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Gene therapy for hearing loss: From mechanism to clinic, volume II

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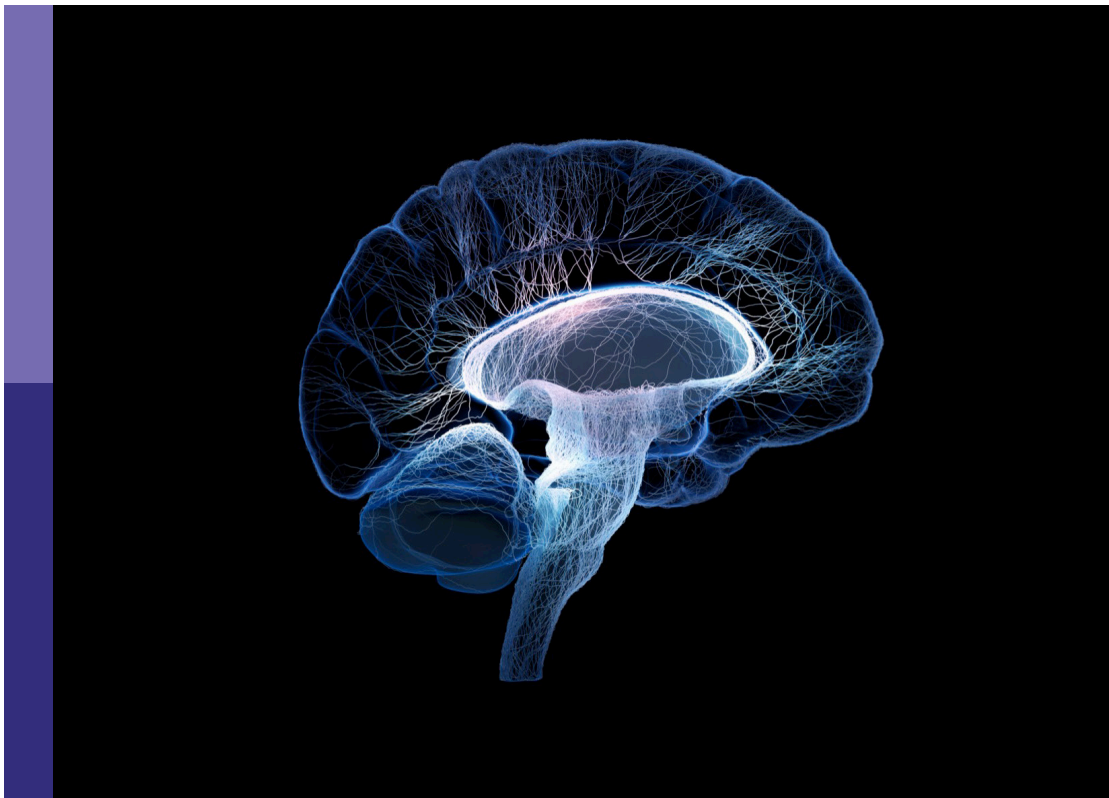

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Gene therapy for hearing loss: From mechanism to clinic, volume II

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Editorial: Gene therapy for hearing loss: from mechanism to clinic, volume II

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Editorial on the Research Topic
Gene therapy for hearing loss: from mechanism to clinic, volume II

Hearing loss affects patients' quality of life and remains a major health problem worldwide due to the failure of damaged inner ear sensory cells to regenerate. This Research Topic presents advanced research progress in hearing. This research includes the application of advanced materials in the inner ear, a new method of hearing testing for congenital inner ear malformation in the clinic, research progress on inner hair cell regeneration and nursing care for deaf patients. This Research Topic introduces recent advances content in gene therapy for the inner ear disorder and describes cases of restored hearing through gene therapy. It also introduces some clinical issues including a new method of evaluating the structural disorder of inner ear through tympanogram, the activity changes of cerebral cortex area in sensorineural hearing loss and the treatment and nursing strategies of sudden sensorineural hearing loss. At the same time, this Research Topic also focuses on the impact of music on the psychological state of adolescents, and emphasizing the importance of fostering a beneficial music environment for adolescents. Meanwhile, this Research Topic introduces the latest research progress in materials science developing, including nanomaterials, magnetic responsive materials, and exosomes, and introduces their roles in the prevention and treatment of sensorineural hearing loss. This Research Topic also introduces some newly pathogenesis and intervention methods of sensorineural hearing loss. This Research Topic provides new ideas and methods for promoting the prevention and treatment of hearing loss.

Wang, Zheng et al. focused on recent advancements in gene therapy for cochlear hair cell (HC) regeneration, highlighting its potential to treat sensorineural hearing loss. These authors identified several transcription factors and downstream signaling pathways involved in HC development and regeneration. The abilities of several new gene editing therapies, CRISPR/Cas9 editing and viral vectors, to improve hearing recovery and HC regeneration are discussed. This review acknowledges the challenges in achieving effective

HC regeneration and emphasizes the need for further research to optimize gene therapy approaches for clinical application.

Li A. et al. compared the difference in wideband acoustic immittance (WAI) between large vestibular aqueduct syndrome (LVAS) patients and the normal population. Compared with patients in the control group, patients in the LVAS group showed an increase in the overall absorbance of the tympanogram. Furthermore, the study compared the frequency-absorbance curves between LVAS patients and controls. The results revealed increased absorbance in the low- and medium-frequency ranges, suggesting that the maximum absorbance on the mean tympanogram could serve as a reliable indicator for evaluating inner ear structure disorders.

Liu et al. reviewed the progress in treating and nursing care for sensorineural hearing loss (SNHL) patients. Current clinical methods for treating SNHL include drug therapy, traditional Chinese medicine, hyperbaric oxygen therapy, and stem cell transplantation. The manuscript introduces some issues that needs paying attention in nursing care. It focuses on psychological care, dietary care, life care, medication care, and traditional Chinese medicine massage. Nurses could help patients establish a positive attitude to cooperate with treatment while also helping them develop healthy dietary plans and daily schedules. Nurses can also monitor potential side effects that patients may experience during treatment and promptly inform doctors to adjust treatment plans. As a characteristic medical treatment in China, they also introduced plans and nursing measures for patients with SNHL receiving traditional Chinese medicine treatment. This review stresses that ongoing research and patient-centered care are essential for advancing SNHL treatment and nursing practices.

Wang Y. et al. conducted a retrospective case–control study to investigate the impact of environmental noise exposure on the prognosis of sudden sensorineural hearing loss (SSNHL). The study compared patients with SSNHL exposed to environmental noise before onset (case group) to those with SSNHL without such exposure (control group). The study revealed that patients who were exposed to noise had a poorer prognosis, a longer duration of treatment, and a lower rate of vestibular dysfunction. The time to treatment and final pure-tone average (PTA) were significant prognostic factors, suggesting that early treatment and the severity of initial hearing loss are crucial in determining outcomes for SSNHL patients exposed to environmental noise.

Chen explored the influence of music on adolescent mental health, discussing both positive impacts, such as emotional expression and social bonding, and potential negative impacts, including the risk of hearing damage from excessive volume. First, they define music and introduce the classification and origin of music. Later, they discussed the benefits and drawbacks that music has to people. It suggests strategies for mitigating risks and enhancing benefits, emphasizing the importance of selecting appropriate music genres, creating a healthy music environment, advocating for positive music education, and encouraging active participation in music activities. This review aims to inform parents, educators, and community workers on fostering a beneficial music environment for adolescents.

Yang et al. provided a review of presbycusis, discussing its pathophysiology, genetic susceptibility, and impact of the environment on ARHL. Furthermore, they described the mechanisms that may induce ARHL, including ROS accumulation, inflammation and lateral wall fibrosis. Prevention strategies and current treatment options, including oral drugs and gene therapy targets, are also discussed. This paper emphasizes the need for further research to explore potential treatments such as antioxidants, anti-inflammatory agents and gene therapy.

Li J. et al. explored alterations in static and dynamic intrinsic brain activity in individuals with unilateral sudden sensorineural hearing loss (SSHL) using fractional amplitude of low-frequency fluctuation (fALFF) analysis. Significant differences in static fALFF patterns were detected between SSHL patients and healthy controls, revealing changes in brain functional areas and compensatory mechanisms in patients with SSHL. Dynamic fALFF analysis also revealed alterations, highlighting the importance of understanding neural changes in SSNHL patients for developing targeted interventions and rehabilitation strategies.

Fang et al. reviewed the use of advanced stem cells and nanomaterials for treating hearing loss, especially research advancements in combining nanomaterials with stem cells for hair cell regeneration. This manuscript describes the functions of various stem cells in hair cell regeneration. The role of exosomes, a newly discovered type of vesicle, in hearing has recently been explored. The author discussed the role of exosomes in hair cell regeneration and drug-induced hearing loss. The mechanism by which nanomaterials enhance the therapeutic effects of stem cells and the promising results of stem cell-derived exosomes in tissue repair are discussed. Despite technical and practical limitations, the findings are encouraging for future clinical applications, highlighting the need for continued research on stem cell therapy for hearing loss.

Wang, Deng et al. reviewed advancements in stem cell (SC) therapy for regenerative medicine. This review describes the classification and function of SCs and the regulatory mechanisms of SCs. In the field of SC therapy, researchers have focused on the gene regulatory mechanism of SCs and the therapeutic approach for SCs transplanted via vectors. The review covered various types of stem cells, including their characteristics, potential applications in cell and cell-free therapies, and technical routes of therapy. This review addresses current challenges in the field, such as safety issues and differentiation control, and highlights the significant therapeutic potential of stem cells in treating a wide range of diseases and traumatic conditions.

Feng et al. reviewed recent advances in the genetic etiology of non-syndromic deafness in children, focusing on the high incidence of genetic hearing loss. This study aimed to assist in personalized diagnosis and treatment by summarizing key findings in genetic research related to non-syndromic deafness. This paper discusses various genes implicated in hearing loss, the pathophysiology, and the molecular mechanisms underlying this condition. This finding emphasized the importance of genetic testing and screening in developing innovative treatment and management strategies for affected children.

Sun et al. explored the potential application value of pigment epithelium-derived factor (PEDF) in treating sensorineural hearing loss (SNHL). As a protein with cellular protection and antioxidative properties, PEDF has protective effects on inner hair cells

and promotes cell differentiation. Subsequently, they analyzed the signaling pathway by which PEDF promotes antioxidant, anti-inflammatory and cell differentiation. This highlights the multifunctional role of PEDF, including its cellular protection and antiangiogenic properties, which might offer new treatment options for inner ear diseases. This review assessed the performance of PEDF in treating SNHL, its mechanisms, and therapeutic prospects, suggesting that further research and clinical trials are needed to establish its safety and effectiveness in treating inner ear disease.

Zhu et al. provided a comprehensive overview of the role of non-coding RNAs (ncRNAs), particularly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), in the pathogenesis of hearing loss. They described the role of miRNAs in various types of hearing loss, including age-related hearing loss, drug-induced hearing loss, noise-induced hearing loss, and the regulation of hair cell regeneration. These findings emphasize the significance of ncRNAs in regulating various physiological and pathological processes that impact hearing loss development and prognosis. This review discussed the potential of ncRNAs as therapeutic targets for precise treatment strategies, addressing the current challenges and future prospects in the study of ncRNAs related to hearing loss.

Lu et al. reviewed the current advances in biomaterials for inner ear cell regeneration, focusing on their application in constructing physiologically relevant 3D culture systems that mimic the stem cell microenvironment. The use of various biomaterials, including hydrogels, conductive materials, magnetoresponsive materials, and photomodulation materials, is highlighted because of their potential to support the regeneration and functional maturation of inner ear cells. This review emphasized the importance of selecting and combining biomaterials strategically based on their physicochemical properties to overcome challenges in inner ear cell regeneration research.

Liu and Xu discussed the role of macrophage-related immune responses in sensorineural hearing loss (SNHL), emphasizing their potential as therapeutic targets. This review described the origin and distribution of inner ear macrophages. Furthermore, they analyzed macrophage activation during acute and chronic cochlear injury. The dynamic changes in macrophages in various inner ear injuries were reviewed, and their potential role in mitigating damage was clarified. This review suggested that gene therapy targeting immune responses could be a promising direction for reconstructing hearing.

For this Research Topic, research on gene therapy in the fields of sensorineural hearing loss, inner ear stem cells, new biological materials, tissue engineering, and other new technologies and methods of development was performed. The content of this Research Topic includes recent research progress in the auditory system and the latest inner ear gene therapy methods and thus could provide a reference for further exploration of auditory disorders.

Author contributions

SZ: Writing – original draft. QZ: Writing – review & editing. YS: Writing – review & editing. XF: Writing – review & editing. WZ: Writing – review & editing. ZH: Writing – original draft, Writing – review & editing.

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Gene therapy: an emerging therapy for hair cells regeneration in the cochlea

Jipeng Wang[1†], Jianwei Zheng[2†], Haiyan Wang[3†], Haoying He[4], Shuang Li[3], Ya Zhang[3], You Wang[5*], Xiaoxiang Xu[3*] and Shuyi Wang[1*]

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Sensorineural hearing loss is typically caused by damage to the cochlear hair cells (HCs) due to external stimuli or because of one's genetic factors and the inability to convert sound mechanical energy into nerve impulses. Adult mammalian cochlear HCs cannot regenerate spontaneously; therefore, this type of deafness is usually considered irreversible. Studies on the developmental mechanisms of HC differentiation have revealed that nonsensory cells in the cochlea acquire the ability to differentiate into HCs after the overexpression of specific genes, such as *Atoh1*, which makes HC regeneration possible. Gene therapy, through *in vitro* selection and editing of target genes, transforms exogenous gene fragments into target cells and alters the expression of genes in target cells to activate the corresponding differentiation developmental program in target cells. This review summarizes the genes that have been associated with the growth and development of cochlear HCs in recent years and provides an overview of gene therapy approaches in the field of HC regeneration. It concludes with a discussion of the limitations of the current therapeutic approaches to facilitate the early implementation of this therapy in a clinical setting.

KEYWORDS

hair cell regeneration, gene therapy, *Atoh1*, inner ear, sensorineural hearing loss

1. Introduction

Deafness is the most common neurological disorder in humans, which has seriously affected the normal life of human beings. According to the World Hearing Report published by the World Health Organization, almost 1.5 billion people worldwide have varying degrees of hearing loss, and 430 million people are at a level of severe hearing loss that requires rehabilitation (Chadha et al., 2021). Deafness can be categorized as conductive, sensorineural, and mixed deafness (Cunningham and Tucci, 2017). The more common type of deafness is sensorineural deafness caused by death or functional loss of cochlear hair cells (HCs). HCs are the most critical cells for sound perception and transmission in the inner ear sensory cells, and their function is to convert the mechanical signals of sound coming in from the environment into electrical signals that the nervous system can perceive (Deans, 2021). HCs are the most critical cells in the mammalian inner ear sensory epithelium. Studies have shown that (Fujioka et al., 2015)

compared with nonmammals (birds and reptiles), HCs cannot regenerate spontaneously in mammals; thus, HC damage often results in permanent hearing loss.

Gene therapy involves transferring an external normal or therapeutic gene, via a vector, to a target cell in the body, causing the target cell to express the relevant gene or to modify the pertinent gene as a therapeutic approach. It has now become a potential treatment for genetic deafness. In several animal models, gene therapy has been used to transfer several genes such as *Syne4* (Taiber et al., 2021), Tmc1 (Marcovich et al., 2022), and *Clarin-1* (Dulon et al., 2018) moved into the cochlea and has significantly improved the degree of hearing impairment in the study animals. During the developmental differentiation and maturation of inner ear HCs, there is also the regulation of multiple genes (Bermingham et al., 1999; Hertzano et al., 2004; Ikeda et al., 2015; Hou et al., 2019; Ding et al., 2020; Jen et al., 2022) and signaling pathways (Benito-Gonzalez and Doetzlhofer, 2014; Waqas et al., 2016; Ebeid and Huh, 2017; Bai et al., 2021). By interfering with these, the normal differentiation of HCs can be restored, and support cells (SCs) can be stimulated to re-differentiate and produce HCs (Menendez et al., 2020). The aim is to treat hearing loss associated with HC damage. In this review, we highlight how gene therapy can promote hair cell regeneration as a way to alleviate the hearing loss in patients and provide an outlook for future research in this area.

2. HC development–related transcription factors

During inner ear development, many transcription factors, including *Atoh1*, are involved in the proliferation and differentiation of HCs (Figure 1). In a mouse model of inner ear development, *Atoh1* was first expressed in the basal progenitor HCs at embryonic stage (E) 13.5 d, and gradually increased until the cochlear spiral matured at E17.5, and gradually decreased after postnatal (P) 0 d. After P7, *Atoh1* expression could not be measured in the spiral (Lumpkin et al., 2003; Cotanche and Kaiser, 2010; Cai et al., 2013). In contrast, the change of *Atoh1*-related downstream targeting factor *Gfi1* was consistent with the change of *Atoh1*, which started to be expressed at E12.5 and also gradually decreased in expression with the end of embryonic stage (Wallis et al., 2003). Conversely, *Pou4f3* and *Barhl1* were detected in cochlear basal HCs only at E13.5 and E14.5, respectively, and continued to be expressed after birth (Xiang et al., 1997; Hou et al., 2019; Figure 2).

2.1. *Atoh1*

Atoh1, also known as *Math1*, is a helix–loop–helix (bHLH) family transcription factor with a coding sequence of 1.053 kb, encoding a protein of size 17.9 kDa. *Atoh1* was the first transcription factor identified in differentiated HC progenitors and is essential for HCs growth and differentiation (Bermingham et al., 1999). In *Atoh1* mutant mice, all inner ear sensory regions do not differentiate to produce HCs (Pan et al., 2011). Further studies revealed that the dependence of HCs on *Atoh1* diminishes as sensory cells in the cochlea develop and mature (Chonko et al., 2013). However, it is not the case that the HCs are unaffected by *Atoh1* after cochlear growth, as *Atoh1* deficiency also disrupts the standard hair bundle structure of the auditory system and eventually leads to the delayed death of HCs (Cai et al., 2013; Cheng et al., 2016). In contrast, the enhanced expression of *Atoh1* promotes the normal development of HCs and improves hearing (Izumikawa et al., 2005; Luo et al., 2022). Thus, the entire auditory system, from the developmental to mature stages, is inseparable from the regulation of *Atoh1*.

2.2. *Atoh1* downstream targeting factors *Pou4f3*, *Gfi1*, and *Barhl1*

Due to the importance of *Atoh1* in HCs, identifying the downstream targeting factors of *Atoch1* is crucial to investigate developmental mechanisms. *Atoh1* target groups were identified in mouse cerebellum and cochlea development was studied using genome-wide *Atoh1* sequencing methods (Klisch et al., 2011; Cai et al., 2015). The direct *Atoh1* target genes *Pou4f3*, *Gfi1*, and *Barhl1* are associated with the normal differentiation and regeneration of HCs (Wallis et al., 2003; Zhong et al., 2018; Chen et al., 2021). The *Atoh1* target group has been identified in the cochlea.

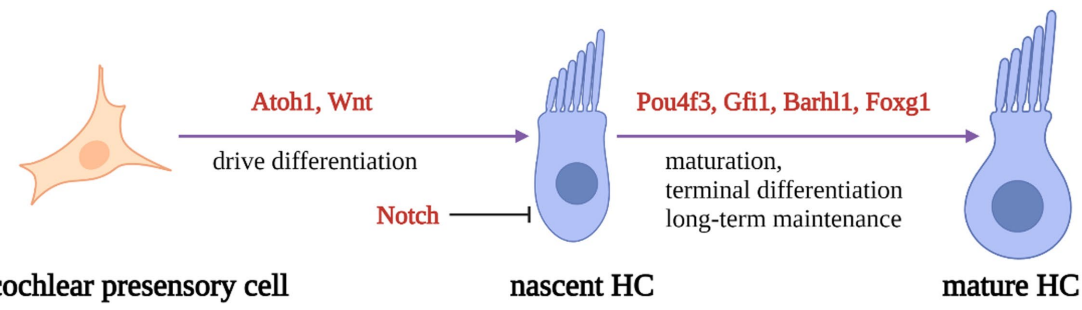

FIGURE 1
Schematic diagram of HC development process. Regulatory factors Atoh1 and Wnt signaling pathways are necessary for presensory cells to differentiate into initial HCs. Atoh1 downstream targeting factors (Pouf4, Gfi1, Barhl3) and Foxg1 play essential roles in nascent HCs maturation and long-term maintenance. At the same time, Notch signal pathway can inhibit the expression of Atoh1 in presensory cells and regulate the differentiation of HCs.

