Acta Otolaryngol 98(sup411):135–139
11. Tye-Murray N, Lowder M, Tyler RS (1990) Comparison of the F0F2 and F0F1F2 processing strategies for the Cochlear Corporation cochlear implant. Ear Hear 11(3):195–200
12. Patrick JF, Clark GM (1991) The nucleus 22-channel cochlear implant system. Sci Publ 5(370):1989–1990
13. Eddington D (1980) Speech discrimination in deaf subjects with cochlear implants. J Acoust Soc Am 68(3):885–891
14. Wilson BS, Finley CC, Lawson DT, Wolford RD, Eddington DK, Rabinowitz WM (1991) Better speech recognition with cochlear implants. Nature 352(6332):236–238
15. Han D, Liu B, Zhou N, Chen X, Kong Y, Liu H, Xu L (2009) Lexical tone perception with HiResolution and HiResolution 120 sound-processing strategies in pediatric Mandarin-speaking cochlear implant users. Ear Hear 30(2):885–891
16. Seligman P, McDermott H (1995) Architecture of the Spectra 22 speech processor. Ann Otol Rhinol Laryngol 8(761):139–141
17. Skinner MW, Arndt PL, Staller SJ (2002) Nucleus 24 advanced encoder conversion study: performance versus preference. Ear Hear 23(1):2–17
18. Patrick JF, Busby PA, Gibson PJ (2006) The development of the Nucleus Freedom Cochlear implant system. Trends Amplif 10(4):175–200
19. Arnoldner C, Riss D, Brunner M, Baumgartner WD, Hamzavi JS (2007) Speech and music perception with the new fine structure speech coding strategy: preliminary results. Acta Otolaryngol 127(12):1298–1303
20. Firszt JB, Holden LK, Reeder RM, Skinner MW (2009) Speech recognition in cochlear implant recipients: comparison of standard HiRes and HiRes 120 sound processing. Otol Neurotol 30(2):146–152
21. Starr A, Picton T, Sininger Y, Hood L, Berlin C (1996) Auditory neuropathy. Brain 119(Pt 3):741–753
22. Hood LJ (2015) Otolaryngologic clinics of North America. Otolaryngol Clin N Am 48:1027–1040
23. House W, Luxford W, Courtney B (1985) Otitis media in children following the cochlear implant. Ear Hear 6:24S–26S
24. Luntz M, Hodges A, Balkany T, Dolan-Ash S, Schloffman J (1996) Otitis media in children with cochlear implants. Laryngoscope 106:1403–1405
25. Morton N (1991) Genetic epidemiology of hearing impairment. Ann N Y Acad Sci 630:16–31
26. House W, Berliner K (1986) Safety and efficacy of the House/3M cochlear implant in profoundly deaf adults. Otolaryngol Clin N Am 19:275–286
27. Mangus B, Rivas A et al (2012) Surgical techniques in cochlear implants. Otolaryngol Clin N Am 45:69–80
28. Iseli C, Adunka OF, Buchman CA (2014) Scala tympani cochleostomy survey: a follow-up study. Laryngoscope 124:1928–1931

29. Kronenberg J, Migirov L, Dagan T (2001) Suprameatal approach: new surgical approach for cochlear implantation. J Laryngol Otol 115:283–285
30. Postelmans JT, Grolman W, Tange RA, Stokroos RJ (2009) Comparison of two approaches to the surgical management of cochlear implantation. Laryngoscope 119:1571–1578
31. Gawecki W, Karlik M, Borucki L, Wróbel M, Stieler O, Szyfter W (2018) Middle fossa approach for cochlear implantation. Otol Neurotol 39:e96
32. Kiratzidis T, Arnold W, Iliades T (2002) Veria operation updated. I. The trans-canal wall cochlear implantation. ORL 64:406–412
33. Kiratzidis T, Iliades T, Arnold W (2002) Veria operation. II. Surgical results from 101 cases. ORL 64:413–416
34. Pau H, Just T, Bornitz M, Lasurashvilli N, Zahnert T (2007) Noise exposure of the inner ear during drilling a cochleostomy for cochlear implantation. Laryngoscope 117:535–540
35. James C, Albegger K, Battmer R et al (2005) Preservation of residual hearing with cochlear implantation: how and why. Acta Otolaryngol 125:481–491
36. Skarzynski H, Lorens A, Piotrowska A, Anderson I (2007) Partial deafness cochlear implantation in children. Int J Pediatr Otorhinolaryngol 71:1407–1413
37. Sun C, Hsu C, Chen P, Wu H (2015) Residual hearing preservation after cochlear implantation via round window or cochleostomy approach. Laryngoscope 125:1715–1719
38. Havenith S, Lammers MJ, Tange RA et al (2013) Hearing preservation surgery: cochleostomy or round window approach? A systematic review. Otol Neurotol 34:667
39. Sennaroglu L, Saatci I (2002) A new classification for cochleovestibular malformations. Laryngoscope 112:2230–2241
40. Sennaroglu L (2009) Cochlear implantation in inner ear malformations – a review article. Cochlear Implant Int 11:4–41
41. Lenarz T (2018) Cochlear implant – state of the art. GMS Curr Top Otorhinolaryngol Head Neck Surg 16:Doc04
42. Farinetti A, Gharbia BD, Mancini J, Roman S, Nicollas R, Triglia J-M (2014) Cochlear implant complications in 403 patients: comparative study of adults and children and review of the literature. Eur Ann Otorhinolaryngol Head Neck Dis 131:177–182
43. Eisenberg LS, Johnson KC, Martinez AS et al (2006) Speech recognition at 1-year follow-up in the childhood development after cochlear implantation study: methods and preliminary findings. Audiol Neurootol 11:259–268
44. Dettman SJ, D'Costa WA, Dowell RC, Winton EJ, Hill KL, Williams SS (2004) Cochlear implants for children with significant residual hearing. Arch Otolaryngol Head Neck Surg 130:612–618
45. Gantz B, Rubinstein J, Tyler R et al (2000) Long-term results of cochlear implants in children with residual hearing. Ann Otol Rhinol Laryngol 185:33–36
46. Dunn CC, Walker EA, Oleson J et al (2014) Longitudinal speech perception and language performance in pediatric cochlear implant users: the effect of age at implantation. Ear Hear 35:148
47. Chen Y, Wong L, Zhu S, Xi X (2016) Early speech perception in Mandarin-speaking children at one-year post cochlear implantation. Res Dev Disabil 49:1–12
48. Geers A, Brenner C, Davidson L (2003) Factors associated with development of speech perception skills in children implanted by age five. Ear Hear 24:24S–35S
49. Lin FR, Chien WW, Li L, Clarrett DM, Niparko JK, Francis HW (2012) Cochlear implantation in older adults. Medicine 91:229
50. Yang Z, Cosetti M (2016) Safety and outcomes of cochlear implantation in the elderly: a review of recent literature. J Otol 11:1–6
51. Bronkhorst A, Plomp R (1988) The effect of head-induced interaural time and level differences on speech intelligibility in noise. J Acoust Soc Am 83:1508–1516
52. Schafer EC, Amlani AM, Seibold A, Shattuck PL (2007) A meta-analytic comparison of binaural benefits between bilateral cochlear implants and bimodal stimulation. J Am Acad Audiol 18:760–776(17)

53. Neuman AC, Haravon A, Sislian N, Waltzman SB (2007) Sound-direction identification with bilateral cochlear implants. Ear Hear 28:73
54. Mosnier I, Sterkers O, Bebear J-P et al (2009) Speech performance and sound localization in a complex noisy environment in bilaterally implanted adult patients. Audiol Neurotol 14:106–114
55. Papsin BC, Gordon KA (2008) Bilateral cochlear implants should be the standard for children with bilateral sensorineural deafness. Curr Opin Otolaryngol 16:69
56. Balkany T, Hodges A, Telischi F et al (2008) William House Cochlear Implant Study Group: position statement on bilateral cochlear implantation. Otol Neurotol 29:107

Chapter 9
Non-implantable Artificial Hearing Technology

Ling Lu, Xiaoli Zhang, and Xia Gao

Abstract A hearing aid is a sound-amplifying device used for aiding hearing-impaired individuals and compensating hearing loss. With the development of science and technology, tremendous strides have been made in hearing aid technology. The history of hearing aids can be divided into five eras: acoustic era, carbon era, vacuum tube era, transistor and integrated circuit era, and digital era. It mainly comprises microphone, amplifier, receiver, battery, volume and tone control buttons, and other electroacoustic components, which can be classified into following types: pocket hearing aids, BTE hearing aids, ITE hearing aids, ITE hearing aids, completely/invisible-in-canal hearing aids (CIC and IIC), and other types. Of them, one bone-anchored hearing aid (BAHA) softband is mainly applicable to children under 5 years old who cannot wear bone-anchored hearing aids in congenital bilateral external auditory canal occlusion. BAHA system comprises three components: a titanium implant (fixture screw), an external abutment (bridging screw), and a sound processor consisting of a microphone and a transducer. The following procedures are included in hearing aid fitting: medical history collection, trial, fitting, ear sample taking, hearing aid refitting, evaluation, practical instruction, and follow-up care. Children should be helped to select hearing aid as early as possible, and for those with congenital hearing loss or prelingual deafness, it can be added assessment of speech development while preventing the child from swallowing hearing aids or batteries by mistake. Speech recognition score (SRS) test is very important in elderly patients, because the speech hearing and pure-tone hearing of presbycusis are often inconsistent. After the hearing aid is selected, the effect evaluation and related rehabilitation should be carried out timely.

Keywords Hearing aids · Bone-anchored hearing aid softband · Amplification

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9.1 Introduction

Artificial hearing devices fall into two categories: implantable and non-implantable. The most typical non-implantable hearing device is hearing aid. This paper briefly describes the development history, main types, working principle, and matching process of hearing aid. It also includes considerations for children and the elderly to choose hearing aids, as well as hearing rehabilitation assessment after wearing hearing aids.

9.2 History of Hearing Aids

A hearing aid is a sound-amplifying device used for aiding hearing-impaired individuals and compensating hearing loss. With the development of science and technology, tremendous strides have been made in hearing aid technology. The history of hearing aids can be divided into eras: sound collector era, carbon era, vacuum tube era, transistor and integrated circuit era, and digital era [1].

9.2.1 Sound Collector Era

According to long-term life experience and production practice, placing cupped hand as well as animal horns, shells, or shell trumpets behind the ear could amplify the sound, which provided a 3–5 dB of gain and reduced behind-the-ear (BTE) noise [2, 3]. With heightened awareness in this domain, people no longer contented themselves with natural hearing aids and began to design various acoustic hearing aids. The more effective versions emerged in the mid-seventeenth century.

9.2.2 Carbon Era

The early carbon hearing aid in its simplest form comprised a carbon microphone, battery (3–6 V), and magnetic receiver. Based on electromagnetism, a carbon hearing aid could generate fluctuating current flow and magnetic field, vibrating the receiver diaphragm to amplify sound [4]. Such early hearing aids were too bulky to be carried around. In those circumstances, to offer higher gains, carbon amplifiers were developed. In the times of carbon amplifiers, it was assumed that amplifications vary with frequencies, which could be achieved using different microphones, amplifiers, and receivers.

9.2.3 Vacuum Tube Era

Vacuum tube amplifiers were invented in 1906 and were applied in hearing aids in 1920 [5]. Several vacuum tubes were connected to make possible amplifiers with greater power, thereby increasing gains. The greatest problem of vacuum tube hearing aids was still the large size. Subsequently, driven by the demands of military applications, the size was rapidly decreased. In the late 1930s, the technology of vacuum tubes and batteries enormously progressed so that it could be employed in hearing aids and allowed for batteries, microphones, and amplifiers mounted in one-piece body-worn hearing aids. Moreover, the advent of multiple novel technologies and materials in World War II led to further significant reduction in the size of one-piece hearing aids [6]. In 1938, the first wearable vacuum tube hearing aid was eventually made in the United Kingdom.