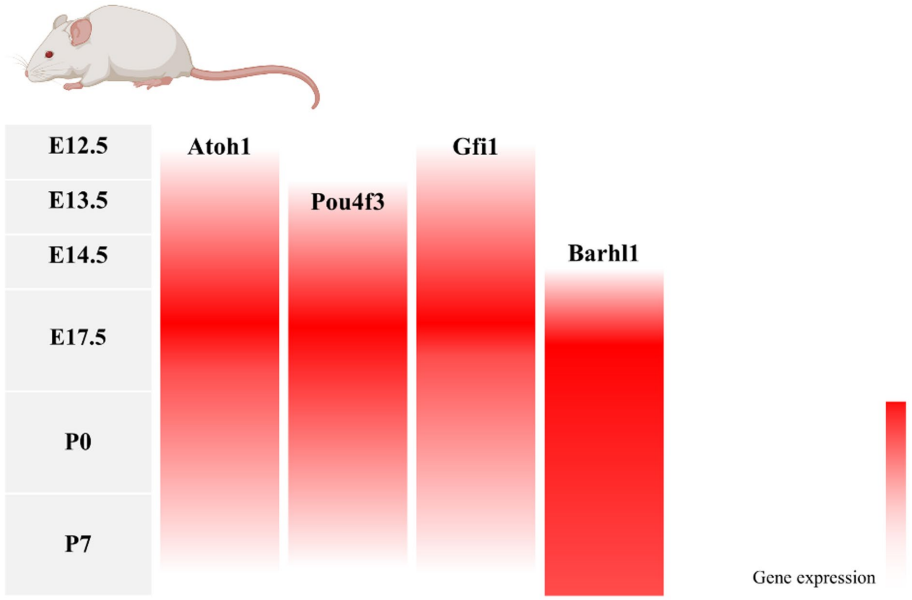

FIGURE 2
Changes in gene expression with age in a mouse model of inner ear development. During mouse embryonic cochlea development, *Atoh1* and its downstream target transcription factors *Pou4f3*, *Gfi1*, and *Barhl1* were successively expressed, with *Atoh1*, *Pou4f3*, and *Gfi1* decreasing in expression after birth as the cochlea matured, and *Barhl1* continuing to be expressed.

Pou4f3, a Pou family transcription factor, is the dominant nonsyndromic deafness 15 (DFNA15) deafness-causative gene (Vahava et al., 1998) and a downstream target of *Atoh1* activation (Ikeda et al., 2015). During HC differentiation, there is a feed-forward synergy between *Atoh1* and *Pou4f3*, with *Atoh1* first stimulating *Pou4f3* expression, which releases *Atoh1*-related elements in a closed state to activate a series of HC-specific enhancers (Yu et al., 2021). *Gfi1* is a zinc-finger transcription factor. Studies have shown that *Gfi1* expression is regulated by *Pou4f3* (Hertzano et al., 2004). *Gfi1* represses neuronal gene expression early in the development of HCs, and in the absence of *Gfi1*, cochlear maturation is stalled (Matern et al., 2020). *Barhl1* is a BarH-like homologous domain transcription factor explicitly expressed in all HCs in the cochlear (Bulfone et al., 2000). Mice lacking *Barhl1* developed severe age-related hearing loss. Further studies have found that HC death in *Barhl1*-null mice begins after 6 days of life and progresses slowly over several months (Li et al., 2002), suggesting that *Barhl1* may be involved in the terminal differentiation and long-term maintenance of HCs.

In conclusion, *Atoh1* is a crucial transcription factor in the formation of HCs, and *Atoh1* mutants lose the ability to generate HC progenitors; *Pou4f3* and *Gfi1*, the genes downstream of *Atoh1*, are required for the late developmental maturation of progenitors into HCs, and delayed degeneration of HCs occurs in *Pou4f3* and *Gfi1* mutants; *Barhl1* is associated with the long-term maintenance of HCs. In *Barhl1* mutants, HCs mature but eventually die within a certain period.

2.3. *Foxg1*

Foxg1, a member of the FOX family, is known to regulate ATP synthesis and metabolism in mitochondria (Pancrazi et al., 2015). *Foxg1* is essential for proper development and formation of the inner ear. In *Foxg1*-null mice, severe inner ear malformations, including shortened cochleae with multiple rows of HCs and supporting cells and reduced or even absent cristae have been reported (Pauley et al., 2006; Hwang et al., 2009). Mechanistically, deletion of *Foxg1* causes inhibition of Notch, Wnt, IGF, and EGF signaling pathways, production of HCs, and induction of their subsequent apoptosis (He et al., 2019). In addition, *Foxg1* regulates auditory degeneration through the regulation of autophagy. In the *Foxg1* downregulated group, the autophagic pathway was significantly inhibited, and reactive oxygen species levels were significantly increased, ultimately leading to the apoptosis of HCs (He et al., 2021). Similarly, *Foxg1* downregulation also considerably increased the sensitivity of HCs to lipopolysaccharide-induced inflammation and accelerated the apoptosis of HCs under inflammatory conditions (He et al., 2020).

3. Gene therapy promotes the regeneration of HCs

3.1. Gene therapy targets for the regeneration of HCs

Due to the critical role that individual genes play in the differentiation and development of HCs, developmental failure during HC differentiation occurs after the deletion of relevant genes. Therefore, inducing re-differentiation to generate new HCs by reprogramming HC-related genes in the inner ear SCs is a potential way to improve HC-related hearing impairment (Costa et al., 2015; Ni et al., 2016; Shibata et al., 2020). HCs regenerate mainly through two pathways: one activates

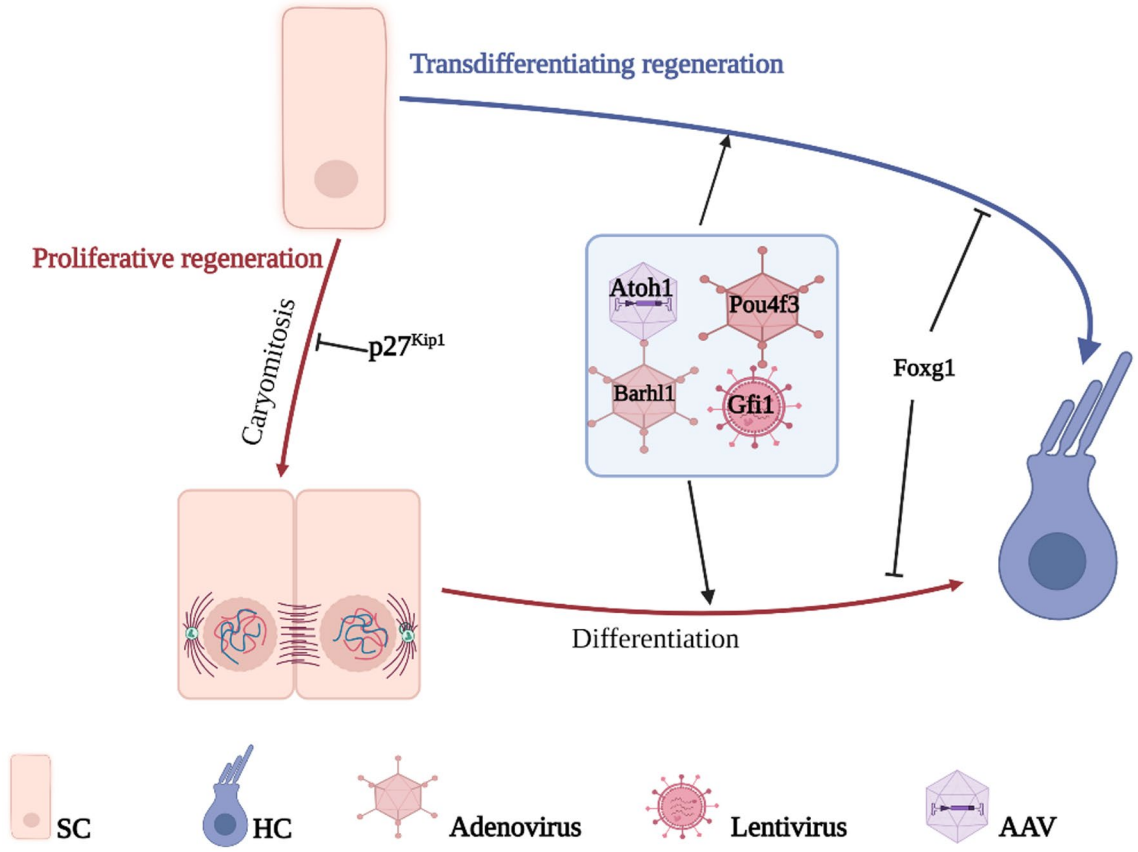

FIGURE 3
Gene therapy promotes HCs regeneration. HCs regenerate mainly through two pathways, direct transdifferentiation of SCs or proliferative differentiation of SCs, the difference between the two is whether mitosis is performed or not. The genes related to the growth, development, and maturation of HCs are transferred to the target cells by gene vector so that the non-sensory cells in the cochlea can differentiate into HCs and realize the regeneration of HCs.

non-sensory cell activation to re-enter the cell cycle and further divide and differentiate into HCs; the other directly induces non-sensory cells to transdifferentiate into HCs without mitosis (Figure 3).

3.1.1. HCs proliferative regeneration

Cell cycle inhibitors are critical for maintaining cells in a quiescent state after mitosis, and therefore activation of non-sensory cells to re-enter the cell cycle requires regulation of the corresponding inhibitors. *P27*^{Kip1} *(p27)*, a member of the Cip/Kip family of cell cyclin-dependent protein kinase inhibitors, is significantly upregulated in dormant cells (Bencivenga et al., 2021) and has been shown to be a common cell cycle inhibitor for sensory and non-sensory cells in the inner ear (Chen and Segil, 1999; Löwenheim et al., 1999). Knocking down *p27* in isolated mouse cochlear cells can effectively activate the proliferation of SCs in cochlea to re-enter the cell cycle, and mitotically generated SCs retain the ability to redifferentiate into HCs (Löwenheim et al., 1999; White et al., 2006; Ono et al., 2009; Maass et al., 2013). Further studies revealed that in *p27* knockout mice, more than just SCs broke out of cell cycle quiescence, HCs also gained some proliferative capacity (Walters et al., 2014), and similar effects were achieved using Retinoic acid to inhibit *p27* (Rubbini et al., 2015). Combined with p27 knockdown, the transdifferentiation of *Atoh1* to produce HCs is not limited to the embryonic period and enables the regeneration of HCs in the mature mouse cochlea (Walters et al., 2017). Unfortunately, the production of HCs by mitotic re-differentiation of SCs does not function properly in mammals, but *p27* remains a potential target for the regeneration of cochlear HCs.

3.1.2. HCs transdifferentiating regeneration

Atoh1, the first HC development–related transcription factor to be identified, plays an irreplaceable role in HC regeneration. In *ex vivo* experiments in normal rats and guinea pigs, *Atoh1* overexpression enabled nonsensory cells of the cochlea to acquire the ability to produce new HCs (Kawamoto et al., 2003; Shou et al., 2003). In a guinea pig model of deafness generated by ototoxic drug-induced HC death, *Atoh1* was injected into the cochlea of deaf animals via an adenoviral vector to increase its expression in nonsensory cells, showing that new HCs were produced at the original site of cochlear trauma. Hearing was restored to some extent in deaf animals as measured based on the auditory brainstem response (ABR) thresholds (Izumikawa et al., 2005). The results showed that new HCs were produced at the original site of trauma in the cochlea and that deaf animals had some hearing recovery as measured using the ABR thresholds.

In contrast, in a model of aminoglycoside-induced profound deafness, although *Atoh1* gene therapy induced the conversion of nonsensory cells in the cochlea into HCs, the resulting HCs failed to mature fully and did not improve hearing in the treated animals (Atkinson et al., 2014). This finding suggests that a combination of gene therapy modalities is required to maximize hearing function in patients. In the Mouse embryonic stem cells cultured *in vitro*, various transcription factors (*Six1*, *Atoh1*, *Pou4f3*, and *Gfi1*) reprogrammed mouse embryonic fibroblasts and expressed the corresponding HC markers. The resulting HCs that were induced were morphologically and physiologically similar to and susceptible to ototoxic drugs as in the case of primary HCs (Costa et al., 2015; Menendez et al., 2020). Similarly, the overexpression of *Gfi1*, *Pou4f3*, and *Atoh1* in human fibroblasts resulted in cells expressing some markers of HCs (Duran Alonso et al., 2018). In drug-treated mouse cochlear sensory epithelial cells, the damage caused by HC death can be reversed by the cotransfection of *Pax2* and *Atoh1*, with *Pax2* promoting the proliferation of SCs and *Atoh1* promoting the regeneration of HCs (Chen et al., 2013). In addition, HC-like cells were generated 4.1-fold more efficiently after cotransfection with *Atoh1* and *Gfi1* than with *Atoh1* alone (Lee et al., 2020); *Atoh1* and *Ikzf2* overexpression induced the transformation of SCs into cochlear outer HCs in the adult mouse cochlea (Sun et al., 2021). The expression of *Atoh1*, *Gfi1*, and *Pou4f3* increased the potency of HC transformation in aged animals (Iyer et al., 2022).

Wnt and Notch pathways play an essential role in cell proliferation and differentiation, including regulating HC differentiation in the cochlea (Ni et al., 2016; Waqas et al., 2016; Wu et al., 2016; Samarajeewa et al., 2019). Disruption of the *Rbpsuh* gene in neonatal mice or treatment of mouse inner ear cells with γ-secretase inhibitor resulted in inhibition of Notch/RBP-J pathway signaling, which in turn led to downregulation of *Hes5* expression and upregulation of *Atoh1* expression, ultimately producing ectopic HCs (Yamamoto et al., 2006; Mizutari et al., 2013; Ren et al., 2016; Luo et al., 2017). Using siRNA to downregulate *Hes1*/*Hes5* can also achieve *Atoh1* upregulation and increase the efficiency of conversion of HCs by SCs (Du et al., 2013; Jung et al., 2013). Adenovirus carrying human *Myc* and *Cre* recombinase genes was injected into the cochlea of adult mice, and an increase in HC numbers was observed along with the inhibition of the Notch pathway (Shu et al., 2019). The expression patterns of the hypermethylated 1 (*HIC1*) transcriptional repressor and *Prox1* genes do not overlap with *Atoh1* and related downstream genes, and studies confirm that both have a repressive effect on *Atoh1* and are responsible for the decrease in *Atoh1* expression in postnatal mice, whereas knockdown of *HIC1* or *Prox1* reverses the repression of *Atoh1* expression and ultimately promotes the differentiation of HCs (Kirjavainen et al., 2008; Abdul-Aziz et al., 2021). Meanwhile, an increase in *Atoch1* expression was induced by the *Atoch1* enhancer or small activating RNA to regulate HC regeneration (Luo et al., 2022; Zhang et al., 2022).

Moreover, recent studies on *Foxg1* have demonstrated its potential as a new target for the regeneration of HCs using gene therapy. In mice with *Foxg1* was knocked out in the inner ear SCs, HC numbers were significantly increased compared to those in normal mice, and the survival time was greatly increased (Zhang Y. et al., 2020; Zhang S. et al., 2020).

In conclusion, with a clear understanding of the mechanism of developmental differentiation of HCs, the regeneration of HCs can be achieved by interference with the relevant genes and pathways, thus reversing hearing loss caused by HC damage.

3.2. CRISPR/Cas9 gene editing system

As the third generation of gene editing technology after zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), the CRISPR/Cas system has the advantages of clear targeting, short RNA sequences, and simultaneous operation of multiple genetic loci, of which the type II CRISPR/Cas9 system is the most widely applied (Bhatia et al., 2023; van der Oost and Patinios, 2023; Wang and Doudna, 2023). Cas9 protein with nucleic acid endonuclease function and single guide RNA (sgRNA) shear the target genome to generate double-strand breaks (DSB), which in turn enables knockdown or knock-in of the target gene by Homology-directed repair (HDR) or Non-homologous end joining (NHEJ) to achieve knockdown or knock-in of target genes (Figure 4). Previous studies have used the CRISPR/Cas9 system to establish transgenic mouse models of deafness to investigate the importance of target genes for the development and maintenance of normal hearing in the inner ear HCs (Li et al., 2018, 2019; Zhu et al., 2018; Cui et al., 2020; Zhang L. et al., 2020; Tu et al., 2021; Xue et al., 2022). CRISPR-Cas9 technology is now also showing great potential in clinically blocking dominant and recessive mutations in deafness and improving hearing impairment (György et al., 2019; Farooq et al., 2020; Ding et al., 2021).

Beethoven deaf mice are deafened by a point mutation (T into A) in the *Tmc1* gene at locus 1,235, causing hearing impairment associated with reduced HCs in the inner ear and successfully targeting the *Tmc1* gene by the lipid-mediated entry of the

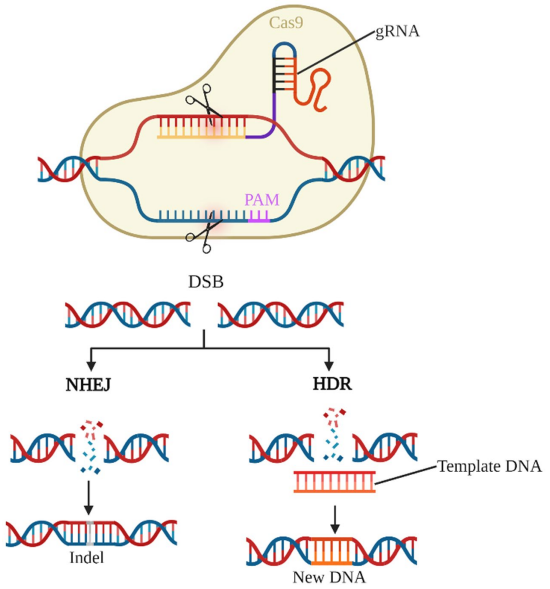

FIGURE 4
CRISPR/Cas9 gene editing system. The gRNA-Cas9 complex enters the cell and identifies the target gene corresponding to the PAM and shears it into a DSB. NHEJ causes the target gene shift mutation to achieve gene knock-out. HDR, on the other hand, repairs the target gene according to the exogenous template DNA and completes the gene knock-in.

Cas9-gRNA complex into the mouse cochlea, resulting in a shift mutation and eventual loss of function due to a random insertion–deletion, which improves the survival of HCs while improved the hearing of mice (Gao et al., 2018). Efficient knockdown of the *Htra2* gene associated with apoptosis by transfection of three gRNAs into *in vitro* cochlear explants and *in vivo* scala medium *via* the CRISPR/Cas9 system improved hearing loss caused by neomycin-induced hair cell apoptosis (Gu et al., 2021). In addition, CRISPR-Cas9 knockdown of *Kcnq4* and myosin VI (*MYO6*) mutant genes have been shown to rescue inherited hearing impairment (Noh et al., 2022; Xue et al., 2022).

Although the CRISPR/Cas9 system can accurately and efficiently edit target genes, it also has limitations in hearing damage rescue studies. The presence of a short DNA sequence called the pro-spacer adjacent motif (PAM) near the complementary region of the gRNA and the target gene. The PAM sequences are mainly used to identify targets, and the presence or absence of PAM sequences in target nucleotides is a critical factor in the precise targeting of the CRISPR/Cas9 system (Manghwar et al., 2019).

3.3. Gene delivery vectors

The two main types of vectors for gene therapy are viral vectors and non-viral vectors, namely, viral vectors including adenovirus (AdV), adeno-associated virus (AAV), lentivirus, and retroviruses; and nonviral vectors including electroporation, liposomes, nanoparticles, and exosomes (Cring and Sheffield, 2022). The choice of gene therapy vector is significant, as it needs to deliver the exogenous gene safely and effectively to the cells of the inner ear without causing a robust immune response and to sustain its action.

AdVs were the first gene delivery vectors to be used; they are now used in various fields for HC regeneration (Syyam et al., 2022). *In vivo* or *in vitro* experiments involving the injection of AdVs carrying different target genes into target cell tissues can effectively transduce nonsensory cells into HCs, with SCs being the main ones transduced (Kawamoto et al., 2003; Shou et al., 2003; Izumikawa et al., 2005; Yamamoto et al., 2006; Chen et al., 2013; Atkinson et al., 2014; Shu et al., 2019; Lee et al., 2020). However, AdVs have a significant immunogenic effect, and their role in gene therapy is somewhat limited. In contrast, AAVs have a much lower immunoreactivity and have gradually become the vehicle of choice for gene therapy in different fields. AAVs have demonstrated their safety and efficacy in gene therapy for the regeneration of HCs. Injecting AAV8 in normal neonatal and adult mice did not cause damage to HCs in the inner ear or hearing loss (Kang et al., 2020). In addition, AAV-mediated gene delivery effectively ameliorates apoptosis and hearing loss of HCs in a drug-induced mouse model of deafness in the long term (Brigande et al., 2009; He et al., 2020; Gu et al., 2021; Xu et al., 2021; Xue et al., 2022). The results of this study are summarized below. Recent studies have shown that AAV-inner ear, a variant of AAV, can more safely and efficiently transduce Atoh1 into SCs and may be the best vehicle for future gene therapy to combat hearing loss (Tan et al., 2019; Tao et al., 2022). In addition, lentiviruses and retroviruses can also be used to deliver HC regeneration–related genes; however, their safety profile needs to be improved (Costa et al., 2015; Menendez et al., 2020).