9.2.4 Transistor and Integrated Circuit Era

In 1952, the transistor was commercially available. Until 1953, it required much lower operating voltages and was small in size compared to the vacuum tube. All new hearing aids were equipped with transistors rather than vacuum tubes.

In the 1960s, remarkable improvements were made in the performance of hearing aid elements. By the early 1980s, most of the elements could be fitted in the ear canal; therefore, in-the-ear (ITE) hearing aids were small. As a result of the progress in battery chemistry, amplifier effect, and microphone technology, the whole hearing aid could be mounted in the ear canal by the early 1990s. Completely-in-the-canal (CIC) hearing aid eventually made the device redundant. They provided acoustic advantages. For the user, the auricle could still pick and filter sound as well as reduce wind noise.

9.2.5 Digital Era

Research on digital processing started from the Bell Labs in 1960 when the digit circuit was first adopted in the hearing aids. However, because computers were running at a low speed, the processing of the input sound signal could not keep up with the output of the sound. By 1970, faster computer running speed permitted simultaneous processing for input and output; by 1980, substantial decrease in energy consumption and further reduction in size made possible wearable digital hearing aids with amplifier circuits. With the digital control circuits, it was convenient for users to set the characteristics of hearing aids. The attached remote control could also serve this purpose, which was appropriate for ITE and CIC types. Digital technology application has brought about a revolution in hearing aids.

9.3 How Hearing Aids Work

Hearing aid is an electroacoustic amplifier that amplifies faint sound until it reaches the intensity to meet human hearing requirement. It mainly comprises microphone, amplifier, receiver, battery, volume and tone control buttons, and other electroacoustic components [7, 8] (Fig. 9.1).

9.3.1 Microphone

Microphone converts sound waves into electric signals. On the basis of its working, it can be classified into electromagnetic induction coil, piezoelectric ceramic, electret, silica gel, directional, etc.

Its frequency response and sensitivity may vary depending on the requirements of the hearing aid. Damper can also be placed to reduce the peak of frequency response. In the technical approaches of modifying specific properties of the microphone frequency response, electronic filter is relatively commonly used. Its popularity rests on the ability to lower the internal noise of the microphone. The microphone can pick sound from all directions (omnidirectional) or a specific direction (directional).

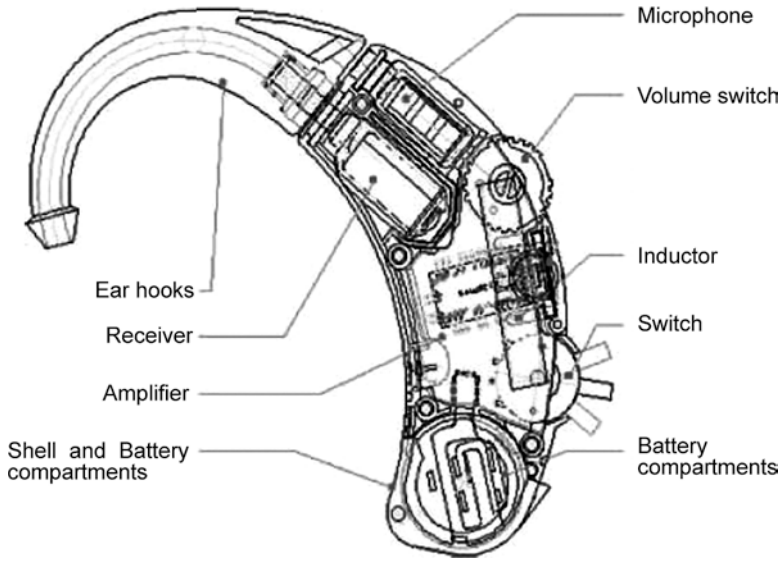

Fig. 9.1 Structure diagram of hearing aids. (Drawn on the basis of network picture)

9.3.2 Amplifier

As the pivot of hearing aid, amplifier is exclusively used for signal processing. The sound gathered by the microphone is processed (e.g., strengthened and filtered) by an amplifier chip. The chip can also control volume, peak clipping, function, signal compression, etc.

9.3.3 Receiver (Earpiece)

Receiver converts enhanced electric signal into sound wave. Its frequency response directly impacts the quality of final audible sounds. Its sensitivity and maximum output are dependent on the size; therefore, the receivers of ITE and CIC types are small at the expense of maximum effects.

9.3.4 Battery

Battery is the power source to make hearing aid works properly. A quality hearing aid battery should feature high capacity, low internal resistance, and prolonged service life. In addition, a premium battery can still work well at low temperatures.

9.3.5 Function Adjustment Knob of Hearing Aids

Analog circuit hearing aids are often configured with adjustment knobs and enable audiologists to customize hearing solution, such as gain preset, filter, output limitation, peak clipping, compression amplitude, compression amplification, etc. Users should not alter the configuration without permission. Certainly, there are some function buttons allowing users to operate and make adjustments as needed, e.g., volume wheel and on/off switch.

9.3.6 Auditory Auxiliary Components

1. Loop System

Loop system includes loop amplifier and loop coil. A well-functioning loop system can produce electromagnetic waves within the loop. The voice of theater actors/actresses or teacher in classroom can be emitted in the loop through an electromagnetic emission device. The switch to a telecoil (T) mode enables the user to clearly

hear these sounds and filters out background noises. Therefore, the sound intensity is not reduced by changes in distance and can be invulnerable to reverberations and background noises.

2. Direct Audio Input

Many BTE hearing aids are equipped with three metal contacts that can fit into a boot-shaped adapter to directly receive sound or audio signals from TV and radio. Therefore, the high-frequency components of a signal are not lost due to excessively long distance, and background noises are not directed into the hearing aid. In addition, signal-to-noise ratio is considerably elevated.

3. Frequency Modulation (FM) System

FM system can be considered as a wireless microphone comprising a directional microphone, FM converter, and FM receiver. Speaker wears a microphone on his/her collar, with a FM converter around his/her waist receiving audio signals transmitted by a connecting lead. The converter then emits the signal with specific FM frequency. The hearing aid on each user is mounted with a FM receiver to reproduce the sound signal. FM system is very suitable in deaf education.

9.4 Types and Characteristics of Hearing Aids

Hearing aids can be classified into following types [9]:

9.4.1 Pocket Hearing Aids

A pocket hearing aid resembles a mini radio. Its major components, which are microphone, amplifier, and battery, are fitted in a pocket. The pocket and earbuds (or earbuds attached onto ear molds) are connected via a fine wire.

The larger size of this type brings about multiple advantages, including great power, wide frequency response, multifunction control, low price, large *on-off* rotary switch, convenient use, and servicing. It is useful in patients with severe-to-profound hearing loss and the elderly with less flexible fingers. Its disadvantages include tendency to produce friction with clothes, unnecessary low-frequency gains, and large size resulting in striking and unpleasant look making it clunky and inconvenient to carry.

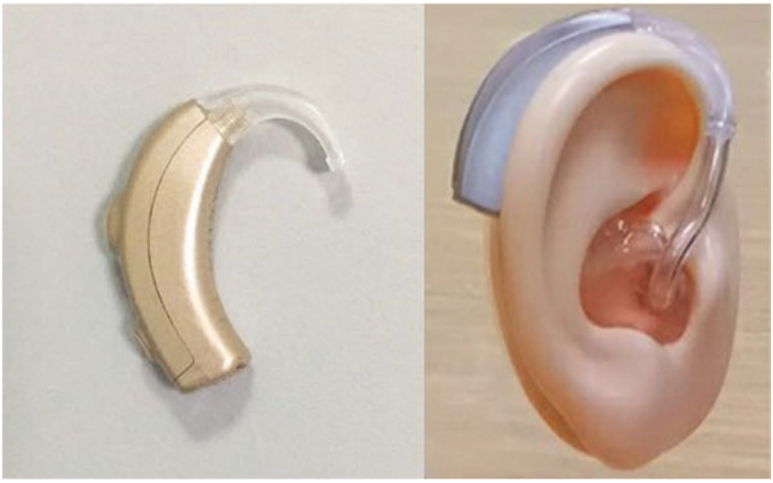

Fig. 9.2 BTE hearing aids

9.4.2 BTE Hearing Aids

A BTE hearing aid is a hearing instrument with primary components housed in a crescent-like casing that rests behind the ear. It is a preferred option for children (Fig. 9.2).

BTE is small and compact, with all the components, including microphone, amplifier, battery, volume control, peak-clipping device, and receivers, encased. Currently, it is an ideal and commonly used hearing device available for children and elderly with hearing loss. It is popular because of high power, small size, low noise, slight distortion, good appearance, and ease of handling.

9.4.3 ITE Hearing Aids

ITE hearing aids are divided into three types according to its location in the ear: ① ITE full shell fitted into the full cavum concha; ② ITE half shell filling half of cavum concha; and ③ ITE low profile. ITE casing is customized according to the user's ear model. Some ITEs are finished products with fixed shape and size, which requires a customized ear mold. The user inserts ITE into the mold and fits it inside the ear (Fig. 9.3).

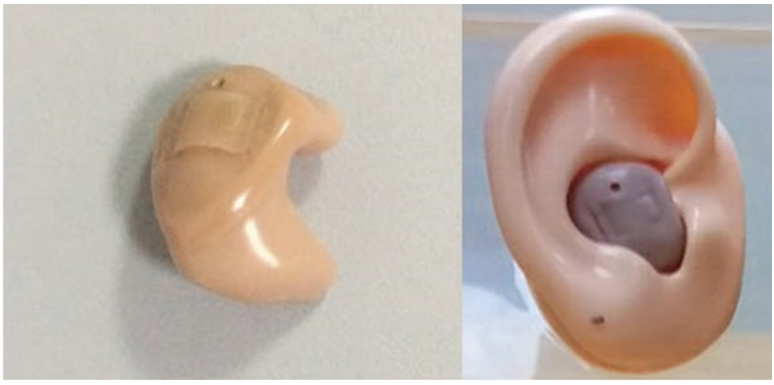

Fig. 9.3 ITE hearing aids

Generally, ITE power ranges from 40 to 110 dB. In consideration of its acoustic characteristics and pros and cons, ITE is normally suitable for the patients who develop moderate-to-severe hearing loss but are unwilling to wear BTEs. In addition, it is convenient to mount more accessories, making it more applicable to the patients looking for high-performance hearing aid. It is also an option for the middle-aged and elderly as well as those with flexible fingers and more severe hearing loss.

9.4.4 In-the-Canal Hearing Aids (ITC)

ITC is a hearing instrument with the shell customized to fit the shape of external auditory canal, sitting from the cavum conchae and extending near the isthmus (Fig. 9.4).

ITC is small, attractive in appearance, and comfortable to wear. In particular, it is physiologically oriented to help improve gains and sound source localization. However, it is also the smaller size that tends to cause acoustic feedback; the intracanal position makes it susceptible to earwax; thus, occlusion effect can easily occur.

Currently, the power of common ITC is normally <80 dB, and some high-power ITC models of certain brands can also have a power of 90–110 dB, but the clinical application is limited. ITC is generally used in following population:

① The young; mild-to-moderate hearing loss; having stringent requirements for hearing aid appearance.
② The middle-aged and elderly; mild-to-moderate hearing loss; flexible hands; having stringent requirements for hearing aid appearance and performance.
③ With average hearing loss lower than 80 dB, patients with high-frequency hearing loss having descending audibility curve. ITC can provide more high-frequency gain compensation.