Other nonviral gene delivery methods have also been used to regenerate HCs. *Hes1* siRNA delivered by propylene-co-glycolate nanoparticles can reduce cochlear *Hes1* mRNA while upregulating *Atoh1* mRNA expression and, in doing so, promote the ability of SCs to acquire redifferentiated HCs (du et al., 2013). In addition, electroporation was influential in transducing plasmids encoding target genes such as *Tub* and *Znf532* into the epithelial progenitor cells of the ear, activating the regeneration of HCs mediated by genes such as *Atoh1* (Brigande et al., 2009; Xu et al., 2021).

4. Summary and perspectives

HCs in the cochlea, as key members of the auditory conduction system, transform incoming mechanical signals into electrical signals for the body to perceive. They do not regenerate spontaneously in mammals, resulting in the associated hearing impairment being poorly treated. An exploration of the developmental maturation mechanisms of HCs reveals that the HC regulatory gene *Atoh1* and its downstream targeting factors activate the ability of nonsensory SCs to differentiate into HCs. Emerging gene therapies can deliver external DNA or RNA into target cells via vectors to alter the gene expression of target cells and improve relevant functions. After addressing congenital hearing impairment, gene-based therapies can be used to treat other types of hearing impairment with the help of HC regeneration mechanisms (Table 1).

Many challenges remain in inducing regeneration of HCs in clinical settings based on gene therapy. First, the growth and development of HCs are regulated by multiple genes and pathways, and a single gene alone cannot bring about the differentiation of SCs into fully functional mature HCs. Second, the choice of vectors for gene delivery is also essential, as it is necessary to deliver the gene to the target cells efficiently and accurately without inducing a robust immune response in the body. Finally, enhancing the efficiency of HCs regeneration while ensuring high targeting requires innovation in multiple steps of gene therapy. Recent studies using the CRISPR/Cas9 system in combination with AAV vectors have shown great advantages (Kang et al., 2020; Zhao et al., 2020).

In conclusion, HC regeneration–based gene therapy shows immense potential in treating sensorineural hearing impairment. It is expected to be used in a clinical setting after further research on the mechanism of HC regeneration and optimizing targeted gene delivery methods.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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TABLE 1 Summary of studies on regenerative gene therapy for HCs.

Experimental subjects	Mode of gene transfer	Contents	Results	References
A model of ototoxic drug-induced deafness in guinea pigs	Adenovirus	*Atoh1*	HCs regenerate, and hearing improves.	Kawamoto et al. (2003), Shou et al. (2003), Izumikawa et al. (2005), and Atkinson et al. (2014)
Mouse embryonic stem cells	Retrovirus/Lentivirus	*Six1 Atoh1 Pou4f3 Gfi1*	Produces induced HCs similar to HCs	Costa et al. (2015) and Menendez et al. (2020)
Human fibroblasts	Lentivirus	*Atoh1 Pou4f3 Gfi1*	Acquisition of cells expressing markers of HCs	Duran Alonso et al. (2018)
Cochlear sensory epithelium in mice after neomycin injury	Adenovirus	*Pax2 Atoh1*	Inducing the production of new HCs with some functional activity	Chen et al. (2013)
Pou4F3^{DTR} mice after diphtheria toxin injury	Adenovirus	*Atoh1 Gfi1*	Induced production of HC-like cells is more efficient than *Atoh1* alone	Lee et al. (2020)
Exosomes of mouse Corti organs carrying the Rbpsuh allele	Adenovirus	Cre recombinase gene	*Rbpsuh* target genes were deleted to obtain cells expressing HC markers	Yamamoto et al. (2006)
A model of ototoxic drug-induced deafness in mice	Physiological saline/propylene-co-glycolic acid nanoparticles	*Hes1*/*Hes5* siRNA	Upregulation of *Atoh1* expression and increase in the number of HCs	du et al. (2013) and Jung et al. (2013)
Rosa-NICD transgenic mice	Adenovirus	*Myc* and *Cre* recombinase genes	Notch pathway inhibition, HC numbers rise	Shu et al. (2019)
C57BL / 6 mice	AAV-ie	*Atoh1*	A large number of infantile HCs appeared compared with the control group	Tan et al. (2019) and Tao et al. (2022)
FVB mice	Electroporation	*Tub Znf532*	Promotes *Atoh1*-mediated regeneration of HCs while ensuring minimal damage to endogenous HCs	Xu et al. (2021)

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Characteristics of large vestibular aqueduct syndrome in wideband acoustic immittance

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Objective: To describe the characteristics of large vestibular aqueduct syndrome (LVAS) in wideband acoustic immittance (WAI) and to explore whether inner ear deformity has an impact on WAI results.

Methods: Subjects with typical LVAS (LVAS group) and control subjects with a normal anatomical structure of the inner ear (control group) were screened from pediatric patients with cochlear implants using thin-slice computed tomography (CT) images of the temporal bone. With inflammation of the auditory canal and middle ear excluded by routine ear examination and 226 Hz acoustic immittance, WAI data were acquired. Then, the maximum absorbance as the major observation indicator on the mean tympanogram was compared between the LVAS group and control group, and a descriptive comparison of the mean tympanogram and frequency-absorbance curve at peak pressure was performed between the two groups.

Results: The LVAS group included 21 cases (38 ears), and the control group included 27 cases (45 ears). All LVAS subjects met the Valvassori criteria, and the VA at the horizontal semicircular canal displayed flared expansion. On the mean tympanogram, the maximum absorbance in the LVAS group (0.542 ± 0.087) was significantly higher than that in the control group (0.455 ± 0.087) ($p < 0.001$). The tympanogram in the LVAS group showed an overall elevation, and the absorbance at all pressure sampling points was significantly higher than that in the control group ($p < 0.001$). The frequency-absorbance curve at peak pressure first increased and then decreased in both groups, and the LVAS group showed higher absorbance than the control group in the frequency range below 2,828 Hz. The absorbance at 343–1,124 Hz was significantly different between the two groups ($p < 0.001$), and 343–1,124 Hz was the major frequency range at which the maximum absorbance on the mean tympanogram increased in the LVAS group.

Conclusion: Large vestibular aqueduct syndrome (LVAS) shows increased absorbance in low and medium frequency ranges in WAI. The maximum absorbance on the mean tympanogram can serve as a reliable evaluation indicator. Inner ear factors must be considered when middle ear lesions are analyzed by WAI.

KEYWORDS

large vestibular aqueduct syndrome (LVAS), wideband acoustic immittance, maximum absorbance, mean tympanogram, frequency-absorbance curve

Introduction

Wideband acoustic immittance (WAI) is a novel middle ear clinical assessment tool to reflect the functional status of the middle ear sound transmission system by measuring the sound energy back to the external auditory canal through the tympanic membrane. WAI has been found to be more sensitive to lesions of the middle ear sound transmission system and can effectively avoid indistinct characteristics of diseases or even missed diagnoses resulting from the offsetting of acoustic compliance in complex lesions in the middle ear, reflecting significant advantages over traditional 226 Hz acoustic immittance (Sanford et al., 2013; Liu et al., 2021; Miehe et al., 2022). WAI are better indicators of conductive hearing loss and middle-ear dysfunction relative to standard 226 Hz tympanometry. The use of a single frequency as in conventional tympanometry cannot investigate middle-ear function at all the frequencies which are critical for human auditory communication. WAI can provide references for the analysis and identification of middle ear diseases and has gradually attracted clinical attention in recent years. During sound transmission, however, sound energy conduction disorders can also be caused by hydrodynamic changes in the inner ear lymphatic fluid, which manifest as conductive hearing loss (Madden et al., 2003). Deficits in the cochlea can also affect middle ear functions. While approaching to patients with inner ear malformations, it is significant to consider their hearing characteristics. Therefore, whether the inner ear components must be considered when analyzing WAI results is worth exploring.

The large vestibular aqueduct syndrome (LVAS) is characterized by the presence of an abnormally large vestibular aqueduct (LVA) generally associated with fluctuating, progressive sensorineural hearing loss (SNHL), often with sudden, stepwise onset or progression secondary to activities involving minor head trauma, large sudden shifts of barometric pressure, and the Valsalva maneuver (Nakashima et al., 2000; Ding et al., 2021; Ota et al., 2021). Large vestibular aqueduct syndrome is one of the most frequent morphogenetic contributors to hearing loss in children, although its prevalence is underestimated. In addition, it is often associated with other congenital inner ear anomalies, the most common being an abnormally large vestibule, an enlarged semicircular canal, or a hypoplastic cochlea (Kei et al., 2013). With large vestibular aqueduct syndrome (LVAS), a typical kind of inner ear deformity, as an example, whether structural deformities generate characteristic manifestations in WAI and the impact of anatomical deformities of the inner ear on WAI absorbance were analyzed in this paper.

Materials and methods

Clinical data

Pediatric patients who received cochlear implantation in Nanjing Drum Tower Hospital from July 2019 to June 2022 (LVAS group) were recruited. The inclusion criteria were as follows: (1) patients who met the computed tomography (CT) imaging diagnostic criteria (Valvassori criteria) for LVAS [i.e., a diameter from the crus commune of the semicircular canal to the first part of the outer orifice of the VA (midpoint measurement, MP) $\geq$ 1.5 mm and a diameter of the outer orifice of the VA (operculum measurement, OP) $\geq$ 2 mm on axial high-resolution CT (HRCT) images of the temporal bone] and (2) patients with unobstructed external auditory canals, intact tympanic membranes on otoscopy, normal middle ear development, and no inflammatory lesions on thin-layer CT images of the temporal bone. Actual CT images from the recruited patients were demonstrated in **Figures 1**, **2**.

The exclusion criteria were as follows: (1) patients complicated with inner ear deformities, such as Mondini malformation or structural abnormalities of the internal auditory canal and (2) patients with a recent history of upper respiratory tract infection or a non-type A tympanogram. Finally, a total of 21 patients (38 ears) met the inclusion and exclusion criteria, including 13 males and 8 females with a median age (interquartile range) of 57 months (35, 96). In addition, pediatric patients of similar ages who received cochlear implant surgery during the same period were recruited as the control group. A total of 27 patients (45 ears) met the inclusion criteria (normal anatomical structure of the cochlea on thin-layer CT images of the temporal bone, no recent history of upper respiratory tract infection, and a type A tympanogram), including 14 males and 13 females with a median age (interquartile range) of 32 months (21.5, 58).

Assessment methods

CT scan of the temporal bone

Computed tomography (CT) scanning of the temporal bone as a routine preoperative examination was conducted using a GE LightSpeed 64-slice spiral CT scanner with the following parameters: slice thickness of 0.45 mm, slice gap of 0.45 mm, pitch of 0.562, 140 kV, and 300 mAs. After image acquisition by bone algorithms, the image was reconstructed bilaterally with

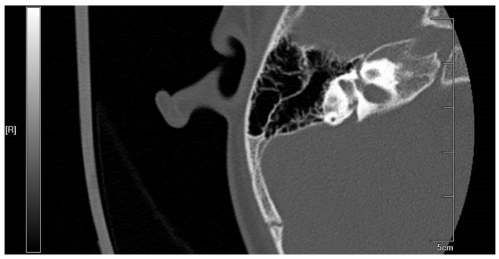

FIGURE 1
Computed tomography (CT) imaging from recruited large vestibular aqueduct syndrome (LVAS) Patient.

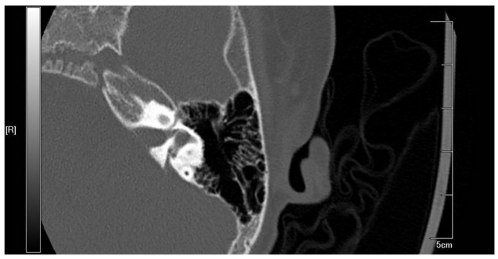

FIGURE 2
Computed tomography (CT) imaging from recruited large vestibular aqueduct syndrome (LVAS) Patient.

the promontorium tympani as the center, and the reconstruction interval was 0.3 mm. In the best plane displayed on the cross-sectional image, the external orifice diameter of the VA (ODVA) inside the ES bone and the midway diameter of the VA (MDVA) (i.e., the diameter of the aqueduct between the midpoint of the crus commune of the vestibulum and the external orifice of the VA) were measured.

WAI

Wideband acoustic immittance (WAI) was conducted on the patients in a quiet state using the Titan WAI meter (Interacoustics, Denmark) in a soundproof room with a background noise < 30 dB (A). The patients were sedated with chloral hydrate, and the external auditory canal and tympanic membrane were assessed prior to the test. Cerumen, if present, should be removed. The test software included 226 Hz acoustic immittance (initial pressure: +200 daPa, end pressure: −400 daPa, positive → negative) and WAI (mixed short sound with an intensity of 85 dB SPL and a frequency of 226–8,000 Hz). The test probe oriented toward the tympanic membrane was plugged into the external auditory canal, and after the sealing test was passed, the start button was clicked to automatically perform 226 Hz acoustic immittance, WAI, and the stapedius reflex test. Then, 3D images were generated by the software according to the absorbance at different pressure and frequency sampling points. Based on the resulting images, frequency-absorbance curves at peak pressure and ambient pressure (226–8,000 Hz, 1/24 octave sampling) and the 375–2,000 Hz mean tympanogram (sampled every 1 daPa at +200 daPa to −400 daPa) were generated from different analysis perspectives, and the sample data were generated in Excel.

The data for subjects who met the inclusion and exclusion criteria were identified, and the maximum absorbance on the mean tympanogram was used as the major indicator for analyzing the characteristic manifestations of LVAS in WAI. A descriptive analysis was conducted on the mean tympanogram and frequency-absorbance curves at peak pressure.

Statistical methods

Normally distributed continuous data are described as the mean ± standard deviation and compared between two groups by the *t*-test. Non-normally distributed data are described as the median (quartile) and compared between two groups by a non-parametric test. Finally, qualitative data are described as numbers and percentages and compared between two groups by the chi-square test or Fisher's exact probability test. $P < 0.05$ was considered statistically significant (SPSS 22.0, Chicago, IL, USA).

Results

The mean tympanogram was unimodal in both groups. The maximum absorbance in the LVAS group (0.542 ± 0.087) was significantly higher than that in the control group (0.455 ± 0.087) ($p < 0.001$). The pressure point where the maximum absorbance was located showed no statistically significant difference between the two groups [LVAS group: (16.079 ± 43.202) daPa vs. Control group: (18.756 ± 29.949) daPa, $p > 0.05$]. The absorbance at each pressure sampling point in the LVAS group was higher than that in the control group, and the difference was statistically significant ($p < 0.05$ or 0.001) (**Figure 3**).

With increasing acoustic frequencies, the frequency-absorbance curve at peak pressure first increased and then decreased in both groups. In the LVAS group, the curve sharply increased at 226–1,029 Hz and slowly increased at 1,029–3,084 Hz. In the control group, the curve sharply increased at 226–1,373 Hz and slowly increased at 1,373–3,174 Hz. The LVAS group had higher absorbance than the control group in the frequency range below 2,828 Hz, while the results were the opposite in the frequency range above 2,828 Hz. In particular, the absorbance at 343–1,124 Hz, 1,943–2,448 Hz, and 3,886–6,727 Hz showed statistically significant differences between the two groups ($p < 0.05$ or 0.001) (**Figures 4–6**).

Discussion

As a common structural deformity of the inner ear, LVAS originates from developmental disorder of the endolymphatic ducts (endolymphatic sacs) at mid-embryo stages, which can maintain the VA and endolymphatic ducts (endolymphatic sacs) in a wide state at early-embryo stages until LVAS occurs after birth (Hunter et al., 2013; Shahnaz et al., 2013; Scheperle and Hajicek, 2020; Eberhard et al., 2022a). Therefore, to better analyze the impact of inner ear structural abnormalities on WAI absorbance, cases were strictly selected according to the Valvassori criteria, i.e., the subjects

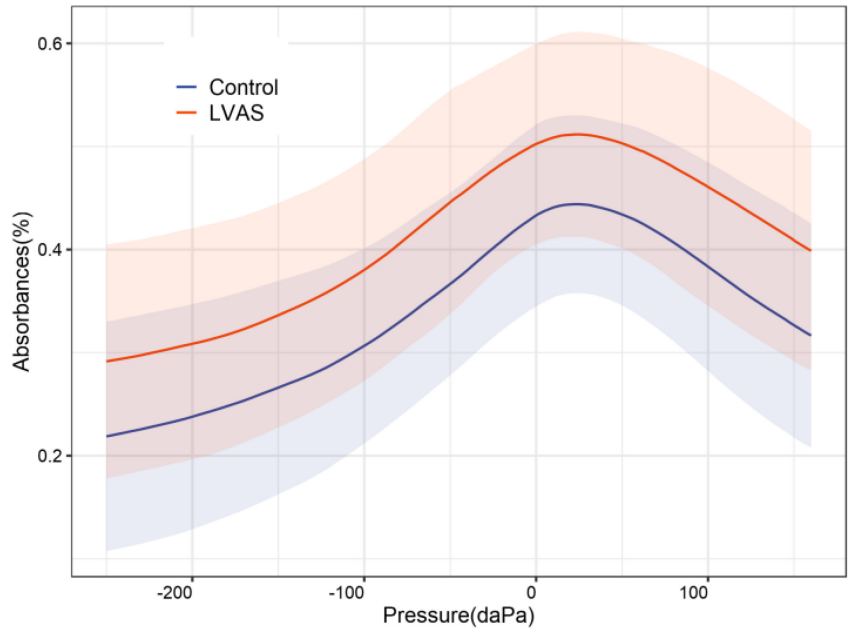

FIGURE 3
2-dimensional comparison of the mean tympanograms between the large vestibular aqueduct syndrome (LVAS) and control groups.

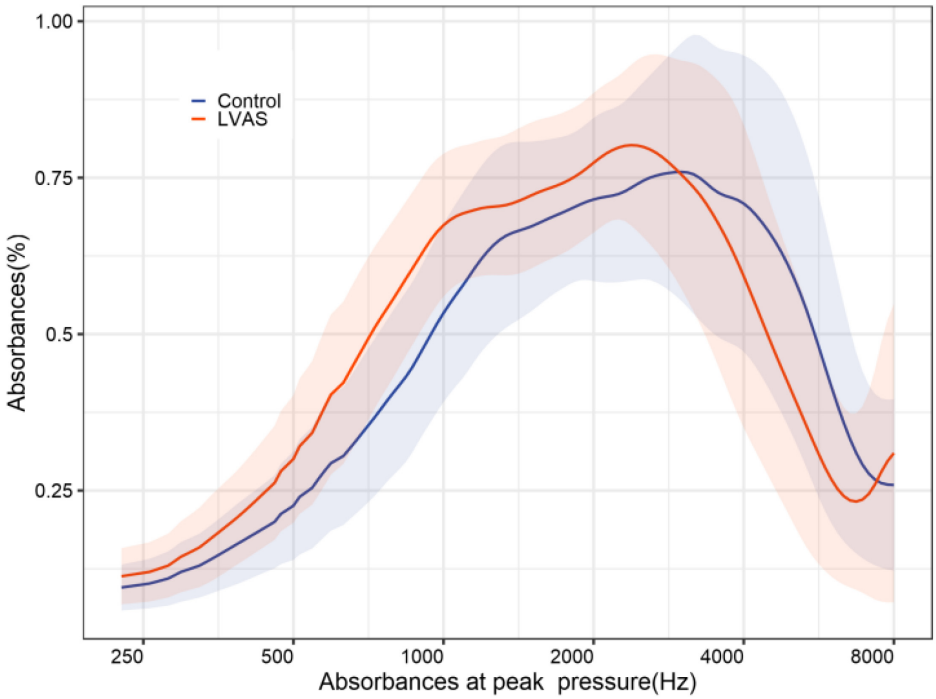

FIGURE 4
2-dimensional comparison of frequency-absorbance curves at peak pressure between the large vestibular aqueduct syndrome (LVAS) group and the control group.

needed to meet the original two criteria, and the VA on the image should demonstrate flared expansion.