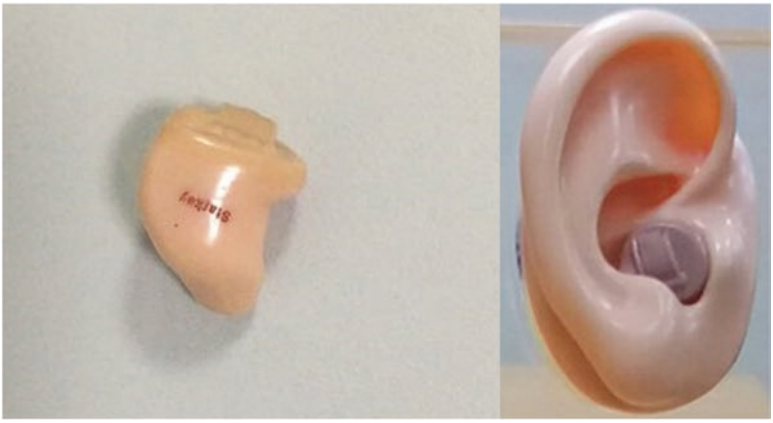

Fig. 9.4 ITC hearing aids

9.4.5 Completely/Invisible-in-Canal Hearing Aids (CIC and IIC)

CIC and IIC are hearing instruments with the shell customized to fit the shape of external auditory canal, sitting from external acoustic porus and extending near the isthmus. They are superior in audible sound transmission, boasting high gains, less occlusion effect, improved sound source localization, and enhanced fidelity. On one hand, it is comfortable and aesthetic and owing to its small size, high invisibility, humanized design, and outstanding immobility. On other hand, it is not recommended for individuals with severe hearing loss as the small size of casing and battery results in limited output power. Lastly, it is expensive because of the customization cost (Fig. 9.5).

9.4.6 Other Types

Apart from the aforementioned types, there are other hearing aids applicable to some special types of hearing loss. In comparison with air conduction hearing aids, they compensate for hearing in other ways, such as contralateral routing of signal, bone conduction, implantable, and soft shell.

9.5 Bone-Anchored Hearing Aid Softband

Aural rehabilitation is always tricky for the children with congenital bilateral atresia of external auditory canal. In addition, the influence of single-sided deafness on sound localization development is still uncertain. Controversy still exists as to the

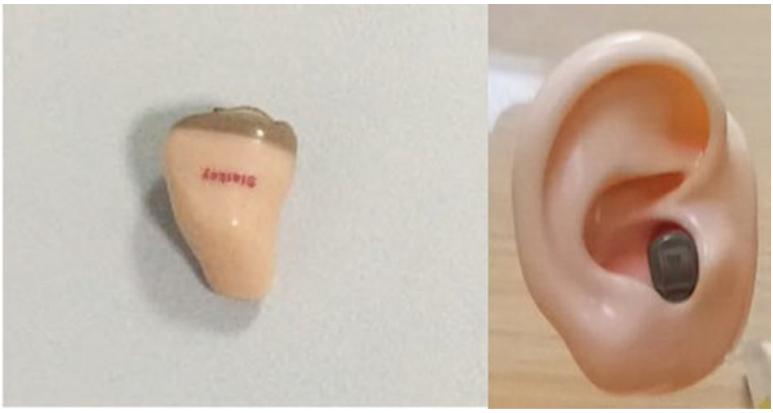

Fig. 9.5 CIC hearing aids

rehabilitation of unilateral hearing loss. The traditional bone-conduction hearing aids are not usually worn and provide less ideal benefits for various reasons. Children aged <5 years cannot wear bone-anchored hearing aids (BAHA) [10, 11]. As clinicians and audiologists become more experienced and knowledgeable about BAHA and have witnessed the advances of BAHA system, softband version has been invented, transforming the standard therapy for such hearing loss. The new BAHA has functions similar to those of traditional ones and overcomes rehabilitation difficulties of the said hearing loss.

9.5.1 BAHA System Components

BAHA system comprises three components: a titanium implant (fixture screw), an external abutment (bridging screw), and a sound processor consisting of a microphone and a transducer. It uses the transducer to transmit the vibrations of the sound (received by the microphone inside sound processor) through the abutment into the implant of the skull, sending the sound directly to the inner ear, thereby bypassing the external ear and middle ear with barriers. A consensus has been reached that implantable BAHA is not suited for children aged <5 years because of low cortical bone thickness and vulnerability to the implant.

BAHA softband is a transition before an implant. An abutment implant is secured in an elasticated headband, which can be adjusted according to head circumference. The headband can also be fixed in different sites of skull, which avoids pressure and discomfort when sound processor always stays in the same place. BAHA softband starts to work after sound processor is connected with abutment. BAHA softband comprises two parts: a headband equipped with an abutment and a sound processor. The abutment is secured onto the head by the headband, so the scalp is included in

the vibration transmission path to the skull; therefore, there is 10–15 dB of sound energy loss compared to BAHA implant.

9.5.2 Indication

The morbidity of congenital malformations of the external and middle ear is approximately 1/10,000, of which the incidence of congenital bilateral atresia of external auditory canal is only approximately 10–25%. In most cases, congenital external auditory canal atresia is accompanied with auricular deformities, which means it is hard for these patients to wear traditional hearing aids. It is difficult for severe cases to improve hearing through surgical reconstruction due to malformations in the middle ear and ossicular chain. Furthermore, the surgery can only be performed after the age of 6 years. Therefore, from birth to the age of 5 years, which is the most important period for speech and language development, it is difficult to effectively rehabilitate children with bilateral ear malformations in hearing and speech ability. These patients are the best candidates for BAHA softband. Myrthe et al. [12] have conducted a study on the language development in children with congenital bilateral atresia of external auditory canal using BAHA softband. They showed that the development of language comprehension and expression was normal before 30 months, was lower than normal levels within 30–46 months, and progressed significantly when the hearing was improved after BAHA was bilaterally worn. It demonstrates that BAHA softband meets the demand of basic language development, but the children at the age of 3 or 4 years need clearer sound to grasp more complex language. An implantable solution can improve the sound transmission, 15 dB more effective than softband. Therefore, it is recommended to progress from a softband to an implant as the patient ages [13].

Some studies reveal that softband can be worn as early as at the age of 3 months. In the early period of speech development, the canonical babbling stage is of prime importance. Canonical babbling normally occurs at 6–10 months. Studies show that the canonical babbling presented after 10 months of age adversely affects speech development. Patients with hearing loss can benefit from wearing softband before canonical babbling appears. Studies indicate that those receiving hearing intervention before 6 months of age perform evidently better in the test of language comprehension and expression (at 3–4 years of age) than those having late intervention.

Compared to traditional bone-conduction hearing aids, BAHA softband noticeably improves with respect to stability and comfort with the use of elastic headband. It has a good appearance and is relatively acceptable. In contrast, it is uncomfortable to wear traditional bone-conduction hearing aids since the vibrator needs to be closely attached on the skin behind the ear, stimulating and damaging the contact area, sometimes causing headache; the shaky vibrator during exertion can affect speech recognition; the unsatisfactory look makes children reluctant to use it.

So far, BAHA softband has become an effective substitute for traditional bone-conduction hearing aids, which can be used until it is appropriate to have hearing surgery or wear surgically implanted BAHA. It provides physicians and patients with more therapy options in terms of method and timing. Meanwhile, for severe unilateral conductive hearing loss, it is generally recommended to wear BAHA softband at affected side as soon as possible to help develop directional stereophonic hearing whose specific peak stage is uncertain [14].

For patients with bilateral ear malformations, a customized softband can be worn on both sides or on one side with better bone-conduction threshold. When the children begin to wear it, the volume should start from 0.5 to 1 and should be gradually increased to 1.5. The user's reactions should be observed. Sound can be made to test if it is too loud. Care should be taken to ensure the patient feels pleasant on the first try. Initially, 10–15 min are enough, and the duration can be gradually extended afterward. It is recommended that the children be accompanied by adults when using BAHA softband. The softband benefits can be examined through pure-tone testing where hearing thresholds before and after wearing are compared to evaluate auditory functions; a behavioral observation audiometry in sound field can also be performed on the children; speech audiometry is at present the best assessment tool of auditory function; the observation of infants' speech development level also provides the information about outcomes.

9.6 Selection of Hearing Aids

The patients with different hearing losses manifest high variability of hearing conditions. The fundamental question and the basis of auditory and language rehabilitation is how to select the right hearing aids and adjust performance parameters of hearing aids for obtaining maximum compensation benefits [15].

9.6.1 Fitting Formulas of Hearing Aids

Audiology professionals have been committed to identifying routine parameters in hearing loss and amplification characteristics of hearing aids, making the output of hearing instruments provides best hearing loss compensation and optimal communication outcomes. These parameters constitute fitting formulas primarily covering linear and nonlinear equations for children and those independently developed by some hearing aid providers [16]. The formulas are generally included in the computer software which will automatically calculate necessary amplification characteristics when the user enters the required data (e.g., audiogram or test volume).

9.6.2 Real Ear Measurement (REM)

REM is the acoustic measurement in a patient's ear [17]. REM provides the objective assessment for the outcomes brought by the amplification when a hearing aid is worn and clearly shows how the hearing aid processes the sound in the ear. The understanding of transition between REM data and standard test data facilitate the clarity of hearing aid formulas and adjustment of hearing aids. REM is usually conducted on REM equipment, but sometimes it can be done in sound field.

9.6.3 Hearing Aid Fitting

1. Medical History Collection

   Medical history collection includes the following:

① Inquire medical history to determine other rehabilitation possibilities (medication and surgery).
② Inquire ear surgery history to judge possible contraindications of hearing aid wearing and whether special approaches and considerations are needed.
③ Inquire patients' expectations and special requirements

2. Trial

   The patient should be guided to try hearing aid after selecting a proper model and ensuring it is worn unilaterally or bilaterally. The purpose of the trial is to examine the gain, slope, and maximum output the patient needs and let him/her experiences the hearing benefits after the instrument is worn.

3. Fitting

   The patient should be instructed to choose the most appropriate type of hearing aid and ear mold depending on key factors, such as evaluation findings, hearing loss conditions, hearing requirements, financial situation, age, preference, education background, and working environment.

4. Ear Sample Taking

   Based on final choice made by the patient, the sample should be taken for making customized hearing aid or BTE ear mold.

5. Hearing Aid Refitting

   When the patient comes to pick up the hearing aid, the professional should slightly adjust it depending on his/her hearing condition and feels. Comfort always outweighs clear hearing.

6. Evaluation

For the evaluation of hearing aid benefits, it could be best to perform sound field test and REM if possible, particularly for hearing-impaired children. By this way we can further identify the appropriateness of gain and frequency response and the capability of speech recognition. The patient should be assessed before and after fitting to acquire comprehensive benefits.

7. Practical Instruction

The patient should be guided as follows:

① Instruct the patient how to use and maintain the hearing aids, including battery installation and service time, approaches of moisture and water resistance, and how to wear the instrument properly.
② Work out the plan of after-sale service and maintenance with the patient.
③ Introduce the four stages about hearing aid adaptation (1–2 months).
④ Remind the patient of preserving dry box, receipt, and warranty card.
⑤ Explain the details about regular and preventive maintenance.

8. Follow-Up Care

The patient should be contacted at a periodic basis, generally once a week in the first month, once every 2 weeks in the second and third months, and once a month thereafter. Some questions about usage should be asked, such as "How does it feel?" "Do you face any problems?" (If there are any problems, the patient should feel free to contact hearing aid fitting center for reassessing benefits and adjusting baseline parameters).

9.7 Fitting Considerations for Children and Seniors

9.7.1 Hearing Aid Fitting for Children [18]

1. Children should be helped to select hearing aid as early as possible because they are at developmental stage, the key period of speech and language development. Hearing loss children using hearing aids can benefit from residual hearing stimulating and utilizing to speak verbal language.
2. Depending on the child's age and cognition level, suitable tests should be taken accordingly to evaluate his/her hearing loss condition. Reliable hearing data is the foundation of proper fitting for hearing loss children and is employed to guide the fitting process.
3. The hearing aid benefits for hearing loss children should be assessed mainly based on aided hearing capability, speech recognition ability, subjective evaluation for the specific outcomes of the instrument, and discomfort assessment. For

the children with congenital hearing loss or prelingual deafness, speech development level assessment can be added, which is also one of the aspects used to assess cochlear implant benefits.