The mean tympanogram of WAI reflected the superposition of the tympanograms in the 3D absorbance diagram at an acoustic frequency of 375–2,000 Hz. Since absorbance was used as the measurement indicator, the unpredictability of the diagram of immittance caused by mutual changes in elastic stiffness and mass with increasing acoustic frequency could be avoided, which is an advantage of WAI. Moreover, the absorbance value corresponding to the pressure axis in the diagram was an average and contained more robust information than the measured value at a specific sampling point in the frequency-absorbance curve. Furthermore,

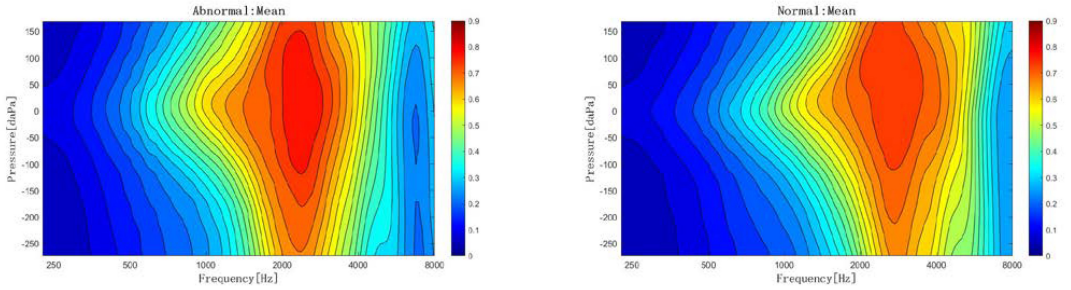

FIGURE 5
2-dimensional comparison of frequency-absorbance curves at peak pressure between the large vestibular aqueduct syndrome (LVAS) group and the control group (**left**: control group; **right**: LVAS group).

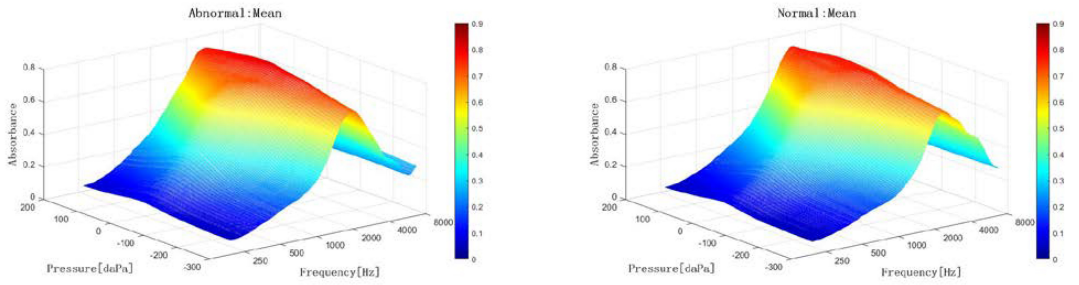

FIGURE 6
3-dimensional comparison of frequency-absorbance curves at peak pressure between the large vestibular aqueduct syndrome (LVAS) group and the control group (**Left**: control group; **right**: LVAS group).

the resulting diagram was similar to a traditional 226 Hz tympanogram (Sun, 2016; Aithal et al., 2019). With reference to the principle of Liden-Jerger classification based on the value and location of the maximum immittance, the maximum absorbance on the mean tympanogram was selected as the major evaluation indicator, which could be better quantified in a diagram analysis (Fu et al., 2017). Additionally, a descriptive analysis was conducted on the change trend and difference between the overall diagram and the diagram in the control group. Finally, the frequency ranges that contributed to the maximum absorbance in the frequency-absorbance curve were analyzed. As a result, the data could be analyzed comprehensively.

In this study, the maximum absorbance in the LVAS group was significantly higher than that in the control group, and the absorbance at all pressure sampling points on the mean tympanogram showed a significant increase. That is, the absorbance curve on the mean tympanogram showed an overall elevation compared to that in the control group. Furthermore, the pressure point where the maximum absorbance was located on the mean tympanogram corresponded to the frequency-absorbance curve at the peak pressure. The absorbance in the frequency range < 2,500 Hz was higher in the LVAS group than in the control group, which was consistent with the frequency superposition range of the maximum absorbance. The absorbance in the frequency range < 1,100 Hz was significantly different between the two groups, which was the major frequency range where the maximum absorbance increased (Aithal et al., 2017; Demir et al., 2020; Eberhard et al., 2022b; Meng et al., 2022; Tanno et al., 2022). At the same time, the absorbance in the frequency range > 3,000 Hz in the frequency-absorbance curve at peak pressure was lower in the LVAS group than in the control group. The influencing factors for middle ear sound transmission include elastic stiffness and mass. Elastic stiffness has a greater impact on the transmission efficiency of low-frequency sound signals, while mass has a greater impact on the transmission efficiency of high-frequency sound signals (Kaya et al., 2020; Downing et al., 2022; Miehe et al., 2022; Aithal et al., 2023). The available results are believed to be related to changes in the anatomical structure and biochemical properties of LVAS and the frequency of the stimulus sound. Although the pathogenesis of LVAS remains unclear, many theories have been proposed. Anatomically, the internal orifice of the VA is located on the medial wall of the vestibulum adjacent to the oval window, which is normally a tiny bony canal. Previous studies reported that structural expansion would lead to the expansion of the endolymphatic ducts and endolymphatic sacs. This increases the pressure of the endolymphatic and perilymphatic fluids, leading to a higher elastic stiffness. As a result, this physical change increases low-frequency sound conduction efficiency (Özdemir et al., 2022).

In addition, excessive pressure can cause rupture of the membrane labyrinth, ion mixing of endolymphatic and perilymphatic fluids, and stria vascularis dysfunction, resulting in ion transport disorders and protein exudation. Low signals on inner ear images indirectly indicate changes in the composition and properties of lymph fluids (Polat et al., 2015; Stuppert et al., 2019; Hougaard et al., 2020; Wu et al., 2022). These changes can damage hair cells and alter sound transmission performance. Due

to the above factors, the absorbance of low-frequency sounds on the mean tympanogram may increase, particularly an overall increase in the entire pressure range. On the other hand, a high-frequency sound has a short wavelength, and its energy decays quickly during transmission, which is more susceptible to mass. Due to the VA enlargement, the volume of the inner ear increases. This reduces the conduction of high-frequency sound (Sundgaard et al., 2022), i.e., a decline in absorbance, suggesting that an increased inner ear volume should be part of the mass in the whole sound transmission system. From the audiology perspective, LVAS often manifests as characteristic low-frequency air-bone conduction differences, but no reports are available on air-bone conduction differences in patients with high-frequency residual hearing, probably suggesting a difference in the absorbance of low-frequency and high-frequency sounds. Of course, the present results remain to be further validated by more studies.

Limitations of our study

The small sample size of our study may have reduced our ability to provide WAI norm data for patients with LVAS. Therefore, studies with a larger sampling size are strongly recommended. Additional work is needed to determine normative data for various age groups since age-related differences in ambient and tympanometric WAI have been observed. Moreover, key indicators should be identified to develop high sensitivity and specificity in WAI analysis.

Conclusion

In conclusion, LVAS shows increased absorbance in WAI's low and medium frequency ranges. The maximum absorbance on the mean tympanogram can serve as a reliable evaluation indicator. Inner ear factors must be considered when middle ear lesions are analyzed by WAI. In the future, whether this conclusion applies to other inner ear deformities will be further explored.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Nanjing Drum Tower Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AL: conceptualization, methodology, formal analysis, visualization, and writing—original draft. HD: data curation, methodology, formal analysis, investigation, and writing—original draft. JG: formal analysis, investigation, resource, and data curation. YXu: formal analysis, investigation, and resource. NZ: data curation and investigation. SG: resources and investigation. YXi: software and validation. XQ and XG: supervision, project administration, writing–review, editing, and resources. YY: project administration, writing–review, editing, and resources. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Research progress on the treatment and nursing of sensorineural hearing loss

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This article provides a comprehensive review of the progress in the treatment and care of sensorineural hearing loss (SNHL), which is a common disease in the field of otolaryngology. In recent years, the incidence of SNHL has been on the rise due to factors such as fast-paced lifestyles, work pressure, and environmental noise pollution, which have a significant impact on the quality of life of patients. Therefore, the study of the treatment and care of SNHL remains a hot topic in the medical community. Despite significant advances in this field, there are still some challenges and limitations. For example, there is currently no single method that can completely cure SNHL, and the effectiveness of treatment may vary significantly among individuals. In addition, due to the complex etiology of SNHL, the prognosis of patients may vary greatly, requiring the development of personalized treatment plans and care strategies. To address these challenges, continuous research is needed to explore new treatment methods and care models to improve the quality of life of patients. In addition, there is a need for health education programs for the general public to raise awareness of SNHL and promote preventive measures to reduce its incidence. The ultimate goal is to ensure the sustainable development of the field of SNHL treatment and care, thus ensuring the health and well-being of affected individuals.

KEYWORDS

sensorineural hearing loss, treatment, nursing, review, progress

Introduction

Sensorineural hearing loss (SNHL) is a common otolaryngologic disorder characterized by damage to the auditory nerve resulting in decreased or complete loss of hearing (Gregory et al., 2023; Matsunaga and Nakagawa, 2023; Zine and Fritzsch, 2023). The incidence of this disease has been increasing in recent years, which may be associated with factors such as fast-paced lifestyles, high-intensity work, and environmental noise pollution in modern society (Al-Azzawi and Stapleton, 2023; Saba et al., 2023). The treatment and care of this disease has been a hot topic in the medical field.

Although there have been many studies on the treatment and care of SNHL, there are still many questions about its pathogenesis and mechanisms. In many cases, the etiology of SNHL is not clear, especially in the absence of specific external stimuli and injuries (Marchioro et al., 2023). Therefore, research on its pathogenesis and mechanisms is still very necessary. From a molecular perspective, SNHL may involve a range of factors such as gene mutations, epigenetic changes, and protein abnormalities (Miguel et al., 2018; Jabbari Moghadam et al., 2023; Kelleci and Golebetmaz, 2023). Some gene mutations may lead to abnormal or disrupted function of

the auditory nerve, leading to the occurrence of SNHL. In addition, epigenetic changes such as DNA methylation and histone modifications have also been found to be associated with SNHL, which may affect gene expression and function (Friedman and Avraham, 2009; Hou et al., 2016; Leso et al., 2020; Flook et al., 2021). Furthermore, protein abnormalities such as synaptic protein deficiency and metabolic enzyme dysfunction are also related to the occurrence of SNHL (Liu et al., 2022). In addition to the molecular level, the etiology and mechanisms of SNHL also involve various aspects such as neurodegenerative diseases, infection, poisoning, and hypoxia. For example, some neurodegenerative diseases such as presbycusis and acoustic neuroma may lead to SNHL (Paciello et al., 2023a, b). Infectious diseases such as rubella, measles, and influenza virus infections may also lead to SNHL (Moseley et al., 2023). Additionally, toxins such as ototoxic drugs and organic solvents may also cause damage to the auditory nerve, resulting in the occurrence of SNHL (Wang et al., 2023).

In terms of the etiology and mechanisms of SNHL, one of the research focuses is to search for potential therapeutic and preventive strategies. For example, treatment methods targeting certain gene mutations and epigenetic changes have been proposed in research. In addition, some studies have suggested that certain drugs and compounds may also have a positive impact on the development and progression of SNHL. For instance, drugs such as antioxidants, neurotrophins, and immunomodulators have been shown to play a role in protecting cochlear and auditory nerve function (Paciello et al., 2023a,b). Furthermore, recent studies have indicated that photoreceptors also play an important role in the occurrence and development of SNHL. Researchers have found that photosensitive proteins are expressed in the neurons and glial cells of the inner ear and are involved in the metabolism and photoresponse processes of inner ear cells. Therefore, the use of phototherapy to regulate inner ear cell metabolism and restore photoreceptor function has become a hot topic of research (Alkén et al., 2019).

In addition to finding treatment methods, preventing the occurrence of SNHL is also one of the research focuses. Although the protection of noise exposure and occupational hazards has received widespread attention, there are still many potential risk factors in areas such as home, entertainment, transportation, and military (Manickam et al., 2023). Therefore, raising public awareness and taking corresponding preventive measures are of great significance in preventing the occurrence of SNHL.

In summary, the etiology and pathogenesis of SNHL are very complex, involving multiple factors and mechanisms. Currently, the treatment and prevention of SNHL mainly rely on drug therapy, auditory rehabilitation, and individualized intervention methods. However, effective treatment and prevention of SNHL still face certain challenges and difficulties. Therefore, in-depth research on the etiology and pathogenesis of SNHL, exploring new therapeutic and preventive strategies, is of great significance in improving patients' quality of life.

Treatment method

Sensorineural hearing loss (SNHL) is a common auditory disorder that affects an increasing number of individuals. Currently, there are several treatment methods available for this condition. Drug therapy, including oral and intravenous methods, is one of the most commonly used treatments. Improvement can be achieved through the use of drugs that improve microcirculation, provide neuronutrition, and hormonal drugs (Erni et al., 2021). The efficacy of drug therapy can be enhanced through methods such as intratympanic injection.

In addition to drug therapy, traditional Chinese medicine (TCM) is also an effective treatment for SNHL (Fei et al., 2019). TCM treatment involves internal medicine, acupuncture, and ear acupressure therapy, which regulate the meridians and promote the exchange of substances between blood and the labyrinth, allowing for repair and regeneration of the inner ear and improvement of the patient's hearing level. TCM treatment usually requires a certain amount of time to take effect. Furthermore, hyperbaric oxygen therapy is also a method for treating SNHL (Rhee et al., 2018; Joshua et al., 2022). Hyperbaric oxygen therapy uses a high-pressure oxygen environment to quickly increase the blood oxygen content, tension, and diffusion in the patient's inner ear, repairing the cochlea and vestibular nerve fibers, and improving hypoxia and ischemia. Hyperbaric oxygen therapy should be performed in a specialized medical institution and under the guidance of a doctor. Recently, stem cell transplantation has also gradually been applied to the treatment of SNHL (Baumgartner et al., 2021). Stem cell transplantation can repair the cell types in the damaged area through the migration and differentiation of stem cells, and has the potential for therapeutic application. Although the efficacy of stem cell transplantation requires further research and validation, it has promising prospects for development.

In summary, treatment methods for SNHL include medication, traditional Chinese medicine, hyperbaric oxygen therapy, and stem cell transplantation, each with unique mechanisms of action and indications. However, these treatments also have limitations and risks. Although medication is one of the main treatments for SNHL, its effectiveness is not always ideal. For example, some patients may not tolerate certain medication side effects, or certain drugs may cause serious adverse reactions. In addition, medication therapy requires long-term use to achieve better efficacy, which may cause psychological and economic burdens for some patients. Although traditional Chinese medicine is an effective treatment method, its efficacy requires accumulation over time, and there is individual variability. Moreover, experienced physicians are needed to ensure the safety and effectiveness of treatment. The mechanism of hyperbaric oxygen therapy is to improve the hypoxic state of the inner ear by increasing the oxygen content of blood. Although this method can bring significant benefits in some cases, it requires treatment in a hyperbaric oxygen chamber, and there may be some discomfort during treatment, such as headaches and dizziness. Stem cell transplantation therapy is an emerging treatment method, and its efficacy needs to be further confirmed by research. Stem cell transplantation therapy also carries some risks and uncertainties, such as potential immune reactions and abnormal cell proliferation. In addition, treatment methods targeting certain gene mutations and epigenetic changes have been proposed in research, which provides new ideas for the treatment of SNHL. Therefore, the treatment of SNHL needs to take into account factors such as the patient's physical condition, the severity of the disease, treatment goals, and risks, and adopt an individualized treatment plan (Löfvenberg et al., 2022). At the same time, healthcare professionals need to establish good communication and trust with

patients, help them solve problems during treatment, and improve patient compliance and treatment efficacy.

Nursing method

For patients with sensorineural hearing loss (SNHL), nursing care should include psychological, dietary, lifestyle, medication, hyperbaric oxygen, and traditional Chinese massage interventions. The specific methods are as follows:

Psychological care

Patients with SNHL often experience dizziness, ear fullness, restlessness, anxiety, and tension (Job et al., 2023). Therefore, it is important to maintain a quiet environment in the hospital room, avoid noise, and arrange single rooms whenever possible. For those who experience dizziness, they should be advised to rest in bed. Communication between the caregiver and the patient should be strengthened to understand the patient's psychological status, provide care, support, and encouragement, and help the patient maintain a positive attitude and cooperate with treatment. At the same time, the caregiver should evaluate the patient's role behavior, classify the patient's role transformation and adaptation into five aspects: lack, conflict, decline, reinforcement, and abnormality, and provide personalized nursing interventions to help the patient adapt to the changes as soon as possible (Chandrasekhar et al., 2019). Nurses can provide patients with disease knowledge education, patiently answer questions, improve the patient's understanding of the disease, establish a good nurse–patient relationship, and improve the patient's treatment compliance and cooperation. Patients should be provided with psychological care and counseling to strengthen their psychological defenses, help relieve negative emotions, and use entertainment activities to divert patients' attention from the disease and relieve their stress response to maintain the best physical and mental state to cooperate with treatment.

Dietary care

During the treatment period, patients should eat low-salt, low-fat, and light foods. They should eat foods containing vitamin E, vegetables, and fresh fruits, such as egg yolk, vegetable oil, beans, and so on. Patients should quit smoking because nicotine in cigarettes can cause vascular spasm, damage endothelial cells, cause platelet adhesion, aggregation, and formation of thrombosis, which affects treatment efficacy. Patients can drink a small amount of alcohol to promote blood circulation and metabolism, and it is recommended to drink light tea because tea contains a variety of vitamins, amino acids, and proteins (Kociszewska et al., 2021).

Life care

Patients with SNHL should be provided with a quiet and comfortable treatment environment to avoid excessive noise. Patients should avoid using headphones or making long phone calls to prevent exacerbation of hearing damage. During treatment, patients should maintain emotional stability and engage in relaxing activities such as listening to music or reading to avoid prolonged phone or television use. Patients should also maintain a regular sleep schedule, prioritize sufficient sleep, and create a relaxing sleep environment. Daily, patients should aim for a minimum of 7 h of restful sleep. Additionally, appropriate exercise should be encouraged to reduce thrombosis, prevent hyperlipidemia, and minimize the impact of slow blood flow on inner ear microcirculation.

Medication care

During treatment with corticosteroids, patients' eating and sleeping patterns should be monitored (Plontke et al., 2022). If there is a noticeable increase in appetite, patients should be advised to control their diet. Many patients also experience sleep disorders, and patients should be informed of the drug's adverse effects and encouraged to minimize daytime sleep to improve nighttime sleep quality. Inhibiting red blood cell and platelet aggregation, lowering blood viscosity, and dissolving blood clots are the goals of batroxobin drugs, which can also increase vascular permeability, improve blood supply, and improve hearing (Wang et al., 2021). However, batroxobin may sometimes cause complications such as increased eosinophils, leukocytosis or leukopenia, decreased red blood cells, and hemoglobin. Digestive symptoms such as nausea, vomiting, stomach pain, and loss of appetite may also occur. In terms of mental and neurological effects, patients may experience dizziness, headache, unsteady gait, or numbness. Therefore, close observation of the patient's condition is essential, and drug administration should be discontinued in case of abnormal reactions. Meanwhile, patients should be monitored for signs of bleeding or other adverse events, and preventative measures should be taken. During medication treatment for SNHL, nurses should increase surveillance and closely monitor changes in patients' condition, vital signs, and blood counts. Any abnormalities should be promptly reported to the physician.

Nursing care for hyperbaric oxygen therapy

When performing hyperbaric oxygen therapy, attention should be paid to the following (Olex-Zarychta, 2020; Ahn et al., 2021; Joshua et al., 2022): (1) Before entering the chamber, analyze the patient's Eustachian tube function and check the patency of the Eustachian tube. For patients with poor Eustachian tube function or allergic rhinitis, nasal spray medication should be used to contract the nasal mucosa and relieve symptoms; instruct patients to chew gum or eat fruit during pressurization to maintain Eustachian tube patency. (2) Due to the airtight environment of the oxygen chamber, it may cause anxiety and worsen depressive symptoms in patients. Individualized psychological counseling should be provided to the patient, paying attention to changes in their psychological state, giving active care and comfort to relieve negative emotions. Introduce the treatment method and expected effects to improve their treatment compliance. Patients may also be shown the treatment environment in advance, and peer education and sharing experiences can be used. (3) During oxygen chamber treatment, maintain the temperature in the chamber, with temperatures ranging from 24°C to 28°C in summer and 18°C to 22°C in winter. Help patients adjust their comfortable position, play soothing

music to ease their nervousness, instruct them on the prevention of common complications, strictly control treatment time and oxygen inhalation time, adjust the oxygen inhalation method, and allow for 30 min of rest inside the chamber after 30 min of oxygen inhalation. The oxygen mask should be in close contact with the face to avoid inhaling mixed air, which may affect the therapeutic effect and cause oxygen poisoning. Instruct patients to breathe independently, and prohibit breath-holding and coughing movements to prevent lung barotrauma. If a patient experiences coughing, chest pain, or restlessness, immediate intervention is required. (4) Before leaving the chamber, control the decompression speed and rate, observe whether the patient has any vomiting, breathing disorders, or restlessness, and stop immediately if abnormalities are found to prevent decompression sickness.

Guidance for traditional Chinese medicine massage

Traditional Chinese medicine massage involves kneading, pushing, pulling, and pulling the earlobe. Kneading the temple and other methods can be performed once a day for 20 min, with a 14-day course, to help improve ear activity, promote blood circulation in the ear, enhance ear blood supply function, and aid in disease recovery. Before the massage, explain the procedure to the patient and obtain their cooperation. Evaluate the patient's skin condition at the massage site, checking for abrasions, nodules, and rashes. The massage pressure should be based on the patient's tolerance, and stopped immediately if any discomfort is experienced.

Discussion

In conclusion, significant progress has been made in the clinical treatment of SNHL (Figure 1). Therefore, high patient cooperation is required during the treatment process, and nurses should focus on patient-centered care, develop a planned and purposeful nursing plan, provide personalized care based on the patient's condition, perform predictive assessments of potential risk factors, and collaborate with disease and medication observations to improve patient treatment compliance, and

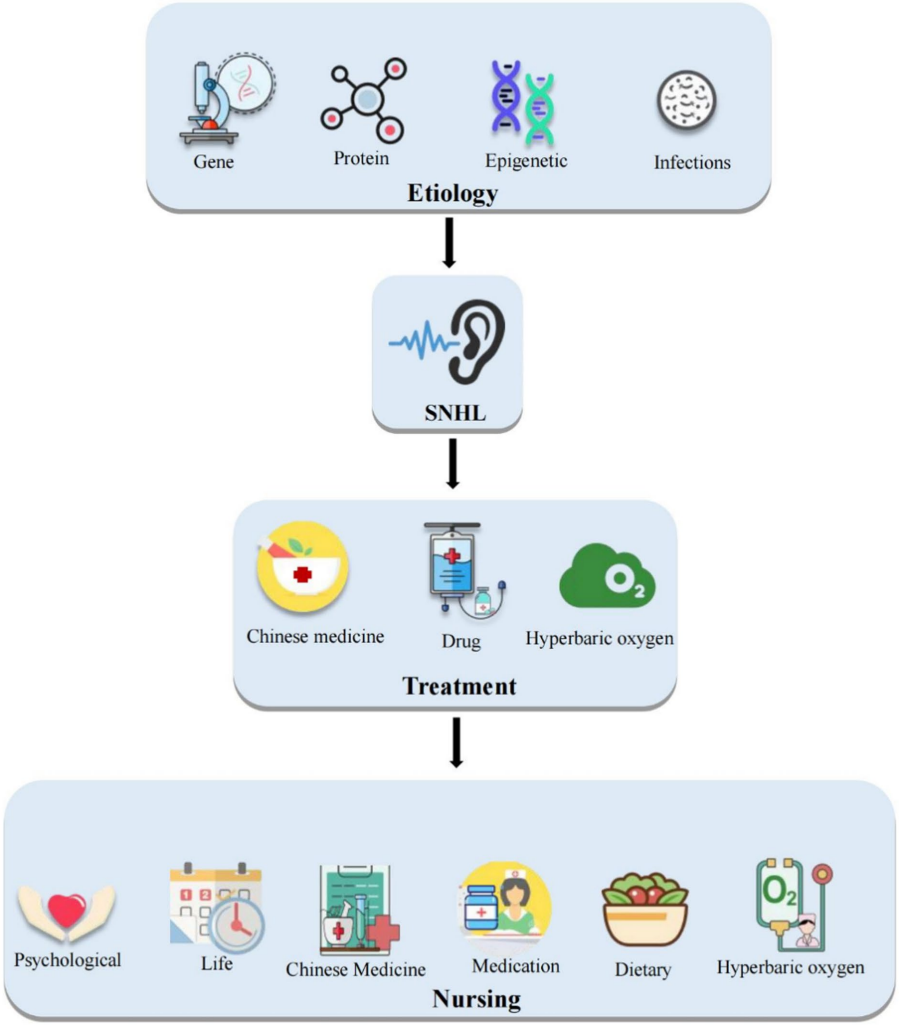

FIGURE 1
Schematic diagram of treatment and nursing research on sensorineural hearing loss.

ultimately enhance treatment outcomes and hearing levels. Moreover, nurses should assist patients in building a positive physical and mental state and correct understanding of their disease to alleviate anxiety and tension and improve treatment compliance to achieve the goal of improving treatment outcomes.

Additionally, SNHL prevention is crucial, and nurses should focus on disease prevention education during the nursing process, such as maintaining healthy lifestyles and avoiding noisy environments to reduce the risk of illness (Rahimi et al., 2023). The development of emerging therapies, new technologies, and ongoing clinical trials has promoted the advancement of treatment and care for SNHL, resulting in improved prognosis. A multidisciplinary approach involving otolaryngologists, audiologists, speech pathologists, and other healthcare professionals has provided optimal guidance for the treatment and care of SNHL patients (Copeland and Pillsbury 3rd., 2004; Gay et al., 2022).

In summary, the treatment and care of SNHL are comprehensive processes that require the joint efforts of medical staff and active patient cooperation. Guided by patient-centered care philosophy, nurses should develop personalized treatment plans based on the patient's actual condition, provide comprehensive management and observation during the treatment process to enhance treatment outcomes and improve patients' quality of life.

Author contributions

FL and BH: conception and design. FL and JH: development of methodology. XZ and SH: analysis and interpretation of data. JH: writing, review, and/or revision of the manuscript. SH: study supervision. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Impact of environmental noise exposure as an inducing factor on the prognosis of sudden sensorineural hearing loss: a retrospective case–control study

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Objective: The study aimed to evaluate the clinical characteristics and prognostic factors associated with unilateral sudden sensorineural hearing loss (SSNHL) related to environmental noise exposure before its onset.

Methods: A total of 50 unilateral SSNHL patients exposed to environmental noise before onset (case group) and 924 unilateral SSNHL patients without any exposure to obvious inducing factors before onset (control group) were enrolled between January 2018 and October 2022. We retrospectively analyzed differences between both groups using the chi-square test, Fisher's exact tests, independent *t*-tests, and Mann–Whitney U-tests as appropriate before and after propensity score matching (PSM) based on sex, age, and initial pure-tone average (PTA). Prognostic factors for the case group were analyzed using univariate and multivariate logistic analyses between the effective and ineffective groups.

Results: Before PSM, significant differences were noted in age, sex, time to treatment, the proportion of combined diabetes mellitus, initial PTA, hearing gain, the incidence of vertigo or aural fulness, the rate of vestibular dysfunction or inner ear MRI abnormalities, the effective rate, the glucose and homocysteine levels, and the proportion of audiogram curve types ($P < 0.05$) between both groups. After PSM, compared to the control group, a longer time to treatment ($Z = -3.02$, $P < 0.05$), higher final PTA ($Z = -2.39$, $P < 0.05$), lower hearing gain ($Z = -3.46$, $P < 0.05$), lower rate of vestibular dysfunction ($\chi^2 = 55.1$, $P < 0.001$), and lower effective rate ($\chi^2 = 4.87$, $P < 0.05$) were observed in the case group. There was a significant difference between the audiogram curve types in both groups ($\chi^2 = 14.9$, $P < 0.05$). Time to treatment (95% confidence interval: 0.692–0.965, $P < 0.05$) and final PTA (95% confidence interval: 0.921–0.998, $P < 0.05$) were associated with the clinical outcomes for the case group.

Conclusion: Unilateral SSNHL patients exposed to environmental noise triggers before onset showed a poorer effective rate and a lower rate of vestibular dysfunction than those who were not. The time to treatment and final PTA were associated with the prognosis of these patients.

KEYWORDS

sudden sensorineural hearing loss, hearing loss, environmental noise, noise exposure, prognosis

1. Introduction

Sudden sensorineural hearing loss (SSNHL) is defined as sudden hearing loss (HL), unilateral or bilateral, of at least 30 dB HL across three sequential frequencies within 72 h (Chandrasekhar et al., 2019). The estimated prevalence rate of SSNHL is 5–30 per 1,00,000 people per year (Schreiber et al., 2010); a study from Germany reported that the annual incidence of SSNHL was as high as 160 per 1,00,000 people per year (Klemm et al., 2009). Recently, SSNHL has gained attention due to its increasing incidence and prolonged negative impact on patients' lives (Härkönen et al., 2017). The exact etiology of SSNHL remains unclear, but recognized etiopathogenesis processes include microcirculatory disorders, viral infection, and autoimmune or inflammatory states (Koçak et al., 2017; Li et al., 2018; Chen et al., 2019). Moreover, the factors contributing toward SSNHL development include exposure to environmental noise, sleep dysfunction, and emotional disorders although most patients presented without obvious inducing factors before the onset of SSNHL. Previous studies have assessed the relationship between sleep dysfunction, mental disorders, and SSNHL (Kim et al., 2018, 2020; Yeo et al., 2022); however, literature reporting exposure to environmental noise as a predisposing factor before the onset of SSNHL is scarce. Therefore, in this study, we retrospectively review the clinical characteristics and laboratory data of patients with unilateral SSNHL with a history of exposure to environmental noise. This study aimed to investigate the clinical characteristics, treatment outcomes, and prognostic factors of patients with SSNHL. This research may help to establish noise stimulation as a risk factor for the onset of SSNHL among patients; therefore, delay in treatment and, subsequently, compromised treatment outcomes may be avoided.

2. Materials and methods

2.1. Design/setting

The medical records of hospitalized patients diagnosed with unilateral SSNHL between January 2018 and October 2022 at our tertiary referral center were reviewed retrospectively. This study followed the STROBE guidelines.

2.2. Participants

The inclusion criteria for the case group were as follows: patients (1) should meet the US diagnostic criteria for SSNHL (Chandrasekhar et al., 2019); (2) with unilateral ear involvement; (3) with exposure to environmental noise triggers before onset; (4) with age between 18 and 70 years; (5) with duration time <30 days; (6) with complete data available for audiology, vestibular function test, and MRI of the inner ear; and (7) with the length of hospital stay >7 days.

The environmental noise incorporated in this study is caused by a variety of sources, such as musical or recreational venues, machinery activity, and community noise. The exclusion criteria for the case group were as follows: patients with (1) co-existing autoimmune disease; (2) congenital or fluctuating deafness; (3)

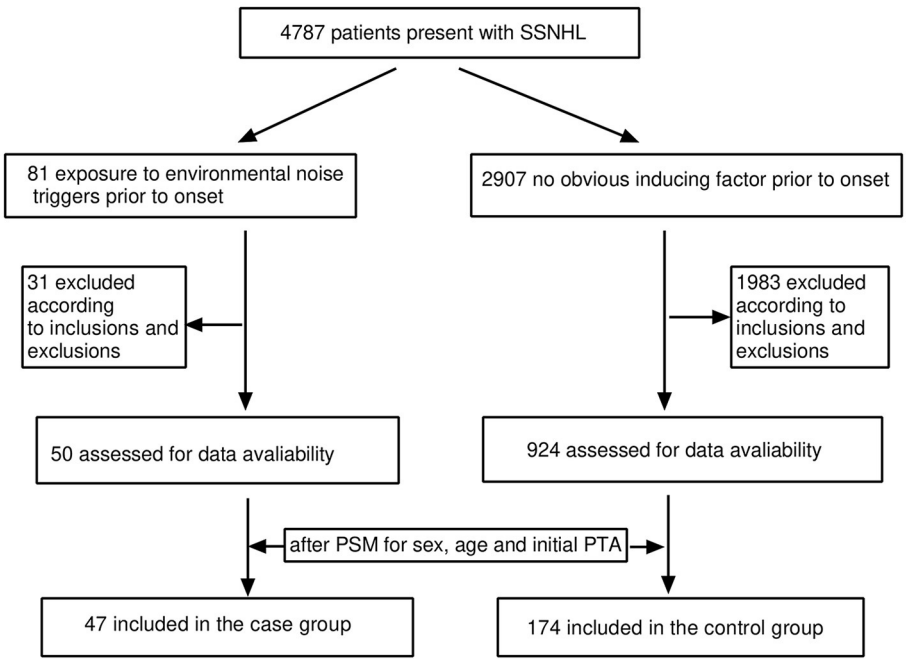

FIGURE 1
Flow diagram for screening the patients included in this study. SSNHL, sudden sensorineural hearing loss; PSM, propensity score matching; PTA, pure-tone average.

TABLE 1 Demographics and clinical variables of patients (before PSM).

Variables	Case group ($n = 50$)	Control group ($n = 924$)	Statistics	*P*-value
Age of onset	36 (30.0, 49.3)	48 (37, 56)	−3.28	0.001*
Males: Females	37:13	465:459	10.65	0.001*
Side (Left: Right)	24:26	475:449	0.22	0.64
Time to treatment (days)	10 (4, 20)	7 (3, 13)	−2.63	0.009 *
Hypertension	5 (10%)	176 (19%)	2.57	0.11
Diabetes mellitus	1 (2%)	120 (12.9%)	5.26	0.02*
Initial PTA (dB)	58.9 ± 3.4	81.3 (56.3, 100)	−4.65	0.00*
Final PTA (dB)	48.3 ± 3.7	52.5 (30, 75)	−1.29	0.19
Hearing gain (dB)	6.25 (1.3, 19.1)	20 (6.3, 38.8)	−4.48	0.00*
Tinnitus	45 (90%)	857 (92.7%)	0.52	0.41
Vertigo	16 (32%)	513 (55.5%)	10.57	0.001*
Aural fulness	39 (78%)	595 (64.4%)	3.86	0.04*
Vestibular dysfunction	35 (70%)	768 (83.1%)	5.64	0.02*
Inner ear MRI (+)	12 (24%)	482 (52.2%)	15.1	0.00*
Thyroid dysfunction	8 (16%)	241 (26.1%)	2.53	0.11
Effective cases	15 (30%)	542 (58.7%)	15.9	0.00*
Blood index				
NLR	1.7 (1.4, 2.5)	1.8 (1.4, 2.5)	−0.38	0.70
MLR	0.19 (0.15, 0.29)	0.19 (0.14, 0.24)	−1.51	0.13
PLR	114.3 (87.9, 135.4)	115.4 (91.4, 148.4)	−0.44	0.66
TBIL (μmol/L)	13.1 (10.7, 16.5)	13.4 (10.5, 17.3)	−0.06	0.95
IBIL (μmol/L)	9.4 (7.5, 11.3)	9.8 (7.4, 12.8)	−0.46	0.64
SOD (U/mL)	158.5 (144.0, 189.5)	164 (139.3, 192)	−0.04	0.97
Fibrinogen (g/L)	2.3 (2.1, 2.7)	2.4 (2.1, 2.8)	−0.42	0.68
Glu (mmol/L)	4.9 (4.6, 5.2)	5.0 (4.6, 5.7)	−1.98	0.04 *
TG (mmol/L)	1.4 (0.9, 1.8)	1.4 (0.9, 2.1)	−1.16	0.25
CH (mmol/L)	4.8 ± 0.1	4.9 (4.2, 5.7)	−0.84	0.39
HDL (mmol/L)	1.3 (1.0, 1.5)	1.3 (1.1,1.5)	−0.48	0.64
LDL (mmol/L)	2.9 ± 0.1	2.9 (2.3, 3.5)	−0.38	0.70
Hcy (μmol/L)	10.7 (7.9, 14.9)	9.4 (7.4, 12.2)	−2.27	0.02 *

PTA, pure-tone average; NLR, neutrophil lymphocyte ratio; MLR, monocyte lymphocyte ratio; PLR, platelet lymphocyte ratio; TBIL, Total bilirubin levels; IBIL, indirect bilirubin; SOD, superoxide dismutase; Glu, glucose; TG, triglyceride; CH, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hcy, homocysteine.
*$P < 0.05$.

Meniere's disease; (4) middle ear malformation, otitis media, or history of middle ear surgery; (5) craniosynostosis or genetic or physical trauma-induced deafness; (6) involvement of bilateral ears; (7) occupying lesions of the pontocerebellar horn; (8) a previous history of hearing loss; (9) pre-onset with other triggers, such as sleep dysfunction, emotional stress, and cold; and (10) a history of ototoxic drug use.

For the control group, the following were the inclusion criteria: patients (1) should meet the US diagnostic criteria for SSNHL (Chandrasekhar et al., 2019); (2) with unilateral ear involvement; (3) with no obvious inducing factors before onset; (4) aged between 18 and 70 years; (5) with duration time <30 days; (6) with complete audiology, vestibular function test, and MRI of the inner ear data; and (7) the length of hospital stay >7 days. The exclusion criteria were as follows: patients with (1) combined autoimmune disease; (2) congenital or fluctuating deafness; (3) Meniere's disease; (4) middle ear malformation, otitis media, or history of middle ear surgery; (5) craniosynostosis or genetic or physical trauma-induced deafness; (6) involvement of bilateral ears; (7) occupying lesions of the pontocerebellar horn; (8) a previous history of hearing loss or contralateral deafness; and (9) a history of ototoxic drug use.

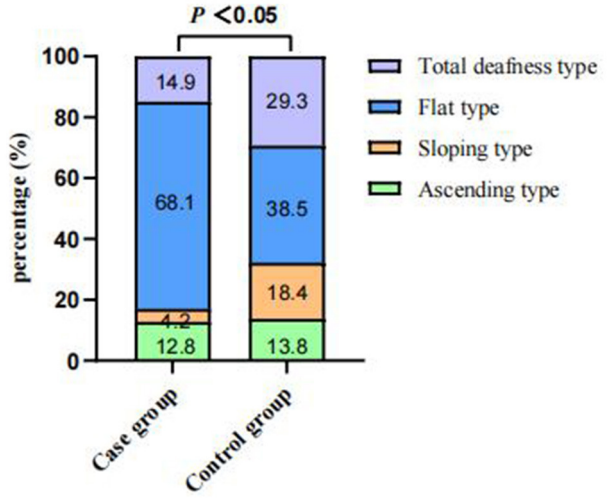

FIGURE 2
Comparisons of audiogram curve types between the case and control groups before PSM. There was a significant difference between the audiogram curve types in both groups ($P < 0.001$). PSM: propensity score matching.

2.3. Collection of data regarding medical history

Data regarding patient histories were collected for time to treatment, sex, deafness side, concomitant symptoms, such as tinnitus, vertigo, and aural fullness, and underlying diseases, such as hypertension and diabetes mellitus.

2.4. Audiological testing

Audiological tests performed to exclude related hearing lesions included pure-tone audiometry (GSI-61, USA), tympanometry (GSI Tympstar, USA), auditory brainstem response (ABR; IHS Smart EP, USA), and distortion product otoacoustic emission tests (DPOAE, IHS Smart EP, USA).

The audiogram curve types were classified as follows: ascending (HL limited to frequencies ≤1.0 kHz), sloping (HL ≥2.0 kHz), flat (HL at all frequencies with the average threshold not exceeding 80 dB HL), or total deafness (HL at all frequencies and ≥80 dB HL). The mean hearing threshold at 500 Hz, 1 kHz, 2 kHz, and 4 kHz was used to calculate the pure-tone average (PTA). The degrees of deafness were classified as mild (26–40 dB HL), moderate (41–60 dB HL), severe (61–80 dB HL), or profound (≥81 dB HL) based on the PTA. The initial PTA was measured before the entry into the study; the final PTA was measured 2 to 4 weeks after treatment. The hearing gain was defined as the difference between the initial and final PTA. Patients with a hearing gain of ≥15 dB were classified as the effective group and those with a hearing gain of <15 dB were classified as the ineffective group (Stachler et al., 2012).

2.5. Vestibular function tests

To assess vestibular function, the caloric test (Ulmer VNG, v. 1.4; SYNAPSYS; Marseille, France), vestibular-evoked myogenic potentials test (Neurosoft LTD; Ivanov, Russia), video head impulse test (Ulmer, Synapsys; Marseille, France), and vestibular autorotation test (Western Systems Research; Pasadena, CA, USA) were performed. Vestibular function was considered to be impaired if the results for any of the aforementioned tests were abnormal.

2.6. Imaging examinations and laboratory tests

Three-dimensional (3D) fluid-attenuated inversion recovery (FLAIR) and contrast-enhanced magnetic resonance imaging (MRI) (PHILIPS, Intera, Netherlands) were performed to exclude lesions and evaluate the inner ear images. 3D T1-weighted, 3D T2-weighted, and 3D T2 FLAIR images were acquired. Inner ear images were categorized into four types: normal, increased protein content, inner ear hemorrhage, and blood–labyrinth barrier. Any of the latter three types were considered positive for an inner ear MRI abnormality.