4. Do not expect immediate reactions and grasp of all the words after a hearing loss child wears a hearing aid. It requires a process of adaptation and learning. Generally, the child's interest in wearing hearing aid should be drummed up. Let him/her practices in a silent environment and perceives familiar sounds, such as water flow and door closing. Step by step, have him/her using the instrument in the surroundings filled with various sounds to develop the ability to adapt to any sounds. The volume should be kept at moderate level from the beginning and should be increased gradually. Meanwhile, prolong the time he/she wears it. If he/she feels tired or uncomfortable, should take it out immediately.
5. The hearing loss children mostly have difficulties in understanding others' spoken words and expressing their own ideas, e.g., poor articulation. Therefore, it is particularly crucial to have auditory training. For the children with profound hearing loss, it is still impossible to provide best compensation for hearing loss after a hearing aid is worn. Effective communication may be barely established using hearing instrument alone. Hence, to make them learn to speak and develop in language, other sensory information (e.g., vision) should assist in conjunction of the full use of auditory information.
6. Safety Concerns

   ① Battery ingestion. Children at any age might risk ingesting batteries. Parents are advised to keep new and old batteries safe from little hands, particularly for children aged <3 years. It is extremely dangerous if the batteries are ingested as toys or snacks.

   ② Hearing aid causes exposure to strong noise, which might exacerbate hearing loss. Children's residual hearing is definitely precious; thus audiologists may protect it by specifying proper gains and OSPL90 values. Hearing aids with low compression ratio should be chosen over linear amplification to lower the risk.

9.7.2 Hearing Aid Fitting for Seniors [19]

1. Speech Recognition Score Test for Seniors in Aural Rehabilitation

For the elderly with hearing loss, inconsistency exists between speech audiometry and pure-tone audiometry. Speech hearing also differs markedly between silent and noisy environment. Thus it is difficult to accurately identify older adults' hearing loss level only depending on pure-tone audiometry. In the circumstance, audiology professionals have paid more attention to speech recognition score test as scientific evaluation tool of auditory perception and rehabilitation outcomes.

2. Post-fitting Consultation and Training for Seniors

The cost-effectiveness of hearing aids is unsatisfactory in the hearing-impaired elderly since the benefits are largely compromised due to pathophysiological features and limitation of hearing aid electroacoustic performance. That is why it is hard to fulfill some patients' overly high expectation, thereby inducing some psychosocial problems and affecting the seniors' aural rehabilitation. In this case, after hearing aid fitting, the older adults should be specifically explained in auditory characteristics of presbycusis and associated facts about hearing aid. Training should be properly provided to make them willingly accept hearing aids to improve its benefits and the aural rehabilitation.

9.8 Evaluation for Rehabilitation and Benefits

After hearing aid fitting, it is necessary to ensure whether the selected amplification route can reach the objective of hearing loss compensation and whether the hearing aid can benefit and satisfy the user [20–22]. Therefore, the benefits of the hearing instrument should be evaluated. The evaluation may proceed in stages with multiple approaches. A satisfied patient should see the outcomes and hearing aid performance consistent with his/her expectations.

9.8.1 Preliminary Evaluation

1. Comparison Between Unaided and Aided Thresholds

Whether hearing aid effectively compensates hearing loss can be verified through REM and sound field audiometry. When REM of gains is unavailable, functional gains may be measured to identify the compensation. The functional gain refers to the difference value between unaided and aided hearing thresholds. The aided hearing threshold can be determined via earphone or sound field. For BTEs, aided hearing thresholds must be measured in sound field where warble tone is tested; for various ITCs, a headphone (over-ear or on-ear headphone) is normally used to measure aided hearing threshold with the premise that the tested hearing aid will not generate audio feedback (squeal) after the headphone is worn. Aided and unaided hearing thresholds should be compared to obtain gain condition provided by the hearing aid through difference value.

2. Speech Test

Speech test is an objective method of directly assessing speech comprehension before and after hearing aid use.

3. Questionnaire Methodology

In a questionnaire, the patient is required to answer questions about the hearing condition in some special scenarios, such as a talk with a salesperson in a supermarket. Additionally, simple drawings can help the patient confirm any environment referred. Each question in the questionnaire offers multiple answers available or only several options to be selected.

4. Self-Report Inventory

There are many questionnaires available in self-report inventory to assess hearing aid outcomes by evaluating disability and impairment. Some questionnaires include both unaided and aided communication ability, which are primarily suited for adults, such as abbreviated profile of hearing aid benefit, hearing handicap inventory for the elderly, client-oriented scale of improvement, etc.

5. Internationally Recognized Outcome Measurement

In 1999, after in-depth discussion, the international outcome items for hearing aids (IOI-HA) was developed by all 12 participants (Robyn Cox et al.) at an international workshop in Denmark, covering a minimal set of core outcome items. It applies to a variety of investigations conducted in different countries across the world. The inventory mainly includes hearing aid usage, such as hours of daily use and impact on quality of life. It is not intended to replace existing outcome measures but to function as an addendum to existing measures in a research context. It might potentially serve as a stand-alone tool for quality assessment.

9.8.2 Mid-stage Evaluation

1. Hearing Aid Usage Consultation

In the earlier period, hearing aid usage and adaptation vary with patients. Some stick to a rehabilitation plan and implement it step by step, giving the instrument into full play, but some only wear it occasionally or even leave it on the shelf due to discomfort on the first try or any other reasons. We should search for root causes if the patient seldom uses it or doesn't wear it at all: it is attributed to patient's selection, or we have made mistakes in hearing aid fitting.

2. Hearing Aid Troubleshooting

The hearing aid outcomes and patients' satisfaction will be inevitably affected when various problems arise out of hearing aids. The professionals should be familiar with the common problems and offer a timely solution to prevent the troubles from having adverse impacts on hearing aids.

3. Evaluation of Patient' Satisfaction with Hearing Aids

A patient's satisfaction with a hearing aid is directed by many factors. The most important decisive factor might be the patient' pre-fitting expectation and the expectation altered by the professionals. A psychological counseling should be provided to dispel wrong notions if it is sky-high expectation that leads to lower satisfaction.

9.8.3 Regular Follow-Up

It is critical to follow up regularly after hearing aid fitting. Different problems occur when the patient begins to use it. Promptly assisting them in addressing problems may boost their confidence in hearing aid usage and make them adapt to it quickly, thereby enhancing their satisfaction.

9.8.4 Influence of Hearing Aids on Health and Life Quality

The hearing aid use can improve the quality of life, thereby promoting physical and psychological health. Patients with hearing loss are poorly capable of communicating owning to auditory limitation. Less social interactions result in growing tendency of self-isolation; therefore, the patients tend to be unsociable, depressive, grumpy, irritable, and suspicious. The intimacy with friends destroyed over time as well as the lack of security in life can do harm to physical and psychological health, particularly for hearing loss children. Adequate studies reveal that for hearing loss individuals, hearing aid users lead a better life than nonusers. The use of hearing aids can increase users' social participation level and elevate sensitivity to make them engage in more recreational activities, thereby uplifting the mood, relieving mania and depression, and improving memory and learning ability.

References

1. Lybarger S (1990) A historical overview. In: Sandlin R (ed) Handbook of hearing aid amplification: theoretical and technical considerations. Singular, San Diego, pp 1–29
2. Apaix A, Decroix G, Olivier JC (1974) La Prothése Auditive. Arnette, Paris
3. Stephens S, Goodwin J (1984) Non-electric aids to hearing: a short history. Audiology 23:215–240
4. Politzer A (1908) Lehrbuch der Ohrenheilkunde, 5th edn. Ferdinand Enke, Berlin
5. Berger K (1974) The hearing aid its operation and development, Revised edn. National Hearing Aid Society, Livonia
6. Hecter G, Pearson H, Dean N, Carlisle R (1953) Recent advances in hearing aids. J Acoust Soc Am 25:1189–1194

7. Thiede O (2005) Principles of hearing aids. Laryngo-rhino-otologie 84(5):357–370
8. Hirsh IJ (1951) Hearing aids: how they work and for whom. Ann Otol Rhinol Laryngol 60(4):1032–1038, disc 1080-71
9. Palmer CV (2009) A contemporary review of hearing aids. Laryngoscope 119:2195–2204
10. Davids T, Gordon KA, Clutton D et al (2007) Bone anchored hearing aids in infants and children younger than 5 years. Arch Otolaryngol Head Neck Surg 133:51–55
11. McDermott AL, Williams J, Kuo M et al (2009) The Birmingham pediatric bone-anchored hearing aid program: a 15-year experience. Otol Neurotol 30:178–183
12. Myrthe KSH, Cremers CWRJ, Coppens-Schellekens W et al (2005) The Baha Softband: a new treatment for young children with bilateral congenital aural atresia. Int J Pediatr Otorhinolaryngol 69(7):973–980
13. Dun CA, Faber HT, de Wolf MJ et al (2011) An overview of different systems: the bone-anchored hearing aid. Adv Otorhinolaryngol 71:22–31
14. Dun CA, de Wolf MJ, Mylanus EA et al (2010) Bilateral bone-anchored hearing aid application in children: the Nijmegen experience from 1996 to 2008. Otol Neurotol 31:615–623
15. Carhart R (1946) Tests for selection of hearing aids. Laryngoscope 6(12):780–794
16. Ching TYC, Zhang VW, Johnson EE et al (2018) Hearing aid fitting and developmental outcomes of children fit according to either the NAL or DSL prescription: fit-to-target, audibility, speech and language abilities. Int J Audiol 57(sup2):S41–S54
17. Marriage JE, Vickers DA, Baer T et al (2018) Comparison of different hearing aid prescriptions for children. Ear Hear 39(1):20–31
18. Streppel M, Betten T, von Wedel H et al (1997) Progressive hearing loss in children with hearing aids. Laryngo-rhino-otologie 76(3):123–126
19. Malinoff RL (1989) Measurement of hearing aid benefit in the elderly. Ear Hear 10(6):354–356
20. Mulrow CD, Tuley MR (1992) Sustained benefits of hearing aids. J Speech Hear Res 35(6):1402–1405
21. Byrne D, Parkinson A (1990) Hearing aid gain and frequency response requirements for the severely/profoundly hearing impaired. Ear Hear 11(1):40–49
22. Pichora-Fuller MK (2006) Effects of age on auditory and cognitive processing: implications for hearing aid fitting and audiologic rehabilitation. Trends Amplif 10(1):29–59

Chapter 10
Stem Cells: A New Hope for Hearing Loss Therapy

Yang Qiu and Jianhua Qiu

Abstract Permanent hearing loss was considered which cannot be cured since cochlear hair cells and primary afferent neurons cannot be regenerated. In recent years, due to the in-depth study of stem cell and its therapeutic potential, regenerating auditory sensory cells is made possible. By using two strategies of endogenous stem cell activation and exogenous stem cell transplantation, researchers hope to find methods to restore hearing function. However, there are complex factors that need to be considered in the in vivo application of stem cell therapy, such as stem cell-type choice, signaling pathway regulations, transplantation approaches, internal environment of the cochlea, and external stimulation. After years of investigations, some theoretic progress has been made in the treatment of hearing loss using stem cells, but there are also many problems which limited its application that need to be solved. Understanding the future perspective of stem cell therapy in hearing loss, solving the encountered problems, and promoting its development are the common goals of audiological researchers. In this review, we present critical experimental findings of stem cell therapy on treatment of hearing loss and intend to bring hope to researchers and patients.