Blood samples were collected from all admitted patients on the morning of their second day in the hospital. Blood indicators were monitored through routine blood tests, analysis of the fibrinogen level, and tests for biochemical indicators, such as the levels of total bilirubin (TB), indirect bilirubin (IB), superoxide dismutase (SOD), glucose (Glu), triglyceride (TG), cholesterol (CH), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and homocysteine (Hcy). The ratios of neutrophils to lymphocytes (NLR), monocytes to lymphocytes (MLR), and platelets to lymphocytes (PLR) are defined as the ratios of neutrophils, monocytes, and platelets to lymphocytes, respectively.

2.7. Treatment process

During hospitalization, all patients received the following combination therapy: *Ginkgo biloba* extract (to improve blood microcirculation), dexamethasone or methylprednisolone sodium succinate (glucocorticoid), batroxobin (to lower the fibrinogen level), and methylcobalamin or mouse nerve growth factor (neurotrophic drug). Dexamethasone was administered intravenously (10 mg/day for 3 days, followed by 5 mg/day for 4 days) when the patient did not have concomitant hypertension or diabetes. Methylprednisolone sodium succinate was administered through an intravenous drip (80 mg/day for 3 days, followed by 40 mg/day for 4 days) when the patient's blood pressure and Glu levels were well-controlled. Thereafter, methylprednisolone sodium succinate post-otic injection was administered and injected continuously (40 mg/day, once every 2 days). Patients were discharged when there was no improvement after two or three consecutive pure-tone audiometry tests (twice in 1 week).

TABLE 2 Demographics and clinical variables of patients (after PSM).

Variables	Case group ($n = 47$)	Control group ($n = 174$)	Statistics	*P*-value
Age of onset	38 (31, 53)	43.5 (31.8, 52.3)	−0.66	0.51
Males: Females	35:12	125:49	0.13	0.72
Side (Left: Right)	22:25	83:91	0.01	0.91
Time to treatment (days)	9 (4, 20)	6 (3.0, 10.3)	−3.02	0.003*
Hypertension	5 (10.6%)	25 (14.4%)	0.44	0.51
Diabetes mellitus	1 (2.1%)	15 (8.6%)	–	0.20
Initial PTA (dB)	61.6 ± 3.3	63.5 ± 2.2	0.43	0.67
Final PTA (dB)	50.5 ± 3.7	38.1 (22.5, 63.8)	−2.39	0.02*
Hearing gain (dB)	6.3 (1.3, 20)	14.4 (5, 31.6)	−3.46	0.001*
Tinnitus	42 (89.4%)	161 (92.5%)	–	0.55
Vertigo	16 (34%)	82 (47.1%)	2.57	0.11
Aural fulness	36 (76.6%)	114 (65.5%)	2.08	0.15
Vestibular dysfunction	12 (25.5%)	142 (81.6%)	55.1	0.00*
Inner ear MRI (+)	12 (25.5%)	70 (40.2%)	3.43	0.06
Thyroid dysfunction	8 (17.4%)	39 (22.4%)	0.55	0.46
Effective cases	15 (31.9%)	87 (50%)	4.87	0.03*
Blood index				
NLR	1.9 (1.4, 2.5)	1.8 (1.5, 2.5)	−0.12	0.91
MLR	0.19 (0.15, 0.29)	0.19 (0.15, 0.24)	−1.53	0.13
PLR	117.2 (88.1, 136.7)	111.5 (91.3, 145.1)	−0.89	0.37
TBIL (μmol/L)	13.1 (10.7, 15.9)	14.1 (11.3, 18.6)	−1.28	0.20
IBIL (μmol/L)	9.3 (7.4, 11.2)	10.2 (7.9, 13.2)	−1.51	0.13
SOD (U/mL)	165.7 ± 4.7	169.5 (141, 198.5)	−1.19	0.23
Fibrinogen (g/L)	2.3 (2.1, 2.7)	2.4 (2.0, 2.8)	−0.68	0.49
Glu (mmol/L)	4.9 (4.6, 5.2)	4.9 (4.5, 5.8)	−0.55	0.58
TG (mmol/L)	1.4 (0.9, 1.7)	1.4 (1.0, 1.9)	−1.15	0.25
CH (mmol/L)	4.8 ± 0.1	4.83 ± 0.08	0.33	0.74
HDL (mmol/L)	1.3 (1.0, 1.5)	1.3 ± 0.02	−0.03	0.97
LDL (mmol/L)	2.9 ± 0.1	2.9 ± 0.07	0.09	0.93
Hcy (μmol/L)	10.8 (8.0, 15.2)	10.4 (8.1, 13.5)	−0.77	0.44

PTA, pure-tone average; NLR, neutrophil lymphocyte ratio; MLR, monocyte lymphocyte ratio; PLR, platelet lymphocyte ratio; TBIL, total bilirubin levels; IBIL, indirect bilirubin; SOD, superoxide dismutase; Glu, glucose; TG, triglyceride; CH, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hcy, homocysteine.
*$P < 0.05$.

Subsequently, the patients were prescribed oral *Ginkgo biloba* extract tablets and methylcobalamin.

2.8. Statistical analysis

Statistical analyses were performed using the SPSS 26.0 statistical package (IBM; Armonk, NY, USA). A 1:4 nearest neighbor matching was performed for sex, age, and initial PTA between the case and control groups using propensity score matching (PSM), and the caliper was set at 0.1. Chi-square and Fisher's exact tests were used to compare the categorical variables, such as sex, involvement side, hypertension, diabetes mellitus, vertigo, tinnitus, aural fullness, vestibular dysfunction, inner ear MRI abnormality, and the effective rate. Normality tests were performed mainly using the Kolmogorov–Smirnov (K-S) and Shapiro–Wilk (S-W) tests. Quantitative variables are described as the mean ± standard deviation or quartiles. Independent *t*-tests and Mann–Whitney *U* tests were used to analyze normally and non-normally distributed variables, respectively. Univariate and multivariate binary logistic regression analyses were used to assess the influence of various clinical variables on the prognosis of the

case group. Differences with P-values < 0.05 were considered statistically significant.

3. Results

3.1. Participant characteristics

Of the 4,787 screened clinical records, 81 patients were exposed to environmental noise triggers before onset and 2,907 patients showed no obvious inducing factors before onset. Finally, 50 patients were enrolled in the case group and 924 patients were selected for the control group, based on the screening criteria. After PSM for sex, age, and initial PTA, 47 and 174 patients were enrolled in the case and control groups, respectively, as shown in Figure 1. Before PSM, there was a significant difference in age, sex, time to treatment, the proportion of combined diabetes mellitus, initial PTA, hearing gain, the incidence of vertigo or aural fulness, the rate of vestibular dysfunction or inner ear MRI abnormalities, the effective rate, Glu and Hcy levels, and the proportion of audiogram curve types ($P < 0.05$), as shown in Table 1 and Figure 2. After PSM, there was a significant difference in time to treatment, final PTA, hearing gain, the rate of vestibular dysfunction, and the effective rate ($P < 0.05$). The time to treatment and final PTA in the case group were larger than those in the control group (Z= −3.02 and −2.39, respectively, both $P < 0.05$). Hearing gain, the rate of vestibular dysfunction, and the effective rate in the case group were lower than those in the control group (Z= −3.46, $P < 0.05$; $\chi^2 = 55.1$, $P < 0.001$; $\chi^2 = 4.87$, $P < 0.05$), as shown in Table 2. After PSM, there was a significant difference between the audiogram curve types in the case and control groups ($\chi^2 = 14.9$, $P < 0.05$), as shown in Figure 3.

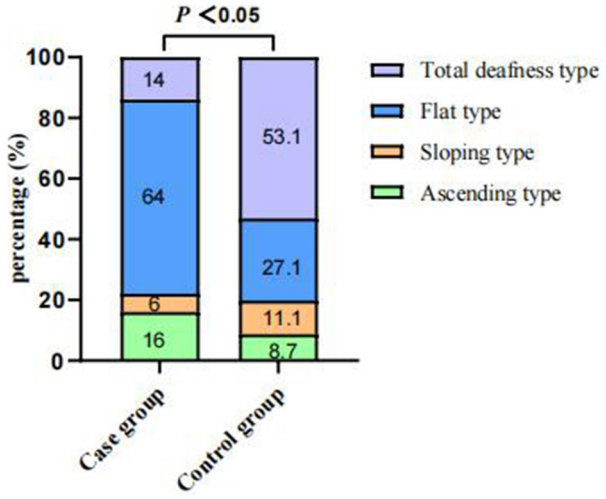

FIGURE 3
Comparisons of audiogram curve types between the case and control groups after PSM. There was a significant difference between audiogram curve types in both groups ($\chi^2 = 14.9$, $P < 0.05$). PSM: propensity score matching.

3.2. Correlations between clinical variables and treatment outcome in the case group

Univariate logistic regression analysis was performed to analyze the age, sex, time to treatment, initial PTA, final PTA, combined hypertension, the incidence of vertigo, tinnitus, aural fulness, the rate of vestibular dysfunction, inner ear MRI abnormality, and various blood parameters. The time to treatment and final PTA were significantly different between the effective and ineffective groups (95% confidence interval: 0.758–0.959 and 0.923–0.988, $P < 0.05$), as shown in Table 3. Clinical variables that yielded a P-value of <0.2 in the univariate logistic regression analysis, such as time to treatment, final PTA, tinnitus, and Glu and Hcy levels, were included in the multivariate binomial logistic regression analysis. Time to treatment (95% confidence interval: 0.692–0.965, $P < 0.05$) and final PTA (95% confidence interval: 0.921–0.998, $P < 0.05$) were associated with the treatment outcomes for the case group (Table 4).

4. Discussion

The exact etiopathogenesis of SSNHL remains unknown; however, the commonly accepted etiological mechanisms for this condition include microcirculatory dysfunction of the inner ear, viral infection, and immune inflammatory states (Koçak et al., 2017; Li et al., 2018; Chen et al., 2019). Most people with SSNHL have no obvious inducing factors before the onset, but some have a variety of high-risk triggers such as noise exposure, insomnia, cold, and emotional stress. To the best of our knowledge, this study is the first to show the differences in outcomes after exposure to environmental noise triggers and the absence of obvious inducing factors before onset in patients with unilateral SSNHL. We found significant differences in the time to treatment, the audiogram curve types, the rate of vestibular dysfunction, and the effective rate between the two groups after PSM for sex, age, and initial PTA. Although a correlation between blood parameters and the prognosis was not found in the case group, the time to treatment and final PTA were positively and independently associated with the treatment outcomes of unilateral SSNHL.

Hypertension and diabetes mellitus are associated with the prognostication of SSNHL (Lin et al., 2016; Fasano et al., 2017). The onset of hypertension and diabetes is closely related to age and blood indicators, such as HDL and bilirubin level, which, in turn, are also associated with sex and age (von Mühlen et al., 2003; Gazzin et al., 2016). The degree of HL before treatment is closely related to the treatment outcomes. Therefore, to better investigate the clinical characteristics and efficacy of the case and control groups of patients with unilateral SSNHL, the effects of sex, age, initial PTA, and the associated confounding factors in this study had been excluded using PSM. Before PSM, the incidence of simultaneous diabetes mellitus, the incidence of vertigo and aural fullness, the rate of inner ear MRI abnormalities, and the Glu and Hcy levels were significantly different; however, these disparities disappeared after PSM. This may be because the PSM achieved a better balance between the confounding factors in the two groups.

TABLE 3 Univariate logistic regression analysis for the case group.

Variables	Ineffective group ($n = 32$)	Effective group ($n = 15$)	OR	95%CI	*P*-value
Age of onset	38.0 (31.0, 53.3)	33.0 (27.0,53.0)	0.990	0.948–1.034	0.657
Males: Females	25:7	10:5	1.786	0.457–6.971	0.404
Time to treatment (days)	15.0 (7.3,24.5)	4.0 (3.0,11.0)	0.853	0.758–0.959	0.008*
Initial PTA	61.4±3.7	61.8±6.8	1.001	0.973–1.029	0.955
Final PTA	57.9±3.9	34.8±6.6	0.954	0.923–0.988	0.007*
Hypertension	29 (90.6%)	13 (86.7%)	1.487	0.221–9.993	0.683
Vertigo	21 (65.6%)	10 (66.7%)	0.955	0.261–3.495	0.944
Tinnitus	2 (6.3%)	3 (20%)	0.267	0.039–1.801	0.175
Aural fullness	8 (25.0%)	3 (20%)	1.333	0.298–5.957	0.706
Vestibular dysfunction	25 (78.1%)	10 (66.7%)	1.786	0.457–6.971	0.404
Thyroid dysfunction	27 (84.4%)	12 (80%)	1.350	0.277–6.585	0.711
Inner ear MRI (+)	25 (78.1%)	10 (66.7%)	1.786	0.457–6.971	0.404
Blood index					
NLR	1.9 (1.4, 2.5)	1.7 (1.3,2.5)	0.844	0.528–1.351	0.481
MLR	0.19 (0.14,0.29)	0.19 (0.16,0.33)	0.581	0.064–5.250	0.629
PLR	118.3 (87.6, 140.0)	117.2 (97.2, 134.9)	1.007	0.993–1.020	0.331
TBIL (μmol/L)	13.1 (10.6, 16.3)	13.1 (10.7, 15.9)	0.965	0.847–1.100	0.597
IBIL (μmol/L)	9.2 (7.4, 11.5)	9.8 (7.7, 11.1)	0.963	0.813–1.141	0.666
SOD (U/mL)	157.5 (147.5, 193.3)	156.0 (133.0, 176.0)	0.989	0.969–1.010	0.305
Fibrinogen (g/L)	2.3 (2.1, 2.7)	2.4 (1.9, 2.8)	0.809	0.313–2.091	0.662
Glu (mmol/L)	4.9 (4.7, 5.3)	4.7 (4.1, 5.2)	0.507	0.186–1.384	0.185
TG (mmol/L)	1.3 (0.8, 1.6)	1.5 (0.9,1.8)	0.867	0.429–1.751	0.690
CH (mmol/L)	4.9 ± 0.2	4.7 ± 0.3	0.818	0.412–1.623	0.565
HDL (mmol/L)	1.3 (1.1, 1.5)	1.2 (0.9, 1.7)	0.401	0.049–3.302	0.396
LDL (mmol/L)	2.9 ± 0.1	2.9 ± 0.2	0.995	0.460–2.153	0.990
Hcy (μmol/L)	10.7 (8.3, 14.6)	11.5 (7.5, 25.1)	1.064	0.979–1.156	0.142

PTA, pure-tone average; NLR, neutrophil lymphocyte ratio; MLR, monocyte lymphocyte ratio; PLR, platelet lymphocyte ratio; TBIL, total bilirubin levels; IBIL, indirect bilirubin; SOD, superoxide dismutase; Glu, glucose; TG, triglyceride; CH, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hcy, homocysteine.
*$P < 0.05$.

Previous studies have demonstrated that vestibular dysfunction is more common in SSNHL patients with profound hearing impairment and that it correlates with hearing recovery in SSNHL (Wang et al., 2020; Chang et al., 2021). The rate of vestibular dysfunction in the case group was significantly lower than that in the control group before or after PSM. This suggests that patients with unilateral SSNHL are less likely to experience vestibular dysfunction after exposure to environmental noise triggers before onset. This may be because the location and extent of inner ear damage vary between subjects with the same degree of HL.

Different audiogram curve types may suggest the existence of different pathogeneses. Flat-type audiogram curves are mostly associated with inner ear vascular spasms or stria vascularis dysfunction; total deafness-type audiogram curves usually occur due to inner ear vessel embolism or thrombosis; sloping-type audiogram curves occur mostly due to hair cell damage; and ascending-type audiogram curves are generally associated with endolymphatic hydrops (Michel, 2011). In this study, flat-type and sloping-type audiogram curves were the most and least commonly observed types of audiogram curves, respectively, in the case group. This suggests that exposure to environmental noise triggers before onset may primarily cause inner ear vascular spasms or stria vascularis dysfunction. In conclusion, the effective rate in the case group was significantly lower than that in the control group despite the matching initial PTA. The prognostic analysis of the case group demonstrated that time to treatment and final PTA was associated with the prognosis. The mean time to treatment in the ineffective group (15 days) was longer than that in the effective group (4 days). Consequently, the treatment efficacy decreases with time. This also suggests, in part, that patients should be more vigilant after exposure to environmental noise.

TABLE 4 Multivariate logistic regression analysis for the case group.

	OR	95%CI	*P*-value
Time to treatment	0.817	0.692–0.965	0.017*
Final PTA	0.959	0.921–0.998	0.039*
Tinnitus	0.270	0.026–2.804	0.273
Glu (mmol/L)	0.391	0.107–1.426	0.155
Hcy (μmol/L)	1.031	0.925–1.149	0.584

PTA, pure-tone average; Glu, glucose; Hcy, homocysteine.
*$P < 0.05$.

Noise damage can cause the depletion of glutathione. Glutathione is an antioxidant that protects cells from damage caused by toxins, such as free radicals (Le Prell et al., 2007). High Hcy levels can affect the production of glutathione, causing further oxidative damage and microvascular ischemia (Lai and Kan, 2015). Hyperhomocysteinemia was demonstrated to serve as a poor prognostic factor for hearing recovery in SSNHL (Passamonti et al., 2015). In this study, the Hcy level in the case group was higher than that in the control group before PSM, but this difference vanished after PSM. This suggests that oxidative stress damage may not be a major etiological mechanism for the case group.

However, this study has several limitations. First, this was a retrospective study based on routine clinical records and data; therefore, some records are incomplete. Second, although most patients were exposed to environmental noise triggers before the onset of SSNHL occasionally as well as transiently, the types of noise were different, and the exposure time was not fixed. However, this study is important because, to the best of our knowledge, it is the first to analyze the differences between unilateral SSNHL caused due to exposure to noise and that with no obvious inducing factors before onset. Moreover, this study matched the relevant variables that could affect baseline characteristics in both the patient groups, such as sex, age, and initial hearing threshold; hence, it can yield highly reliable results. Nevertheless, further studies are necessary to analyze more variables and prognostic factors in a larger number of patients.

5. Conclusion

Patients with unilateral SSNHL with exposure to environmental noise triggers before onset mainly exhibited flat-type hearing loss and showed a poorer effective rate, longer time to treatment, and lower rate of vestibular dysfunction than those in the unilateral SSNHL patients for whom there was an absence of obvious inducing factors before onset. Time to treatment and final PTA were associated with the prognosis of unilateral SSNHL with exposure to recreational or lifestyle noise triggers before onset.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Shandong Provincial ENT Hospital (XYK20180102). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YW and MW designed the study and wrote the manuscript. WX, XS, KL, and FD undertook the research and analyzed the data. HW funded the research. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1210291/full#supplementary-material

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Influence of music on the hearing and mental health of adolescents and countermeasures

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This review elaborates on the influence of music on the psychological well-being of adolescents, covering the potential advantages, drawbacks, and necessary strategic interventions associated with music exposure. Initially, we characterize music and delve into a multifaceted classification system. Music, as a pervasive art form, is categorized based on regional and national parameters, and it also distinguishes through the assorted genres and traits. The mental well-being of adolescents is significantly influenced by music through mechanisms such as the facilitation of emotional expression and regulation, fortification of social bonds and the sense of belonging, as well as the fostering of creativity and cognitive development. Nonetheless, music, if misused or associated with inappropriate content, could elicit a spectrum of issues ranging from auditory impairment, diversion of attention, addiction tendencies, to the induction of negative emotions. To counteract these potential hazards, we propose several mitigation strategies including the selection of appropriate music styles, the establishment of a wholesome music environment, the promotion of the constructive role of music education, and fostering active participation in music activities among the youth. In conclusion, we underscore the necessity of a collaborative endeavor from all sectors of society to ensure a healthy music environment for the youth, which in turn would enhance the positive influence of music on the mental health development of this population.

KEYWORDS

adolescents, mental health, music influence, hearing, review

1. Introduction

Music, as an immersive and broad artistic medium, plays a pivotal role in the lives of adolescents. This stage of life, commonly known as adolescence, is viewed as a crucial period for psychological, social, and cognitive development; the role of music within this framework is significant. As per the research conducted by Ouergui et al. (2023), youth display a high frequency of interaction and deep engagement with music. In the contemporary digital age, music is ubiquitous. Whether via streaming services, social media platforms, or traditional radio and television, music is woven into the fabric of young people's lives. This pervasive presence of music provides adolescents with a means to express themselves and articulate their emotions; furthermore, it has emerged as a crucial avenue for their social interactions.

Numerous adolescents opt to convey their identities and emotions by curating and sharing music playlists (Taruffi, 2021; Feneberg et al., 2023). This behavior extends beyond the expression of their musical esthetics; it mirrors their quest for resonance and the establishment of social relationships through music. For instance, a melancholic playlist might communicate an adolescent's low mood, whereas an energetic playlist can depict their elation and positivity.