Keywords Hearing loss · Stem cells · Cell transplantation · Hair cell regeneration

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10.1 Introduction

In the mammalian inner ear, the cochlear hair cells and primary afferent neurons are terminal sensory cells, which do not regenerate after injury; this also is the leading cause of permanent hearing loss [1]. The effect of clinical treatment is unsatisfactory also due to this kind of irreversible damage. For sensorineural deafness caused by hair cell damage, the feasible treatment mainly includes drug therapy, the different types of hearing-aid device, and cochlear implants, but the effect is uncertain and has issue with low population compliance [2]. For auditory neuropathy caused by cochlear spiral ganglion neuron damage, unfortunately there is no effective treatment [3]. In recent years, stem cell therapy and cellular reprogramming technology become the most popular of the forefront of medical research. The high capacity of differentiation ability and low immunogenicity of stem cells provide huge application potential in the disease treatment. At present, the researchers focused on stem cell treatment technology used in the research of cochlear auditory nerve cell regeneration/replacement; its strategies are mainly for two kinds: endogenous stem cell activation and exogenous stem cell transplantation.

10.2 Endogenous Stem Cell

In the study of birds, fish, and amphibians, the vestibular and cochlear hair cells can self-regenerate and repair after injury and also can restore vestibular and auditory functions [4, 5]. Researchers believe that their hair cells and supporting cells involved in the repairing process after injury, but there was no clear evidence of the role of stem cells involved in this process in the inner ear at that stage. In 2003, Li et al. found that there were stem cells which have differentiation potential presented in adult mice inner ear, which provides a possibility of application for endogenous stem cell to treat hair cell damage [6]. In 2007, Oshima et al. isolated stem cells from the organ of Corti and vestibular sensory epithelium of newborn mice. They found that after in vitro induction, these stem cells could differentiate into hair cell-like cells which expressed various hair cell markers and functional ion channels similar to normal hair cells [7]. However, endogenous stem cells from adult mammalian cochlear basilar membrane are in a state of "silent," which cannot spontaneously activate their differentiation potential in response to hair cell damage [8]. And, as the growing of the age, the number of endogenous stem cells also gradually reduced [7]. These problems make the therapeutic potential of cochlear endogenous stem cells only limited to the neonatal mammals with hair cell damage.

In recent years, researchers have successfully isolated cells with proliferation ability and directional differentiation potential from neonatal mammalian cochlea, which is called the cochlear progenitor cells. Cochlear progenitor cells are in a transition state between stem cell and its progeny terminal differentiation cells; they have a stable cell phenotype, preserve limited ability of mitosis, and are considered

to be the important seed cells in hair cell regeneration research [9]. Cochlear progenitor cells are normally in the stationary state, which do not have ability to spontaneously differentiate into the new hair cells; it is probably due to the lack of promoting factor to initiate the progenitor cell differentiation [8]. Therefore, breaking the "silent" state of the progenitor cells, activating the expression of key molecules in the process of hair cell differentiation, and thus promoting the cochlea progenitor cell differentiation into the hair cells are key points to solve the problem.

In the previous study of inner ear development, many genes were considered to participate in regulation of hair cell differentiation process. Their expression products include transcription regulatory factors, growth factors, tyrosine kinase receptor, cell division factor-dependent kinase inhibitors and Notch receptor, Notch activation molecules, and signaling proteins on the cell membrane surface [10]. Members of the family of the bHLH (basic helix-loop-helix) transcription factor (Atoh1, Hes1, and Hes5) and their interaction molecules have been reported to play an important role in the process of hair cell differentiation [10, 11]. Atoh1 (Math1) has proven to be positive regulating factor of hair cell differentiation. As negative regulating factors of hair cell differentiation, the absence of Hes1 can lead to an increased number of inner hair cells, and the absence of Hes5 can lead to an increased number of outer hair cells [12–14]. Through in vitro culture of cochlea tissue of neonatal rat, researchers found that overexpression of Hes1 and Hes5 could have antagonism effects to Atoh1 function of induced ectopic hair cells; this finding indicated that Hes1 and Hes5 inhibited the cochlea progenitor cells differentiating into hair cells by antagonizing Atoh1 function [13]. Other interaction molecules, such as transcription factor Sox2 with its antagonism effect to Atoh1 function during hair cell differentiation process, can inhibit the progenitor cells differentiate into hair cells. Downregulation of Sox2 expression in cochlea and hair cell development stage has been proved to cause precocious hair cell differentiation and an increasing number of inner hair cells [15]. Notch signaling pathway has also been considered to play a key role in the development of sensory epithelium. In mammalian inner ear, the expression of Notch transmembrane receptor during hair cell differentiation is required to activate the downstream signaling pathway, thereby increasing the expression of Hes1 and Hes5 to restrict the number of cell that can adopt the hair cell fate [16]. Canonical Wnt signaling pathway has been proved as a key signal to regulate stem cell proliferation and differentiation [17]. Similarly in inner ear, Wnt pathway mediator, beta-catenin, regulates Atoh1 expression to control hair cell differentiation [18]. In addition to these large molecules, microRNAs also play an important role in regulating stem cell differentiation. MicroRNA-183 family members (microRNA-183, microRNA-96, and microRNA-182) have been reported to have crucial roles in cell-fate determination during inner ear development [19]. The dynamic change in their expression during inner ear progenitor cell differentiation has been assessed and compared to neural stem cells [20]. In vitro studies suggested that these microRNAs would promote inner ear progenitor cell differentiation into a hair cell-like fate, which functions may be associated with transcription factors Tbx1 and Sox2 [20, 21]. These molecules and signaling pathways make a complex

network to regulate hair cell differentiation from stem cells during inner ear development stage. Researchers believe that they found the target molecules to promote progenitor cell differentiation to repair hair cell damage.

In 2011, a stem cell marker and Wnt target gene, Lgr5 was found to be expressed in mouse cochlear duct during embryonic and postnatal periods [22, 23]. After several years of studies, researchers precisely positioned Lgr5 expression in inner pillar cells and third Deiters' cells during neonatal period (Fig. 10.1) and proved that Lgr5+ progenitor cells were capable of differentiating into hair cell by regulating Wnt or Notch signaling pathway [24, 25]. Lgr5+ progenitor cells therefore became the target cells as precursors to hair cells in further researches. Studies around Wnt and Notch signaling pathway in Lgr5+ progenitor cell regulating hair cell regeneration became the focus of the inner ear endogenous stem cell therapy of hair cell damage [26–28]. Although these studies provided inspiring results which observed hair cell-like cell regeneration in vitro by co-regulating Wnt and Notch signals, a series of problems still remain to be solved. These problems include insufficient number of newly regeneration cells; hair cell-like cells do not have normal function as mature hair cells; newly regeneration cells cannot survive for a long period. These problems indicate that it is difficult to regenerate functional hair cells by regulating single or only two signaling pathways. Further researches should focus on multi-genes synergic regulation in order to increase the efficiency of hair cell regeneration; to promote functional maturity of newly regenerated cells; and to increase the survival period of newly regenerated cells.

The studies of inner ear endogenous stem cells demonstrated its application prospect in the treatment of sensorineural deafness, but at present the most effective clinical treatment of sensorineural deafness is still cochlear implants, and the therapeutic effect depends on the quantity and quality of residual spiral ganglion neurons.

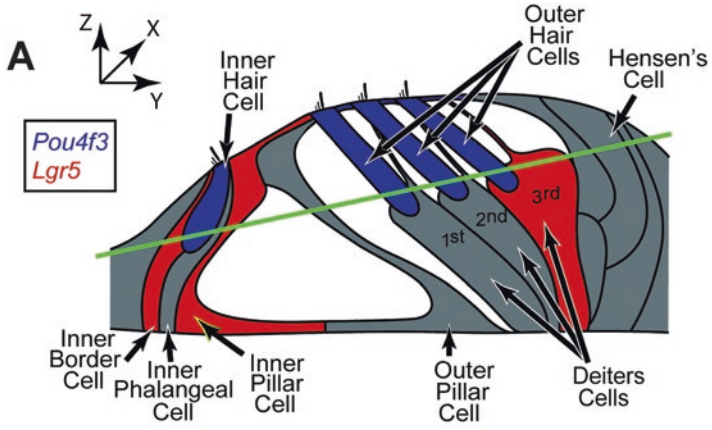

Fig. 10.1 Illustration of Lgr5-positive cells in the organ of Corti. Lgr5-positive cells were considered as inner pillar cells and third Deiters' cells, which were thought to be able to differentiate into hair cells after injury [25]

Neural degeneration has been considered as a secondary consequence of hair cell damage after noise exposure [29, 30]. This consequence greatly limits the functionality of cochlear implants. Primary damage of auditory neurons leads to auditory neuropathy, caused by gene mutation, aging, and trauma, and is another clinic difficult disease with no effective treatment.

The primary approach for functional recovery of neuron loss is to activate endogenous auditory progenitor/stem cell differentiation into neurons. This type of tissue-specific progenitor/stem cells may be closer to the cells' differentiation process in body. Researchers have been isolated cells from spiral ganglion and showed differentiation property [7, 31, 32]. These cells have been demonstrated, after being induced, able to differentiate into neurons with electrophysiological properties of spiral ganglion neurons in vitro. Neurotrophins (NT-3 and BDNF) have been proved to have great effect on neural fiber regrowth to make neurons reconnect to hair cells [33]. However, studies on endogenous stem cells in regeneration of spiral ganglion neurons were limited on in vitro stage and lacked the support of in vivo studies. Other mesenchymal cells in spiral ganglion, Schwann cells, and satellite cells have been shown that did not spontaneously differentiate into neurons [34].

10.3 Exogenous Stem Cells

The other approach is to use exogenous stem cell transplantation to replace damaged neurons or regenerate new neurons. This approach has been deeply investigated in CNS disease [35–37]. And because of low immunogenicity of stem cells, it was considered to be suitable for inner ear application. There are several potential candidates for transplantation to regenerate spiral ganglion neurons. Embryonic stem cell is one of sources for transplantation. Researchers successfully transplanted non-differentiated and partially differentiated embryonic stem cells in the scala media; however there were no regenerated neurons found in spiral ganglion [38]. Sekiya et al. transplanted embryonic stem cell at the internal auditory meatus of an atrophic auditory nerve and found that stem cells migrated along the nerve fibers to the modiolus [39]. In 2008, after in vitro study of inducing neurogenin 1 (neurog1) expression followed by BDNF and GDNF treatment, embryonic stem cells could differentiate into glutamatergic neurons. Researcher brought their findings to in vivo study and received similar result that 50% to 75% of transplanted embryonic stem cell expressed early neural cell marker Tuj1 and most of these cells have glutamatergic phenotype [40]. These findings suggest that embryonic stem cells need to be induced prior to transplant, to increase their differentiation efficiency. Embryonic stem cell-derived neural progenitor cell is another potential candidate of transplantation. Transplantation of partially differentiated embryonic stem cell, induced with basic fibroblast growth factor (bFGF) and insulin-transferrin-sodium selenite (ITSS) to form neuroectoderm-containing embryoid bodies (EBs), only found few amount of cells alive at the transplantation site and target damaged site [41]. Embryonic stem cell-derived neural progenitor cells were injected into the

cochlear nerve trunk in a ouabain-induced primary neuronal degeneration animal and shown that transplanted cells expressed neuron-specific markers and the neurites grew through Rosenthal's canal into denervated organ of Corti [42]. There are several types of mesenchymal stem cell that could be used for transplantation, such as bone marrow-derived mesenchymal stem cell, nasal mesenchymal-like stem cell, and adipose-derived mesenchymal stem cell [43–48]. Among various types of mesenchymal stem cells, bone marrow-derived mesenchymal stem cells are one of the most promising candidates compared to others for cell replacement therapy. However, most of these researches were in vitro studies. In 2011, Cho et al. transplanted neural differentiated mesenchymal stem cells into the cochlea, but only limited number of regenerated neurons were observed and mild hearing function was recovered [46]. Due to lack of convincible in vivo studies, mesenchymal stem cells for sensory cell replacement therapy remain to be further confirmed. Compared with other types of stem cells, neural stem cells are thought to have a better ability of directional differentiation. Parker et al. transplanted c17.2 cNSCs into damaged cochlea and found these stem cells had characteristics of both neuron tissues (spiral ganglion neurons and glial cells) and cells of the organ of Corti (hair cells and supporting cells) [49]. Hu et al. transplanted embryonic dorsal root ganglion (DRG) neurons and applied with chronic electrical stimulation (CES) and exogenous neurotrophic growth factor (NGF) into damaged cochlea, found that transplanted DRG cells expressing neuronal cell marker and positioning close to Rosenthal canal, and extensive neurite outgrowth observed to reach the spiral ganglion region. However, there was no significant difference of hearing function between treated animals and control animals [50]. The emergence of induced pluripotent stem cells (iPSCs) opens a new era of stem cell research. Induced embryonic stem cells from patient's own cells have a huge impact on stem cell therapeutic strategy. The induced cells can be used for autologous transplantation, thus avoiding the immunosuppression and ethical debate. Through a relatively simple process of overexpressing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under embryonic stem cell culture condition, adult fibroblasts could be induced into pluripotent stem cells [51]. After transplanting neurons derived from iPSCs into the cochlea, the cell settlement and neurite projected toward hair cells were observed. Some of these transplanted cells expressed glutamatergic neuron marker, vesicular glutamate transporter 1 (VGLUT1) [52]. The therapeutic effect of induced pluripotent stem cells, however, was not clear. The in vivo research results were only the phenotype changes of cells and there were no significant changes in hearing function [53].