Moreover, sharing these playlists encourages youth to find shared interests among friends and peers, thereby enhancing social connections. Concurrently, music plays a key role in shaping adolescents' identity and self-perception (McFerran et al., 2010; Haeyen and Noorthoorn, 2021). During this phase, adolescents engage in the process of discovering and molding their identities; the music they select and appreciate often reflects their values, beliefs, and self-concepts. This journey of self-recognition and self-expression through music significantly affects adolescents' mental health and development.

In recent years, there has been an escalating focus on research exploring the influence of music on adolescents' mental health. For example, Knoerl R and Neal-Barnett A discovered that youth frequently utilize music as a coping mechanism for their emotions, such as alleviating stress and enhancing mood (Neal-Barnett et al., 2019; Knoerl et al., 2022). Further, several studies have indicated that collaborative musical activities (like choir participation or orchestra) can boost adolescents' social skills and self-esteem (Porter et al., 2017). However, some research has also highlighted potential risks associated with music, including hearing damage and distraction (Halevi-Katz et al., 2015). These studies collectively underscore the profound influence of music on the mental health of adolescents.

The aim of this article is to review the existing research on the effect of music on the psychological health of adolescents, with a specific emphasis on the benefits and potential hazards of music and strategies for ensuring that adolescents can safely enjoy the psychological benefits of music. This review hopes to offer practical suggestions for parents, educators, and community workers and to guide future research in this domain.

2. Definition and classification of music

2.1. Definition of music

Music, as a universal form of human expression, has a well-known definition, but it has multiple interpretations in terms of substance and artistic features. In its essence, music is a conscious and organized expression of sound and silence (Tervaniemi, 2023). It involves elements such as melody (a musical phrase formed by a series of notes), rhythm (the organization and duration of notes), harmony (two or more notes sound simultaneously), and color (the characteristics and sensations of music, often contributed by specific instruments or sound sources).

In terms of artistic features, music is regarded as a universal human language that can convey emotions, express ideas, provide comfort, and cross cultural boundaries. It is an art form with high expressiveness and depth, through which we can experience various aspects of life (Mastnak, 2016).

2.2. Classification of music

The diversity of music is reflected in its broad classification. Classified by region and country, music from different regions reflects their respective cultural characteristics and traditions. For example, Chinese music is famous for its unique pentatonic scale, strong expressiveness, and rich variety of instruments (Wang et al., 2021; Su and Kong, 2023). The characteristics of Indian music are complex rhythmic structures, such as Tala, and improvisation based on the scale (Raga; Fletcher, 1915; Sharma et al., 2021). Western classical music is known for its rigorous formal structure, rich harmony, and complex note textures (Georges, 2017; Lepping et al., 2019).

Another classification method is based on the genre and characteristics of music. Classical music usually refers to music created between 1650 and 1900, with carefully crafted compositions and rich expressive power. Pop music is the most common type of music since the mid-20th century, characterized by simple melodies, easily accepted lyrics, and strong rhythms (Ren, 2021). Jazz originated in the early 20th century in the United States and is known for its unique rhythm (such as swing rhythm) and extensive improvisation (Roe and Lysaker, 2023). Rock music originated in the United States in the 1950s, and it has had a widespread impact worldwide due to its strong rhythm and gripping guitar solos (Bogt et al., 2021).

Each type of music has its unique characteristics and forms of expression, providing people with various ways of expression and experience. The richness and diversity of music not only reflect human creativity but also provide a powerful tool that can influence and shape our psychological state.

3. Benefits of music

3.1. Facilitates expression and regulation of emotions

Music has long been recognized as a potent medium for expressing emotions. It enables individuals to convey a wide spectrum of complex emotions. For instance, fast-paced, high-intensity music may signify happiness and excitement, whereas slow-paced, low-key music can indicate sadness and frustration (Dovorany et al., 2023). Through creating and listening to music, adolescents can easily comprehend and manage their emotions. Moreover, music serves as an instrument for emotional regulation, assisting individuals in alleviating stress, mitigating anxiety, and bolstering self-confidence (Aalbers et al., 2017). Research indicates that listening to music can lower cortisol levels (a stress hormone), thereby reducing stress (Emami et al., 2023). The theory of self-efficacy suggests that participation in music activities (such as learning an instrument or singing) can enhance individual self-confidence and self-esteem (Paolantonio et al., 2020).

3.2. Enhances social connections and sense of belonging

Music also provides social benefits, as it serves as a shared interest and experience and fosters a sense of connection and belonging. For instance, sharing musical tastes can strengthen friendships among young people and promote the formation of social identity (Yifan Zou and Wang, 2021). Additionally, participation in group music activities (such as orchestra or choir) can help adolescents develop teamwork skills and provide a robust sense of belonging and shared accomplishment (Cores-Bilbao et al., 2019).

3.3. Fuels creative and intellectual development

Music is considered a vital source of creativity. Through music composition and improvisation, teenagers can freely explore and express their thoughts, emotions, and imagination (Xia et al., 2023). Moreover, studies have shown that music training can positively affect intellectual development, particularly improvements in musical training correlated with mathematical, logical, and spatial intelligence (Kim and Chung, 2023). Although the specific influence of music on intellectual development continues to be studied, evidence suggests that adolescents involved in music activities perform better academically than those who are not.

4. Potential drawbacks of music

Despite the widely researched and acknowledged benefits of music, its potential disadvantages, particularly when improperly used, must be recognized.

4.1. Inappropriate usage of music

Excessive and improper use of music may contribute to hearing damage. Long-term exposure to high-volume music, especially through headphones or earbuds, increases the risk of harmful noise exposure, potentially resulting in temporary or permanent hearing damage (Hanson and Fearn, 1975). As a result of their limited self-regulation, adolescents may unconsciously listen to loud music, endangering their hearing health. Consequently, teenagers must be educated on safe music listening habits, including reducing volume, limiting listening duration, and using safe listening devices (Gopal et al., 2019).

Additionally, excessive music listening may distract attention, thereby affecting academic and work performance (Chen et al., 2023). Some argue that music enhances work efficiency, but research suggests that listening to lyrical music can distract attention, particularly when performing tasks requiring language processing (Vasilev et al., 2018). Furthermore, overreliance on music might lead to habitual addiction, which is characterized by an uncontrollable urge to listen to music, potentially affecting daily life negatively.

4.2. Potential negative effect of music content

The content of music may induce or exacerbate negative emotions and behaviors. For instance, lyrics containing violence, sex, discrimination, and negativity may negatively influence adolescents (Wang and Jiang, 2022). Studies have demonstrated that young people often interpret and internalize lyrics more than adults do; thus, the youth are more susceptible to these negative influences than adults (Martino et al., 2006).

The emotional tone of music may influence the listeners' emotional state. Some studies have indicated that listening to melancholic and intense music may induce or exacerbate negative emotions (Hahn et al., 2022). Though young individuals may use such music to regulate or express their emotions, in the long run, excessive exposure to such music may detrimentally affect their mental health (Figure 1).

5. Implementing effective coping strategies

5.1. Selecting appropriate music genres

For adolescents, the choice of an appropriate music genre is critical, as it can satisfy both their personal preferences and psychological needs. This selection process should prioritize the adolescents' preferences and psychological needs and consider the esthetic value and educational significance of music. For instance, according to Maslow's hierarchy of needs theory, music can fulfill physiological needs (such as relaxation and stress relief), safety needs (like fostering a sense of security), social needs (enhancing belongingness and interpersonal relationships), esteem needs (expressing oneself and receiving acknowledgment), and self-actualization needs (such as creation and performance; Healy, 2016).

Furthermore, the esthetic value and educational implications of music should not be overlooked. Esthetic studies illustrate that music can trigger profound emotional experiences and spiritual exchanges and offer beauty appreciation. Education research suggests that music education can enhance holistic adolescent development, including intellectual, emotional, social, and moral dimensions (Ouergui et al., 2023). Thus, we should motivate teenagers to choose music of high esthetic and educational value, including classical music, ethnic music, and world music.

5.2. Establishing a healthy music environment

To safeguard adolescents' auditory health, parents must monitor volume and duration and avoid prolonged exposure to loud music. In addition, the content of music, particularly those expressing negative emotions, violence, discrimination, and other harmful elements, should be regulated. These responsibilities require the collaborative efforts of families, schools, and society at large. The home, being a teenager's first classroom, should see parents educating adolescents about the appropriate use of music, including volume control, time management, and selection of positive and healthy music content. Schools, as the secondary classroom, should guide adolescents toward appreciating high-quality music, thereby positively impacting music education. The society, being the broader classroom, should offer abundant music resources and services to cultivate a healthy musical environment for adolescents (Kortesoja et al., 2023).

5.3. Advocating positive music education

Music education plays a pivotal role in fostering adolescents' mental health. First, we should prioritize the design and teaching methods of music courses. Music syllabi should encompass diverse facets of music, including music theory, instrumental performance, vocal training, music appreciation, and music creation. Teaching

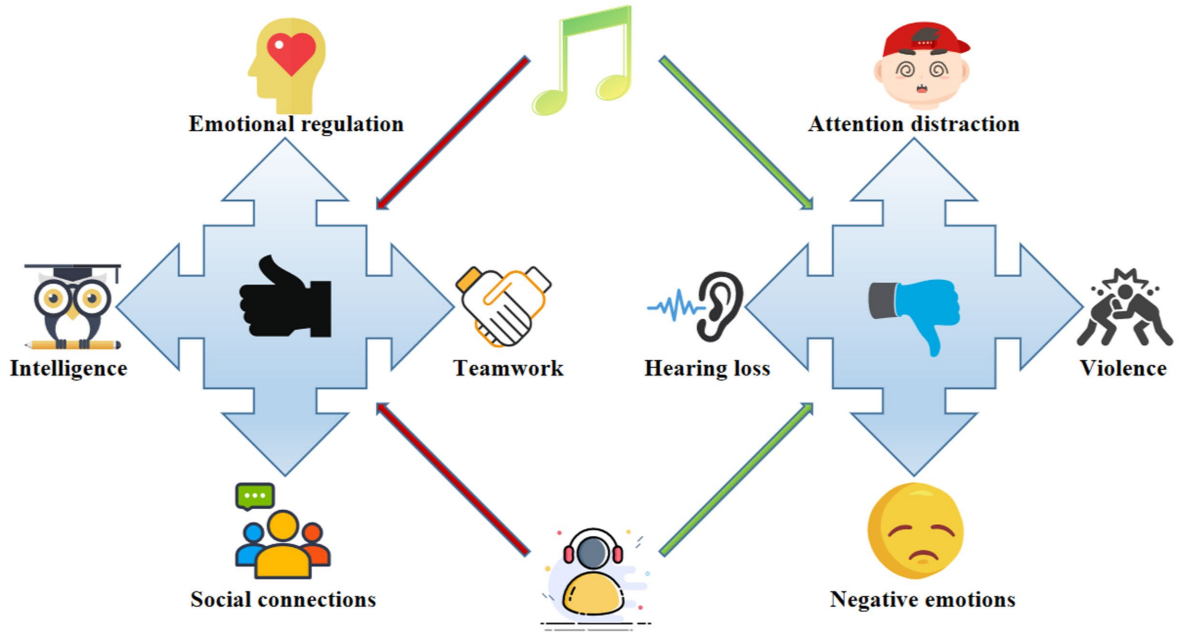

FIGURE 1
Schematic of music on the mental health of youth.

methods should emphasize student-centric approaches and inspire active participation, exploration, and creative thinking. Innovative teaching techniques, such as project-based learning, problem-based learning, and cooperative learning can pique adolescents' interest and enhance their learning efficacy (Lei, 2022). Second, we should expand the accessibility of music education resources. This expansion includes providing a plethora of resources like music books, music software, and music websites and organizing a variety of music activities such as concerts, music competitions, and music festivals.

5.4. Encouraging active participation in music activities

Music activities offer an excellent platform for teenagers to hone their musical skills, showcase their talents, and relish the joy of music. Adolescents should be encouraged to actively participate in various music activities, like learning musical instruments, partaking in performances, and organizing music clubs. These activities not only enhance the music literacy of adolescents but also boost their social, organizational, and leadership skills. Moreover, the innovative spirit in adolescents must be stimulated, and they should be encouraged to engage in music creation, adaptation, and research. These activities allow adolescents to comprehend music profoundly, express music freely, and actively engage in music, thereby enhancing their mental health (Perkins et al., 2016).

6. Conclusion

In this systematic review, we explored the intricate relationship between music and the mental health of adolescents. The influence of music on adolescent mental health is significant, encompassing areas such as emotional expression and regulation, fostering social connections and a sense of belonging, and promoting creativity and intellectual growth. Music offers adolescents an avenue to articulate their identity and emotions while assisting in the development of their social networks, creativity, and cognitive abilities. Conversely, the mental health status of adolescents influences their interaction with and perception of music. Adolescents may select different music genres in alignment with their emotional state and psychological requirements. For instance, during periods of stress or unhappiness, they may gravitate toward music that provides comfort or reflects their current emotional state.

This bi-directional relationship underscores the significance of music in the realm of adolescent psychological health, and it contributes fresh perspectives and ideas for future research. Possible future research areas could include studying how adolescents utilize music to manage their psychological stress or investigating ways to enhance their mental health through music education (Wang et al., 2022). Although music confers a multitude of benefits on adolescent mental health, inappropriate utilization of music may have detrimental effects. High-volume music can lead to auditory damage, and excessive music engagement may cause attention dispersion or contribute to addictive tendencies. Moreover, some music content can instigate negative emotional responses in adolescents, encompassing themes of violence and discrimination (Wang and Jiang, 2022). Thus, adolescents should enjoy music in moderation and proactively mitigate possible risks. Parents and teachers bear the responsibility of instructing adolescents on appropriate music consumption, including adjusting suitable volume, managing listening duration, and selecting positive and health-promoting music content. These strategies can help adolescents evade the negative implications of music while maximizing its positive influences, thereby enhancing their mental health status.

The establishment of a healthy music environment is pivotal in promoting adolescent mental health; this goal can be achieved through the collective efforts of families, schools, and society. Homes should provide safe and inviting music environments for adolescents. Schools should be equipped with music education resources and opportunities. Society should facilitate music activities and services to cater to adolescents' musical needs. Further, adolescents should be encouraged to actively engage in music activities, such as learning instruments and partaking in musical performances. These activities not only foster adolescents' musical aptitude and knowledge but also hone their social skills, boost self-confidence, and stimulate creativity (Phillips et al., 2019). In this process, the role of music educators is paramount. They bear the responsibility of imparting not only musical knowledge and skills to the adolescents but also helping them understand the spiritual value of music. They should facilitate the understanding of how music affects their emotions and mental states, as well as how to employ music as a tool to enhance their mental health (Steele and Young, 2011).

In conclusion, music significantly influences adolescent mental health. A concerted effort at all levels is required to cultivate a healthy musical environment for adolescents to optimally harness the positive influence of music and avert its potential negative effects.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Progress on mechanisms of age-related hearing loss

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Age-related hearing loss, or presbycusis, is a common cause of hearing loss in elderly people worldwide. It typically presents as progressive, irreversible, and usually affects the high frequencies of hearing, with a tremendous impact on the quality of life. Presbycusis is a complex multidimensional disorder, in addition to aging, multiple factors including exposure to noise, or ototoxic agents, genetic susceptibility, metabolic diseases and lifestyle can influence the onset and severity of presbycusis. With the aging of the body, its ability to clean up deleterious substances produced in the metabolic process is weakened, and the self-protection and repair function of the body is reduced, which in turn leads to irreversible damage to the cochlear tissue, resulting in the occurrence of presbycusis. Presently, oxidative stress (OS), mitochondrial DNA damage, low-grade inflammation, decreased immune function and stem cell depletion have been demonstrated to play a critical role in developing presbycusis. The purpose of this review is to illuminate the various mechanisms underlying this age-related hearing loss, with the goal of advancing our understanding, prevention, and treatment of presbycusis.

KEYWORDS

age-related hearing loss, presbycusis, oxidative stress, mitochondrial DNA damage, inflammation

Introduction

Age-related hearing loss (ARHL), or presbycusis, is the most common sensory deficit affecting aging adults (Agrawal et al., 2008). ARHL typically presents as a progressive, irreversible sensorineural hearing loss that increases with age, mainly involving high frequency hearing, and gradually spreads to low frequency hearing (Wang and Puel, 2020). Presbycusis is not only associated with damage to the cochlear organs but is often accompanied by central nervous system dysfunction that reduces the ability to process auditory information (Basner et al., 2014). Therefore, while auditory sensitivity decreased, patients may also experience reduced understanding of speech in noisy environments, as well as slowed central processing of acoustic information and impaired localization of sound sources (Boettcher, 2002; Bielefeld et al., 2010; Fetoni et al., 2011).

According to a 2012 estimate by the World Health Organization, about 164.5 million people over the age of 65 suffer from hearing impairment worldwide, accounting for about 33% worldwide in adults older than 65 years (Sheffield and Smith, 2019). The prevalence of hearing loss increases with age, and it can reach 84.3% among people over 80 years old (Yamasoba et al., 2013). With the aggravation of population aging and the extension of life expectancy, it is

estimated that more than 500 million individuals over the age of 60 will affected by presbycusis by 2025 (Wang and Puel, 2020). Presbycusis often presents with communication difficulties, reduced quality of life, and social isolation (Kay et al., 1964). In recent years, some studies have shown that presbycusis can lead to cognitive decline and is an independent risk factor for a series of neuropsychiatric diseases such as depression, dementia and Alzheimer's disease (Lin et al., 2011, 2013). Those creates a large socioeconomic burden.

Presbycusis is a complex chronic aging disease that results from the gradual accumulation of deleterious biological lesions and auditory system dysfunction (Eckert et al., 2021). With the aging of the body, a series of changes occur in the auditory organs. For example, the irreversible degeneration of the cochlear stria vascularis, spiral ligament, hair cells, and auditory nerve fibers can lead to blockage of ion and signal transmission, the exhaustion of stem cells weakens the regenerative repair ability of fibroblasts in the spiral ligament, and the decline of immune function leads to persistence of chronic inflammation, reduced enzyme activity can weaken the body's ability to remove toxic substances and ultimately leading to hearing loss (Seidman et al., 2002; Lang et al., 2006; Watson et al., 2017; Tawfik et al., 2020; Eckert et al., 2021). However, the age of onset and the degree of hearing loss vary greatly among individuals. Presbycusis is influenced by multiple factors and represent the interaction of numerous intrinsic and extrinsic factors. In addition to aging, many factors such as exposure to noise, or ototoxic agents, genetic susceptibility, metabolic diseases and lifestyle can all contribute to the development of presbycusis alone or in combination (Keithley, 2020; Wang and Puel, 2020). Those factors mainly lead to the occurrence of hearing loss through the mechanism of oxidative stress (OS), mitochondrial DNA damage, low-grade inflammation and reduced vascularisation in the cochlea (Seidman et al., 2002; Watson et al., 2017). The purpose of this review is to illuminate the pathophysiological features, etiology and pathogenesis of presbycusis, with the goal of advancing our understanding, prevention, and treatment of presbycusis.

Pathophysiological features of presbycusis

The cochlea is a spiral-shaped cavity composed of three liquid-filled compartments, scala media, scala tympani and scala vestibuli, which can convert the mechanical vibration caused by sound waves into electrical signals during sound transmission and is an important target organ for presbycusis. Scala media contains endolymph with high potassium and low sodium and is sandwiched by scala tympani and scala vestibuli containing perilymph with low potassium and high sodium. The endolymph in the scala media contains a positive voltage of +80 to +100 mV (endocochlear potential: EP), which is generated by the potential difference between the endolymph in scala media and the perilymph in scala tympani (Hibino et al., 2010; Bazard et al., 2021a). The stria vascularis and spiral ligaments located on the cochlear lateral wall have an intact ion channel transport system that transports potassium ions into the endolymph to maintain the high potassium status of the endolymph and the highly positive EP, which is essential for hair cell transmission (Wangemann, 2006).

Auditory information transmission is a complex process, and the cochlea is an important organ in this process. The shear motion caused by acoustic vibration causes the cilia of hair cells to bend or deflect, which in turn opens potassium channels at the top of hair cells, allowing potassium ions in the endolymph to flow into the hair cells to produce depolarization. Depolarization of hair cells causes calcium channels to open in cells and calcium ions to influx, which in turn prompts hair cells to release neurotransmitters into the synaptic cleft of the hair cells and auditory neurons (Bazard et al., 2021a). Damage to cells and tissues associated with auditory information conduction, as well as changes in the cochlear microenvironment, may contribute to the development of presbycusis (Noble et al., 2022).