For stem cell transplantation, it should not only be considered the characteristics of different types of cells but also differentiation status of cells. The undifferentiation stem cells in the cochlea have strong abilities of proliferation and migration. Neural stem cells and neural progenitor cells transplanted in the cochlea have strong ability to differentiation into neurons. Auditory neural stem cells are easy to integrate into cochlear tissue and to project neurites to the organ of Corti. However, auditory neural stem cells tend to stay in their transplanted site, rather than migrate toward the Rosenthal's canal. Mesenchymal stem cells have strong ability to differentiate into mesoderm cells and are more suitable for the treatment of hearing loss caused by connective tissue damage.

The optimal strategy of cell transplantation is a complex process. There are many factors to consider in each study, such as the use of different stem cells or cell lines, cell differentiation status, the differences of host species and tissues, and the strategy of cell transplantation techniques, surgical approaches, and transplantation site, which are likely to affect the effect of the transplantation.

10.4 Cell Transplantation Approaches

It is a huge challenge of transplanted cells into delicate cochlea. The main purpose of stem cell transplantation is to deliver cells to the damaged area; firstly, this depends on the cochlear damage status and locations (hair cells or spiral ganglion neurons). Another challenge is to make the transplanted cells reach to the all damaged location of cochlea, at the same time, as far as possible to reduce additional damage caused by transplantation surgery.

The scala tympani of lymphatic system transplantation approach is most commonly used for delivering stem cell into the cochlea. Compared with other approaches, this approach has a larger transplantation space; on the other hand, perilymph flow is the best carrier to bring transplanted cells distributed to whole cochlea. Moreover, scala tympani of lymphatic system transplantation approach can only cause minimum damage to the cochlea; by cochlear dissection nearing round window or directly through the round window, stem cells can be transplanted into the cochlea. However, in most of studies, after researchers delivered embryonic stem cell or mesenchymal stem cells into scala tympani, most of transplanted cells existed in spatia perilymphaticum, and only few number of cells were distributed close to Rosenthal's canal [41, 54, 55]. Transplanted cells migrated into cochlear modiolus is considered to through canaliculae perforantes of Schuknecht, which provided large window for transplanted cells to migrate to Rosenthal's canal [56]. In 2007, with the aid of perfusion system, Parker et al. monitored the distribution status of scala tympani transplanted cells. The results showed that transplanted cells migrated into the organ of Corti, Rosenthal's canal, and even spiral ligament. These cells could differentiate into hair cells, supporting cells, spiral ganglion neurons, satellite cells, and spiral ligament cells. Despite the increase in cell migration rate, this method also might bring potential complication, such as cell diffusion into the cerebrospinal fluid through aqueduct of the cochlea [49].

The main purpose of directly transplanted stem cells into scala media is for hair cell replacement; however there are several biological obstacles that need to be considered. Most of exogenous cells cannot survive under high concentration of potassium ion of endolymph fluid. A previous study showed that high potassium concentration of artificial endolymph fluid, greater than 50 mM, would cause apoptosis and necrosis of transplanted stem cells, whereas low potassium concentration, less than 30 mM, would improve the survival rate of transplanted stem cells [57]. Furthermore, the complex structure of the organ of Corti is another challenge. It is difficult for transplanted cells to cross through the adhesive connection between hair

cells and supporting cells. Iguchi et al. transplanted stem cells into the cochlea through lateral wall and found that stem cells distributed into all three scala with no differentiation and integration into cochlear tissue. There was no significant recovery of hearing function of treated animals [58]. The scala media-transplanted approach through cochlear lateral wall would impair cochlear function, internal ionic environment, and endolymphatic potential. Lesions caused by transplantation approach would also damage stria vascularis and thus affect cochlear blood supply [59].

Cochlear modiolus and auditory nerve trunk approach is mainly used to deliver stem cells to replace degenerated spiral ganglion neurons. Several research teams thought that this approach to deliver stem cell to Rosenthal's canal is more reasonable compared with other two approaches. However, embryonic stem cells transplanted at auditory nerve trunk showed that they migrated from transplanted site along auditory nerve trunk to the peripheral and central nervous system and only few cells migrated to Rosenthal's canal [60]. Evidence showed that transplanted neuroblasts near internal auditory meatus to undamaged auditory nerve trunk differentiated into spiral ganglion neuron-like cells [56]. In order to explore similar technique, neural progenitor cells derived from embryonic stem cells have been used on animal models, transplanted at bony wall between round window niche and auditory nerve. However, there were only few transplanted cells found in Rosenthal's canal, but they formed ectopic ganglion at the transplantation site and projected neurites toward Rosenthal's canal and organ of Corti [42]. Although auditory nerve trunk approach seemed to be suitable for transplanted cells, cells still could not migrate into Rosenthal's canal [61]. However, due to the anatomic characteristics and adjacent relationship of the cochlea, the exposure of the cochlear modiolus in the process of the auditory nerve trunk transplantation would cause damage to the anatomical structure of the cochlea, in which was difficult to maintain the integrity of cochlear function and which was not conducive to auditory function recovery after stem cell transplantation.

In 2013, Zhang et al. demonstrated a new transplantation route by injecting neural stem cell, derived from mouse olfactory bulb, at cochlear lateral wall, rather than penetrating into scala media (Fig. 10.2). They found the transplanted cells migration along basilar membrane and into Rosenthal's canal with high migration and differentiation efficiencies [62]. This study demonstrated a novel approach for stem cell transplantation and found a novel cell migration route, from cochlear lateral wall through basilar membrane to reach Rosenthal's canal, and it revealed that basilar membrane might have crevices permitting stem cells migration.

10.5 Cochlear Internal Environmental Factors and External Stimulation Factors

Cochlear internal environment influences survival, migration, differentiation, and function of transplanted cells [39, 55, 63, 64]. The complexity of ionic environment of the cochlea affects the survival rate of transplanted cells. High potassium

concentration of endolymph caused apoptosis and necrosis of transplanted stem cells, therefore limiting the use of exogenous stem cells to replace the damaged hair cells [57]. It has been reported that potassium ion and potassium channel play important role in stem cell apoptosis. Potassium ion efflux and substantial potassium ion loss contributed to cell apoptosis [65–68]. However, endogenous progenitor cells laying on the basilar membrane have adapted environment of high potassium concentration, which have become the first choice of treatment of hair cell damage. Transplantation of exogenous stem cells to scala tympani, auditory nerve trunk, and lateral wall to avoid high potassium concentration could greatly increase the survival rate of the transplanted cells. The main components of perilymph are high concentration of sodium ion and glucose, which lack growth factor and neurotrophins for stem cells' long-term survival and differentiation [69]. Transplanted stem cells into scala tympani coupled with neuritrophin GDNF and/or BDNF supply increased stem cell long-term survival and therefore were able to differentiate into Tuj1-positive neurons [40, 70]. Previous studies have shown that there were high migration and differentiation ratio after transplanting stem cells into damaged cochlea compared to undamaged cochlea [39, 55, 71]. These findings indicated that surrounding environment change might enhance the stem cell migration and differentiation. Migration toward the neural degeneration site is a crucial step after stem cell transplantation; migration efficiency has a decisive influence on its therapeutic effect. One of the best-studied mediators for stem cell-directed migration and homing is CXC chemokine receptor 4 (CXCR4) and its ligand stromal cell-derived factor 1 (SDF-1 or CXCL12) [72–76]. SDF-1/CXCR4 signal acts as chemotactic factor and promotes concentration-dependent pathology-directed chain migration of stem cell movement toward the pathology region [72, 73, 75, 76]. Research has shown that there was a regional increase of SDF-1 expression derived from glial cells around degenerated spiral ganglion neuron in the early injury microenvironment of the cochlea [77]. This upregulation of SDF-1 increased attraction of CXCR4 expressed stem cells moving to the injury region and therefore increased potential therapeutic efficiency of stem cell transplantation. Wnt signal is another critical factor for which mediates transplanted stem cell differentiation. Research has reported an increase in Wnt expression in glial cells after spiral ganglion neuron degeneration in the cochlea. In vitro study has shown a significant increase in MAP 2-positive neuronal differentiation of stem cells co-cultured with Wnt1-upregulated Schwann cells using a Transwell system [71]. This finding indicated the autonomous upregulation of Wnt signal in the microenvironment of injury of the cochlea promoted stem cell differentiation into neurons.

In addition to the internal environment factors, external stimulation factors can also affect the migration and differentiation of transplanted stem cells. External acoustical stimulus can promote development and functional maturation of auditory system [78–80]. Researchers have shown that augmented acoustical stimulus exposure (75 dB) after stem cell transplantation increased the survival rate of transplanted cells and upregulated SDF-1 expression in spiral ganglion, hence assisting stem cell migration [77, 81]. Electrical stimulation is also considered as one of the important factors influencing cell proliferation, differentiation, and migration [82–85].

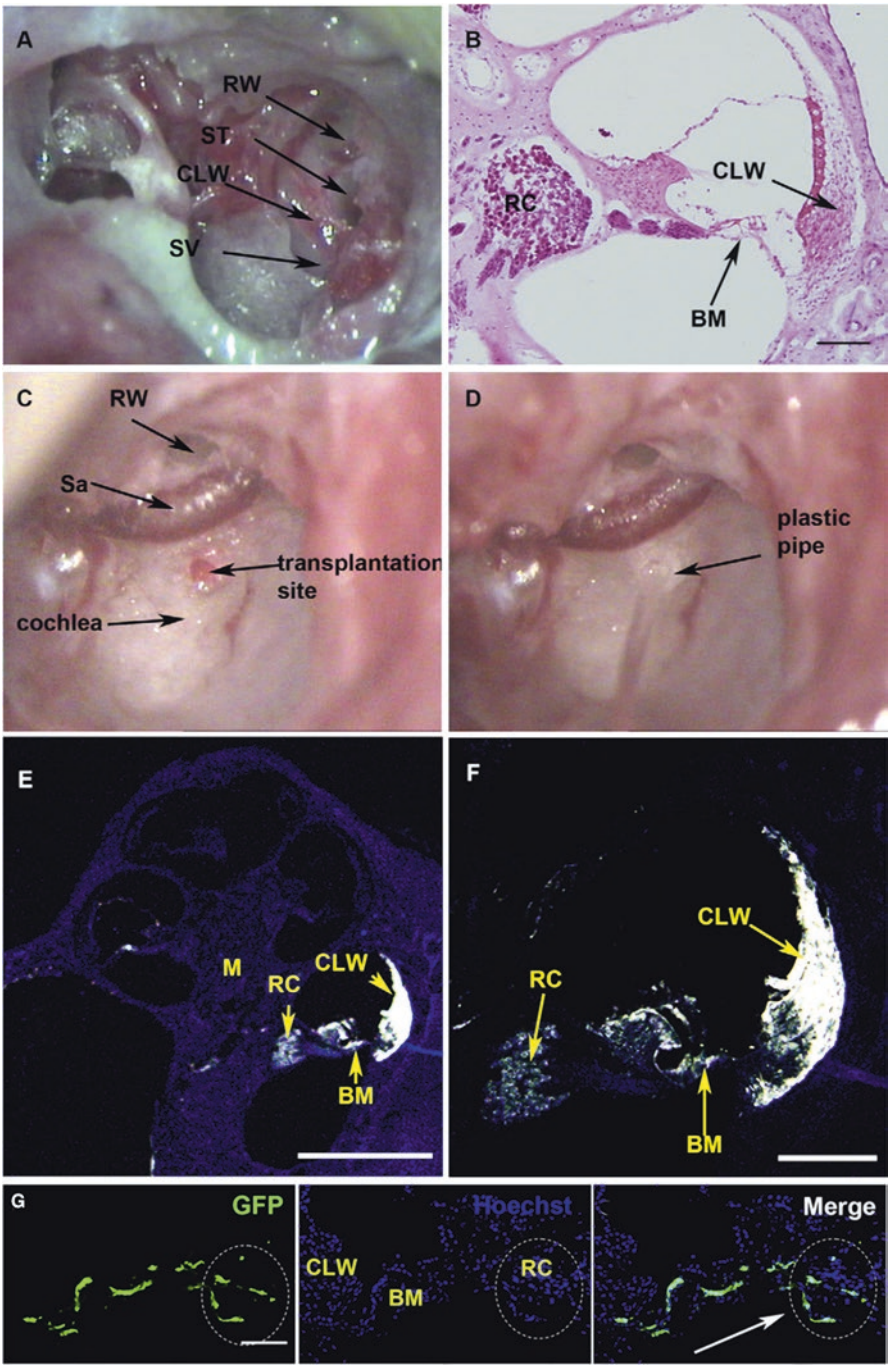

Fig. 10.2 Cochlear anatomy, injection site, and migration route tracks. (**a**) Cochlear anatomy exposed after partially removing the bone capsule. (**b**) H&E staining of cochlear radial section.

Especially within developing and damage neural tissues, it increased neuronal differentiation of neuroblasts, induced branch formation of spiral neurons, assisted axon regeneration, and enhanced neural stem cell function [86–89]. Recent study has demonstrated that applying electrical stimulation to neural stem cell culturing on conductive neural interfacing material graphene enhanced neural stem cell differentiation into neurons [90].

10.6 Functional Restoration of Hearing

In order to restore auditory function, transplanted cells have to establish a connection between hair cells and cochlear nucleus. Several groups have done in vivo studies of neuron replacement; results showed neurite projection of transplanted cells through Rosenthal's canal into the organ of Corti [39, 42, 52, 91]. Fully functional restoration of hearing also needs implanted cells to build up connection to central nerve. In vitro study has proved that implanted auditory stem cells with a high degree of survival rate projected axons toward cochlear nucleus in co-culture system with brain stem [92]. However, a study showed that there was only partial restoration of hearing function after stem cell transplantation in vivo [46]. Due to lack of evidences of in vivo study, especially the establishment of the synaptic connection between transplanted cells and hair cells, as well as the neurotransmitter exchange, complete restoration of auditory function still needs further investigations.

10.7 Challenges and Perspectives

As one of the most popular technologies in the biological treatment, stem cell therapy has great potential in its application on hearing loss. However, due to the complexity of physiological structure and internal environment of the cochlea and complexity of mechanisms of neurogenesis, the therapeutic effect of in vivo

Fig. 10.2 (continued) Bar = 100um. (**c**) After the cochlea was exposed, a hole is made at the cochlear lateral wall of the basal turn which was transplantation site of stem cells. (**d**) A plastic pipe inserted into the hole for Fluorogold injection. (**e** and **f**) Fluorogold distribution after injection. Injected Fluorogold migrated through basilar membrane into Rosenthal's canal. The intensity decreased from cochlear lateral wall to Rosenthal's canal. Bar (E) = 1 mm; Bar (F) = 200um. (**g**) Migration of GFP-labeled stem cell after injected at lateral wall. Injected cells migrated from lateral wall through basilar membrane and finally into Rosenthal's canal. Bar = 100um. *RW* round window, *ST* scala tympani, *CLW* cochlear lateral wall, *SV* scala vestibule, *RC* Rosenthal's canal, *Sa* stapedial arteria, *BM* basilar membrane, *M* cochlear modiolus [62]

application of stem cell therapy is not satisfactory. Another concern for stem cell in vivo application is that the complex internal environment makes precise regulation of stem cells relatively challenging, which may increase the risk of inducing other lesions, such as cancer formation because of powerful proliferation ability of some types of stem cells. In recent years, precise genetic programming/editing technology is developing rapidly; its application for hereditary deafness treatment in animal model has also made certain progress. Researchers have used CRISPR/Cas9 gene-editing technology to treat autosomal dominant hearing loss in animal model [93]. Combination of stem cell therapy and genetic programming/editing technology used in the treatment of deafness is the direction of future development. Genetic programming/edit technology may be able to achieve precise regulation of stem cells in vivo, so as to improve the applicability of stem cell therapy. Likewise, combination of stem cell therapy with existing methods, such as cochlear implants, may improve their therapeutic outcomes for each other. The electrical field generated from implanted device may help stem cells to find the right path through the "chaos" to reach their fate of our hope.

References

1. Jacob S, Johansson C, Fridberger A (2013) Noise-induced alterations in cochlear mechanics, electromotility, and cochlear amplification. Pflugers Arch 465(6):907–917
2. Geleoc GS, Holt JR (2014) Sound strategies for hearing restoration. Science 344(6184):1241062
3. Starr A, Rance G (2015) Auditory neuropathy. Handb Clin Neurol 129:495–508
4. Tucci DL, Rubel EW (1990) Physiologic status of regenerated hair cells in the avian inner ear following aminoglycoside ototoxicity. Otolaryngol Head Neck Surg 103(3):443–450
5. Cotanche DA, Dopyera CE (1990) Hair cell and supporting cell response to acoustic trauma in the chick cochlea. Hear Res 46(1–2):29–40
6. Li H, Liu H, Heller S (2003) Pluripotent stem cells from the adult mouse inner ear. Nat Med 9(10):1293–1299
7. Oshima K, Grimm CM, Corrales CE et al (2007) Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. J Assoc Res Otolaryngol 8(1):18–31
8. Martinez-Monedero R, Oshima K, Heller S, Edge AS (2007) The potential role of endogenous stem cells in regeneration of the inner ear. Hear Res 227(1–2):48–52
9. Chen J, Streit A (2013) Induction of the inner ear: stepwise specification of otic fate from multipotent progenitors. Hear Res 297:3–12
10. Bermingham-McDonogh O, Reh TA (2011) Regulated reprogramming in the regeneration of sensory receptor cells. Neuron 71(3):389–405
11. Mulvaney J, Dabdoub A (2012) Atoh1, an essential transcription factor in neurogenesis and intestinal and inner ear development: function, regulation, and context dependency. J Assoc Res Otolaryngol 13(3):281–293
12. Su YX, Hou CC, Yang WX (2015) Control of hair cell development by molecular pathways involving Atoh1, Hes1 and Hes5. Gene 558(1):6–24
13. Zheng JL, Shou J, Guillemot F, Kageyama R, Gao WQ (2000) Hes1 is a negative regulator of inner ear hair cell differentiation. Development 127(21):4551–4560
14. Zine A, Aubert A, Qiu J et al (2001) Hes1 and Hes5 activities are required for the normal development of the hair cells in the mammalian inner ear. J Neurosci 21(13):4712–4720

15. Dabdoub A, Puligilla C, Jones JM et al (2008) Sox2 signaling in prosensory domain specification and subsequent hair cell differentiation in the developing cochlea. Proc Natl Acad Sci U S A 105(47):18396–18401
16. Lanford PJ, Lan Y, Jiang R et al (1999) Notch signalling pathway mediates hair cell development in mammalian cochlea. Nat Genet 21(3):289–292
17. Van Camp JK, Beckers S, Zegers D, Van Hul W (2014) Wnt signaling and the control of human stem cell fate. Stem Cell Rev 10(2):207–229
18. Shi F, Hu L, Jacques BE, Mulvaney JF, Dabdoub A, Edge AS (2014) beta-Catenin is required for hair-cell differentiation in the cochlea. J Neurosci 34(19):6470–6479
19. Li H, Kloosterman W, Fekete DM (2010) MicroRNA-183 family members regulate sensorineural fates in the inner ear. J Neurosci 30(9):3254–3263
20. Hei R, Chen J, Qiao L et al (2011) Dynamic changes in microRNA expression during differentiation of rat cochlear progenitor cells in vitro. Int J Pediatr Otorhinolaryngol 75(8):1010–1014
21. Wang XR, Zhang XM, Du J, Jiang H (2012) MicroRNA-182 regulates otocyst-derived cell differentiation and targets T-box 1 gene. Hear Res 286(1–2):55–63
22. Barker N, van Es JH, Kuipers J et al (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449(7165):1003–1007
23. Chai R, Xia A, Wang T et al (2011) Dynamic expression of Lgr5, a Wnt target gene, in the developing and mature mouse cochlea. J Assoc Res Otolaryngol 12(4):455–469
24. Chai R, Kuo B, Wang T et al (2012) Wnt signaling induces proliferation of sensory precursors in the postnatal mouse cochlea. Proc Natl Acad Sci U S A 109(21):8167–8172
25. Bramhall NF, Shi F, Arnold K, Hochedlinger K, Edge AS (2014) Lgr5-positive supporting cells generate new hair cells in the postnatal cochlea. Stem Cell Rep 2(3):311–322
26. Zak M, Klis SF, Grolman W (2015) The Wnt and notch signalling pathways in the developing cochlea: formation of hair cells and induction of regenerative potential. Int J Dev Neurosci 47(Pt B):247–258
27. Ni W, Lin C, Guo L et al (2016) Extensive supporting cell proliferation and mitotic hair cell generation by in vivo genetic reprogramming in the neonatal mouse cochlea. J Neurosci 36(33):8734–8745
28. Ni W, Zeng S, Li W et al (2016) Wnt activation followed by notch inhibition promotes mitotic hair cell regeneration in the postnatal mouse cochlea. Oncotarget 7(41):66754–66768
29. Hakuba N, Koga K, Gyo K, Usami SI, Tanaka K (2000) Exacerbation of noise-induced hearing loss in mice lacking the glutamate transporter GLAST. J Neurosci 20(23):8750–8753
30. Kujawa SG, Liberman MC (2009) Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J Neurosci 29(45):14077–14085
31. Martinez-Monedero R, Yi E, Oshima K, Glowatzki E, Edge AS (2008) Differentiation of inner ear stem cells to functional sensory neurons. Dev Neurobiol 68(5):669–684
32. Rask-Andersen H, Bostrom M, Gerdin B et al (2005) Regeneration of human auditory nerve. In vitro/in video demonstration of neural progenitor cells in adult human and Guinea pig spiral ganglion. Hear Res 203(1–2):180–191
33. Wang Q, Green SH (2011) Functional role of neurotrophin-3 in synapse regeneration by spiral ganglion neurons on inner hair cells after excitotoxic trauma in vitro. J Neurosci 31(21):7938–7949
34. Lang H, Li M, Kilpatrick LA et al (2011) Sox2 up-regulation and glial cell proliferation following degeneration of spiral ganglion neurons in the adult mouse inner ear. J Assoc Res Otolaryngol 12(2):151–171
35. Lunn JS, Sakowski SA, Hur J, Feldman EL (2011) Stem cell technology for neurodegenerative diseases. Ann Neurol 70(3):353–361
36. Drago D, Cossetti C, Iraci N et al (2013) The stem cell secretome and its role in brain repair. Biochimie 95(12):2271–2285
37. Murrell W, Palmero E, Bianco J et al (2013) Expansion of multipotent stem cells from the adult human brain. PLoS One 8(8):e71334

38. Hildebrand MS, Dahl HH, Hardman J, Coleman B, Shepherd RK, de Silva MG (2005) Survival of partially differentiated mouse embryonic stem cells in the scala media of the Guinea pig cochlea. J Assoc Res Otolaryngol 6(4):341–354
39. Sekiya T, Kojima K, Matsumoto M, Kim TS, Tamura T, Ito J (2006) Cell transplantation to the auditory nerve and cochlear duct. Exp Neurol 198(1):12–24
40. Reyes JH, O'Shea KS, Wys NL et al (2008) Glutamatergic neuronal differentiation of mouse embryonic stem cells after transient expression of neurogenin 1 and treatment with BDNF and GDNF: in vitro and in vivo studies. J Neurosci 28(48):12622–12631
41. Coleman B, Hardman J, Coco A et al (2006) Fate of embryonic stem cells transplanted into the deafened mammalian cochlea. Cell Transplant 15(5):369–380
42. Corrales CE, Pan L, Li H, Liberman MC, Heller S, Edge AS (2006) Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti. J Neurobiol 66(13):1489–1500
43. Bas E, Van De Water TR, Lumbreras V et al (2014) Adult human nasal mesenchymal-like stem cells restore cochlear spiral ganglion neurons after experimental lesion. Stem Cells Dev 23(5):502–514
44. Boddy SL, Chen W, Romero-Guevara R, Kottam L, Bellantuono I, Rivolta MN (2012) Inner ear progenitor cells can be generated in vitro from human bone marrow mesenchymal stem cells. Regen Med 7(6):757–767
45. Duran Alonso MB, Feijoo-Redondo A, Conde de Felipe M et al (2012) Generation of inner ear sensory cells from bone marrow-derived human mesenchymal stem cells. Regen Med 7(6):769–783
46. Cho YB, Cho HH, Jang S, Jeong HS, Park JS (2011) Transplantation of neural differentiated human mesenchymal stem cells into the cochlea of an auditory-neuropathy Guinea pig model. J Korean Med Sci 26(4):492–498
47. Lee JH, Kang WK, Seo JH et al (2012) Neural differentiation of bone marrow-derived mesenchymal stem cells: applicability for inner ear therapy. Korean J Audiol 16(2):47–53
48. Peng T, Zhu G, Dong Y et al (2015) BMP4: a possible key factor in differentiation of auditory neuron-like cells from bone-derived mesenchymal stromal cells. Clin Lab 61(9):1171–1178
49. Parker MA, Corliss DA, Gray B et al (2007) Neural stem cells injected into the sound-damaged cochlea migrate throughout the cochlea and express markers of hair cells, supporting cells, and spiral ganglion cells. Hear Res 232(1–2):29–43
50. Hu Z, Ulfendahl M, Prieskorn DM, Olivius P, Miller JM (2009) Functional evaluation of a cell replacement therapy in the inner ear. Otol Neurotol 30(4):551–558
51. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
52. Nishimura K, Nakagawa T, Ono K et al (2009) Transplantation of mouse induced pluripotent stem cells into the cochlea. Neuroreport 20(14):1250–1254
53. Chen J, Guan L, Zhu H, Xiong S, Zeng L, Jiang H (2017) Transplantation of mouse-induced pluripotent stem cells into the cochlea for the treatment of sensorineural hearing loss. Acta Otolaryngol 137(11):1136–1142
54. Matsuoka AJ, Kondo T, Miyamoto RT, Hashino E (2006) In vivo and in vitro characterization of bone marrow-derived stem cells in the cochlea. Laryngoscope 116(8):1363–1367
55. Hu Z, Wei D, Johansson CB et al (2005) Survival and neural differentiation of adult neural stem cells transplanted into the mature inner ear. Exp Cell Res 302(1):40–47
56. Sekiya T, Holley MC, Kojima K, Matsumoto M, Helyer R, Ito J (2007) Transplantation of conditionally immortal auditory neuroblasts to the auditory nerve. Eur J Neurosci 25(8):2307–2318
57. Wang M, Qiu J, Mi W, Wang F, Qu J (2011) In vitro effect of altering potassium concentration in artificial endolymph on apoptosis and ultrastructure features of olfactory bulb neural precursor cells. Neurosci Lett 487(3):383–388
58. Iguchi F, Nakagawa T, Tateya I, et al (2004) Surgical techniques for cell transplantation into the mouse cochlea. Acta Otolaryngol Suppl (551):43–47

59. Izumikawa M, Minoda R, Kawamoto K et al (2005) Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nat Med 11(3):271–276
60. Hu Z, Ulfendahl M, Olivius NP (2004) Central migration of neuronal tissue and embryonic stem cells following transplantation along the adult auditory nerve. Brain Res 1026(1):68–73
61. Palmgren B, Jin Z, Jiao Y, Kostyszyn B, Olivius P (2011) Horseradish peroxidase dye tracing and embryonic statoacoustic ganglion cell transplantation in the rat auditory nerve trunk. Brain Res 1377:41–49
62. Zhang PZ, He Y, Jiang XW et al (2013) Stem cell transplantation via the cochlear lateral wall for replacement of degenerated spiral ganglion neurons. Hear Res 298:1–9
63. Lang H, Schulte BA, Goddard JC et al (2008) Transplantation of mouse embryonic stem cells into the cochlea of an auditory-neuropathy animal model: effects of timing after injury. J Assoc Res Otolaryngol 9(2):225–240
64. Matsuoka AJ, Kondo T, Miyamoto RT, Hashino E (2007) Enhanced survival of bone-marrow-derived pluripotent stem cells in an animal model of auditory neuropathy. Laryngoscope 117(9):1629–1635
65. Yu SP, Yeh C, Strasser U, Tian M, Choi DW (1999) NMDA receptor-mediated K+ efflux and neuronal apoptosis. Science 284(5412):336–339
66. Schulz JB, Weller M, Klockgether T (1996) Potassium deprivation-induced apoptosis of cerebellar granule neurons: a sequential requirement for new mRNA and protein synthesis, ICE-like protease activity, and reactive oxygen species. J Neurosci 16(15):4696–4706
67. Wang SP, Wang JA, Luo RH, Cui WY, Wang H (2008) Potassium channel currents in rat mesenchymal stem cells and their possible roles in cell proliferation. Clin Exp Pharmacol Physiol 35(9):1077–1084
68. Yu SP, Canzoniero LM, Choi DW (2001) Ion homeostasis and apoptosis. Curr Opin Cell Biol 13(4):405–411
69. Mothe AJ, Kulbatski I, Parr A, Mohareb M, Tator CH (2008) Adult spinal cord stem/progenitor cells transplanted as neurospheres preferentially differentiate into oligodendrocytes in the adult rat spinal cord. Cell Transplant 17(7):735–751
70. Altschuler RA, O'Shea KS, Miller JM (2008) Stem cell transplantation for auditory nerve replacement. Hear Res 242(1–2):110–116
71. He Y, Zhang PZ, Sun D et al (2014) Wnt1 from cochlear schwann cells enhances neuronal differentiation of transplanted neural stem cells in a rat spiral ganglion neuron degeneration model. Cell Transplant 23(6):747–760
72. Imitola J, Raddassi K, Park KI et al (2004) Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A 101(52):18117–18122
73. Bagri A, Gurney T, He X et al (2002) The chemokine SDF1 regulates migration of dentate granule cells. Development 129(18):4249–4260
74. Klein RS, Rubin JB, Gibson HD et al (2001) SDF-1 alpha induces chemotaxis and enhances sonic hedgehog-induced proliferation of cerebellar granule cells. Development 128(11):1971–1981
75. Reiss K, Mentlein R, Sievers J, Hartmann D (2002) Stromal cell-derived factor 1 is secreted by meningeal cells and acts as chemotactic factor on neuronal stem cells of the cerebellar external granular layer. Neuroscience 115(1):295–305
76. Belmadani A, Tran PB, Ren D, Assimacopoulos S, Grove EA, Miller RJ (2005) The chemokine stromal cell-derived factor-1 regulates the migration of sensory neuron progenitors. J Neurosci 25(16):3995–4003
77. Zhang PZ, He Y, Jiang XW et al (2013) Up-regulation of stromal cell-derived factor-1 enhances migration of transplanted neural stem cells to injury region following degeneration of spiral ganglion neurons in the adult rat inner ear. Neurosci Lett 534:101–106
78. Zhang LI, Bao S, Merzenich MM (2001) Persistent and specific influences of early acoustic environments on primary auditory cortex. Nat Neurosci 4(11):1123–1130

79. Percaccio CR, Pruette AL, Mistry ST, Chen YH, Kilgard MP (2007) Sensory experience determines enrichment-induced plasticity in rat auditory cortex. Brain Res 1174:76–91
80. Zhou X, Nagarajan N, Mossop BJ, Merzenich MM (2008) Influences of un-modulated acoustic inputs on functional maturation and critical-period plasticity of the primary auditory cortex. Neuroscience 154(1):390–396
81. Chen Y, Qiu J, Chen F, Liu S (2007) Migration of neural precursor cells derived from olfactory bulb in cochlear nucleus exposed to an augmented acoustic environment. Hear Res 228(1–2):3–10
82. Nuccitelli R (2003) A role for endogenous electric fields in wound healing. Curr Top Dev Biol 58:1–26
83. Nuccitelli R (2003) Endogenous electric fields in embryos during development, regeneration and wound healing. Radiat Prot Dosim 106(4):375–383
84. Stump RF, Robinson KR (1983) Xenopus neural crest cell migration in an applied electrical field. J Cell Biol 97(4):1226–1233
85. Babona-Pilipos R, Droujinine IA, Popovic MR, Morshead CM (2011) Adult subependymal neural precursors, but not differentiated cells, undergo rapid cathodal migration in the presence of direct current electric fields. PLoS One 6(8):e23808
86. Hinkle L, McCaig CD, Robinson KR (1981) The direction of growth of differentiating neurones and myoblasts from frog embryos in an applied electric field. J Physiol 314:121–135
87. Pomeranz B, Mullen M, Markus H (1984) Effect of applied electrical fields on sprouting of intact saphenous nerve in adult rat. Brain Res 303(2):331–336
88. Borgens RB, Roederer E, Cohen MJ (1981) Enhanced spinal cord regeneration in lamprey by applied electric fields. Science 213(4508):611–617
89. Zhu W, Ye T, Lee SJ et al (2017) Enhanced neural stem cell functions in conductive annealed carbon nanofibrous scaffolds with electrical stimulation. Nanomedicine 14:2485–2494
90. Park SY, Park J, Sim SH et al (2011) Enhanced differentiation of human neural stem cells into neurons on graphene. Adv Mater 23(36):H263–H267
91. Shi F, Corrales CE, Liberman MC, Edge AS (2007) BMP4 induction of sensory neurons from human embryonic stem cells and reinnervation of sensory epithelium. Eur J Neurosci 26(11):3016–3023
92. Thonabulsombat C, Johansson S, Spenger C, Ulfendahl M, Olivius P (2007) Implanted embryonic sensory neurons project axons toward adult auditory brainstem neurons in roller drum and Stoppini co-cultures. Brain Res 1170:48–58
93. Gao X, Tao Y, Lamas V et al (2018) Treatment of autosomal dominant hearing loss by in vivo delivery of genome editing agents. Nature 553(7687):217–221