According to postmortem histopathological studies, a variety of pathological changes occur in the inner ear of patients with presbycusis, such as the atrophy of the stria vascularis (SV) and loss of fibrocytes of the spiral ligament (SL) in the lateral wall of the cochlea (strial presbycusis, also known as metabolic presbycusis), decrease of sensory hair cells (sensory presbycusis) and degeneration of the auditory nerve (neural presbycusis) (Ohlemiller, 2004; Bowl and Dawson, 2019). The cochlear lateral wall degeneration is an important pathological change in in aging. Even the quiet-aged gerbil that raised under strictly controlled experimental conditions will experience degeneration of the stria vascularis and spiral ligament at both ends of the cochlear duct with aging (Spicer and Schulte, 2002). Deterioration of the lateral wall of the cochlea reduces the number and function of the sodium-potassium pump (Na-K-2Cl cotransporter NKCC1 and Na+, K+-ATPase), resulting in impaired potassium circulation and reduced EP (Chen and Zhao, 2014; Bazard et al., 2021a). Hair cells (HCs), which transduce mechanical stimuli into electrical activity through the hair bundle on their apical surface, degenerate with age and are also susceptible to factors such as noise exposure and ototoxic drugs (Bazard et al., 2021a). HCs damage is predominantly outer hair cells (OHCs), and it begins both apical and basal ends of the cochlea and progresses throughout the length of the organ of Corti, while inner hair cells (IHCs) damage is less and restricted to the extreme basal end of the aging cochlea (Kujawa and Liberman, 2019). The EP and OHCs have the function of cochlear amplification, which can provide 50–70 dB of gain in the basal turn of the cochlea (high-frequency hearing threshold region), while the apical of the cochlea (low-frequency hearing threshold region) can only gain 20 dB (Lang et al., 2010). This may explain the greater impairment of high-frequency hearing thresholds when the cochlear lateral wall and OHCs are damaged. IHCs are sensory receptors that transmit amplified information to the brain via spiral ganglion neurons and auditory nerve. Aging or noise-accumulating lesions can lead to degenerative changes in spiral ganglion neurons (SGNs), in which low-spontaneous rate (SR) fibers are more susceptible to damage and lesions usually involve both apical and basal ends of the cochlea (Lang et al., 2010). The low-SR fibers mainly contribute to encoding transient stimuli in the background of noise, while not threshold detection in quiet situations (Furman et al., 2013). Therefore, neural presbycusis is mainly manifested as reduced understanding of speech in noisy environments, while the hearing threshold nearly normal in quiet. With aging, the cochlear vascular also exhibits pathological changes, such as merged capillaries, reduced red blood cell velocity and vascular plasticity, and thickened basement membrane, resulting in weakened oxygen and nutrient delivery and waste elimination (Ohlemiller et al., 2008; Eckert et al., 2021). Secondly, the permeability of the strial microvasculature increases, allowing harmful substances to enter the cochlea (Seidman et al., 1996).

However, in reality, affected by various pathogenic factors, presbycusis can cause lesions in multiple parts of the cochlea, showing a "mixed" pathology (Tawfik et al., 2020). In addition, presbycusis may have no obvious histopathological changes under light microscopy, but submicroscopic structural changes may occur, such as stereociliary lesions or reduced synapses between inner hair cells and afferent fibers (Sliwinska-Kowalska and Davis, 2012). Liu et al. found that aging mouse had loss of stereocilia and shrinkage of hair cell soma precede hair cell loss. After acoustic overstimulation, synaptic connections also disappear before hair cells in presbycusis (Liu et al., 2022).

Risk factors of presbycusis

Genetic susceptibility

Presbycusis is a disease with genetic susceptibility. According to twins studies and longitudinal studies of family cohorts, its heritability is small to moderate, with a heritability indices of between 0.35 and 0.55 (Bowl and Dawson, 2019). Unlike the single-gene genetic pathogenic pattern of congenital deafness and early-onset deafness, it is generally believed that presbycusis involves multiple genetic variants, each of which has a small impact (Wells et al., 2020).

Genome-wide association study (GWAS) is widely used for genetic composition analysis of presbycusis, and many candidate genes have been found to be associated with presbycusis (Bowl and Dawson, 2019). These genes may play an important role in signaling and maintenance of the cochlear microenvironment. Several independent studies have reported that the gene encoding glutamate metabotropic receptor 7 (GRM7) is associated with presbycusis. Mutations in *GRM7* may lead to the accumulation of neurotransmitters in synaptic connections, thereby altering the susceptibility to presbycusis (Van Laer et al., 2010; Newman et al., 2012). Genetic polymorphisms in the genes coding detoxification enzymes are also linked to presbycusis, such as Uncoupling protein 2 (*UCP2*), Superoxide dismutase 2 (*SOD2*) and N-acetyltransferase 2 (*NAT2*) (Arsenijevic et al., 2000; Unal et al., 2005). Additionally, genetic variation may also contribute to increased susceptibility to presbycusis by environmental factors such as noise and ototoxic drugs (Prezant et al., 1993; Sliwinska-Kowalska and Pawelczyk, 2013).

Monogenic deafness-causing genes, especially those that cause delayed-onset deafness, may also be associated with presbycusis. Wells et al. (2019) performed GWAS for self-reported hearing loss adults in UK Biobank and reported 10 of the 44 associated loci included monogenic deafness genes. *TMC1* variant was previously thought to be associated with progressive postlingual hearing loss and profound prelingual deafness. However, in a recent study, Boucher et al. (2020) demonstrated through *in vitro* transfection and *in vivo* animal models that the heterozygous pathogenic variants of *TMC1* can cause presbycusis in a single-gene form, which updates our understanding of the inheritance pattern of presbycusis and provides a basis for potential inner ear treatments.

Noise exposure

Noise is the second most common cause of hearing loss other than old age, and noise-induced hearing loss (NIHL) is considered a common occupational disease, with a high incidence in occupational workers who have been exposed to noise for a long time, such as workers in textile, mining, and heavy engineering industries (Nandi and Dhatrak, 2008; Natarajan et al., 2023). Both aging and acoustic trauma can lead to loss of hair cells at the base end of the cochlea. Aged animals raised in quiet environments show did not lose hair cells until well past the middle of the lifespan, and the loss was small, whereas human temporal bone specimens have found stable and large loss of hair cells throughout the life (Kujawa and Liberman, 2019). This suggest that noise exposure synergizes with aging in the development of presbycusis.

Noise damage to the auditory system is affected by intensity and duration of noise exposure and can cause permanent threshold shifts (PTS) or temporary threshold shift (TTS) (Natarajan et al., 2023). Both long-term high-intensity noise exposure and one-time exposure to hazardous noise levels can lead to PTS (Liberman, 2016; Ryan et al., 2016). Due to the repair function of the stereocilia tip links, moderate noise damage often leads to temporary hearing loss, which recovers within 24–48 h (Gerhardt et al., 1987; Jia et al., 2009). Although low-intensity noise stimulation does not directly cause hair cell loss, it may causes permanent damage to the stereocilia bundles on the hair cells and to the synaptic connections between the auditory nerve fibers (ANF) and the IHC, which results in a blockage of auditory signal transmission and decreased ability to distinguish speech in noisy background (Kujawa and Liberman, 2019). Therefore, noise exposure has a cumulative effect on damage to the auditory system, prolonged exposure to 70 dB of noise may also cause hearing damage, and long-term lower noise and short-term louder noise have the same effect on hearing (Natarajan et al., 2023).

Ototoxic agents

Aminoglycoside antibiotics and chemotherapy drugs such as cisplatin and carboplatin can cause degenerative changes in the cochlea and hearing loss (Jiang et al., 2017). Additionally, through a large longitudinal cohort study lasting 10 years, Joo et al. (2020) found that loop diuretics and nonsteroidal anti-inflammatory drugs were associated with risk of progressive hearing loss, and may contributor to the incidence and severity of age-related hearing loss.

Metabolic diseases

Metabolic diseases are a cluster of diseases or disorders that disrupt normal metabolism, including high blood sugar (hyperglycemia), increased blood pressure (hypertension), excess fat around the waist (obesity), and abnormal levels of cholesterol or triglycerides (dyslipidemia). In recent years, under the influence of unhealthy diet and lifestyle, the incidence of metabolic diseases and its components are on the rise, and it is most common in the elderly (Saklayen, 2018). Presbycusis and metabolic diseases are both chronic diseases with a high prevalence, and many older people suffer from them at the same time (Guo et al., 2022). In a large cohort study of 94,223 people in Korea, Rim et al. (2021) reported that obesity, hypertension, hyperglycemia and dyslipidemia were all strongly associated with hearing loss, and the number of components of the metabolic diseases is positively correlated with the rate of sensorineural hearing loss. In two cohort studies in Europe and Korea, high body mass index (BMI) and low BMI were found to be associated with hearing loss, respectively (Fransen et al., 2008; Lee et al., 2015). Furthermore, Nguyen et al. (2022) found that the mouse model of diabetes and dyslipidemia had higher hearing impairment and degeneration of the cochlear spiral ganglion and stria vascularis. Some studies have found mitochondrial dysfunction occurs in both

metabolic diseases and presbycusis, and Guo et al. (2022) suggested that metabolic diseases may increase susceptibility to presbycusis by causing mitochondrial dysfunction.

Lifestyle

Lifestyle effects on hearing are diverse, with studies showing that both smoking and passive smoking increase the risk of hearing loss, while moderate alcohol consumption has a protective effect on hearing (Dawes et al., 2014). Diet and exercise may also play a role in aging and hearing. A high antioxidant diet can reduce mitochondrial dysfunction, thereby decreasing the magnitude of the vascular atrophy and cochlear auditory nerve degeneration (Le and Keithley, 2007). Han et al. (2016) found that increasing exercise in mice was effective in attenuating cochlear degeneration and hearing loss.

Age-related changes and pathogenesis of presbycusis

Age-related reactive oxygen species accumulation and mitochondrial DNA damage

Reactive oxygen species (ROS) are highly reactive chemicals produced during mitochondrial respiration or cellular response to endogenous and exogenous factors, mainly including superoxide anion (O2-), hydrogen peroxide (H2O2), hydroxyl radical (OH-) and nitric oxide (NO-) (Pizzino et al., 2017). ROS can serve as critical signaling molecules in cell proliferation and survival, but their excessive production and accumulation can lead to oxidative stress (OS), which in turn leads to macromolecular damage, promoting diseases such as presbycusis, aging and cancer (Ray et al., 2012). In the cochlea, ROS can damage DNA, break down lipid and protein molecules, and lead to cochlear cell apoptosis (Paplou et al., 2021). Increased plasma levels of ROS in humans are associated with hearing loss, while a high antioxidant diet can reduce cochlear degeneration and hearing loss (Le and Keithley, 2007; Lasisi and Fehintola, 2011). In fact, there are antioxidant enzymes in the body that can remove ROS, such as glutathione reductase (GSR), superoxide dismutase (SOD), catalase (CAT) and methionine sulfoxide reductase (MSR). The balance of the body's antioxidant enzymes and ROS can avoid damage to cells and tissues caused by OS (Seidman et al., 2002; Paplou et al., 2021).

The cochlea is an energy-intensive organ in which mitochondria provide energy for their sodium-potassium pump activity and ion transport through oxidative phosphorylation, while producing large quantities of ROS. The aggregation of ROS in the cochlea can lead to mutations in the mitochondrial genome, resulting in mitochondrial DNA (mtDNA) damage (Seidman et al., 2002). Mitochondrial DNA mutation is an important component of auditory system damage, and its characteristic 4,977 bp deletion occurs frequently in temporal bone tissue samples from patients with presbycusis (Zhong et al., 2011). In addition, postmortem analysis of the temporal bones of patients with presbycusis revealed defects in the expression of mitochondrial aerobic metabolism-related enzymes (Markaryan et al., 2009). For the damaged mitochondria, cells can clear and renew them through the autophagy and mitochondrial dynamics (fission and fusion events) (Wang and Puel, 2018).

However, as the body ages, the production and function of antioxidant enzymes decrease, and ischemic and hypoxic damage from local vascular lesions in the cochlea leads to increased production of ROS (Ray et al., 2012). As a result, the original balance is broken, and OS will cause cumulative damage to mitochondria and cochlear cells. In addition, mitochondrial biogenesis in the elderly is weakened, autophagy and mitochondrial dynamics are reduced, so that mitochondria are constantly depleted, so that normal cells and cochlear function cannot be maintained (Seidman et al., 2002; Wang and Puel, 2020; Figure 1).

Inflammaging and decreased immune function

Inflammaging is chronic and low-grade inflammation of tissues and organs that occurs during aging and can lead to conditions as diverse as cardiovascular disease, diabetes, and neurodegenerative diseases (Bazard et al., 2021b; Kociszewska and Vlajkovic, 2022). Various stimuli, including cellular debris, nutrients and pathogens, can drive sterile inflammation (Franceschi et al., 2018). The body's immune function declines with age, mainly manifested by shift in T-cell subpopulation distribution (the number of naive T cells (especially CD8+) decreases and homeostatically proliferate into memory T cells), impaired calcium-mediated signaling and thymic atrophy, resulting in weakened immune surveillance and clearance of pathogens (Goronzy and Weyand, 2013; Yousefzadeh et al., 2021). In turn, it leads to the accumulation of inflammatory stimuli, which continuously stimulates the body to produce chronic inflammatory responses. The cochlea is not an immune-privileged organ. Systemic inflammation is associated with presbycusis, and changes in the morphology and number of macrophages can occur in the aging cochlea (Watson et al., 2017).

Macrophages are a key part of the innate immune system, presented in the cochlear spiral ligament, the auditory nerve, and the organ of Corti. Activated macrophages transform from highly branched morphology to amoeboid shape and can phagocytose cellular debris or pathogens, which are critical for maintaining the homeostasis of the cochlear microenvironment (Noble et al., 2022). In addition, the vascularization function of macrophages can regulate the permeability of the blood-labyrinth barrier (BLB) of the strial microvasculature (Noble et al., 2022). Noise trauma activates macrophages, causing changes in their shape and number (Presta et al., 2018). Analysis of temporal bone specimens of different ages revealed that aging cochlear macrophages were highly activated (Noble et al., 2019). The inflammatory response driven by macrophage activation plays an important role in the development of presbycusis by leading to cochlear degeneration and increased stria vascular permeability (Noble et al., 2022).

The intestinal tract contains a large number of microorganisms and their genetic material, and gut dysbiosis may also contribute to inflammaging. In addition to the high-fat diet can lead to the development of cochlear inflammation (Kociszewska et al., 2021). As the body ages, a series of changes occur in the intestinal tract, such as the reduced microbiota diversity, more pro-inflammatory microbiota such as LPS-producing Gram-negative bacteria, and the permeability of the intestinal barriers increases (Ragonnaud and Biragyn, 2021). Therefore, pathogens and metabolites of intestinal microorganisms can be transported to the cochlea through the systemic circulation, leading to chronic inflammation of the cochlea and the occurrence of presbycusis (Kociszewska and Vlajkovic, 2022; Figure 1).

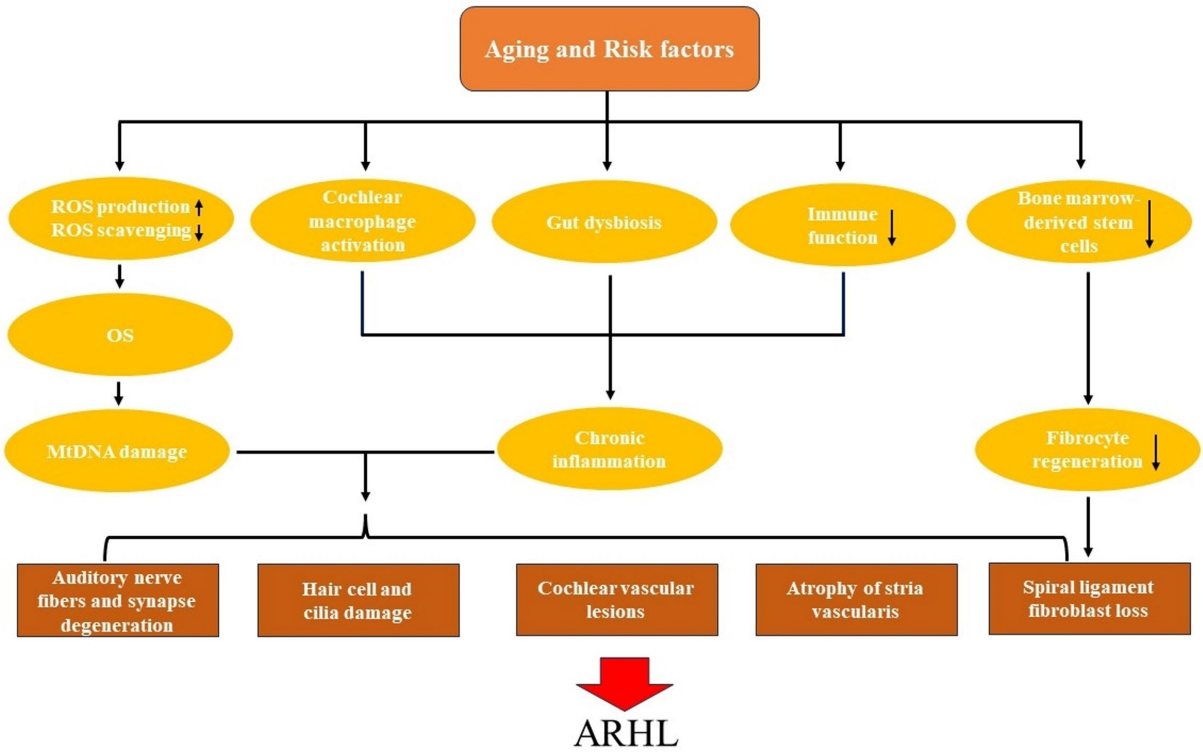

FIGURE 1
The pathogenesis and pathological changes of age-related hearing loss (ARHL).

Fibrocyte regeneration and stem cell depletion

The spiral ligament is a component of the potassium ion transport system in the lateral wall of the cochlea, which contains five types of fibrocytes, and its normal function is crucial for the maintenance of the EP (Spicer and Schulte, 1991; Lang et al., 2003; Eckert et al., 2021). Unlike sensory hair cells and neurons, which do not regenerate, spiral ligament fibrocytes typically recover rapidly after ototoxic drug and noise damage (Roberson and Rubel, 1994; Lang et al., 2003). The regenerative repair ability of fibrocytes relies on bone marrow-derived stem cells (Lang et al., 2006). However, as the body ages, stem cells are continuously depleted and their differentiation potential and proliferation rate decrease (Fehrer and Lepperdinger, 2005). Therefore, the renewal and repair ability of fibrocytes in the elderly is weakened, the spiral ligament atrophies, and metabolic presbycusis occurs (Eckert et al., 2021; Figure 1).

Prevention and treatment of presbycusis

Prevention of presbycusis

The management of presbycusis should first focus on prevention and avoid exposure to risk factors. While we cannot prevent aging, nor can we change our genetic background, we can minimize noise exposure, wear earplugs in noisy environments, and avoid ototoxic medications (He et al., 2019). Elderly patients should actively treat metabolic diseases and ear infections to avoid damage to their hearing (Guo et al., 2022). A good lifestyle is also essential for the prevention of presbycusis, and a reduced intake of fatty foods and a diet high in antioxidants can reduce hearing loss (Le and Keithley, 2007; Kociszewska et al., 2021). In addition, proper exercise not only strengthens immune function, but also reduces free radicals in the body. Studies have shown that long-term exercise can delay the progression of presbycusis by reducing age-related capillary loss associated with inflammation (Han et al., 2016).

Treatment of presbycusis

Treatment of presbycusis still relies clinically on hearing amplification and cochlear implantation. Air conduction hearing aids are commonly worn in patients with mild to moderate hearing loss, active middle ear implants can be used in patients with moderate to severe hearing loss, and cochlear implants should be considered in patients with severe to profound hearing loss (Seidman et al., 2019). However, it is estimated that only 15% of eligible patients use them due to multiple factors including cost, appearance, discomfort, and lack of perceived benefit (Chien and Lin, 2012; Mahboubi et al., 2018).

In recent years, some researchers have begun to explore the treatment of presbycusis with antioxidants, anti-inflammatories, neurotrophins and other drugs (Wang and Puel, 2020). Studies by Benkafadar et al. (2019) show that EUK-207, a synthetic superoxide dismutase/catalase mimetic, reduced hair cell degeneration and age-related hearing loss in senescence-accelerated mouse-prone 8 (SAMP8) mice. Serra et al. (2022) believe that although the antioxidant melatonin cannot completely prevent presbycusis, it can delay its occurrence. Aspirin displays anti-inflammatory and antioxidant properties, and studies have shown that it can effectively reduce hearing loss in aged mice (Cazals, 2000). An ongoing clinical trial is attempting to assess its potential therapeutic effect on presbycusis in humans (Lowthian, et al., 2016). Cassinotti et al.

(2022) overexpressed neurotrophin-3 (Ntf3) in mouse cochlea starting at middle age, thereby preventing age-related inner hair cell synaptopathy and slowing age-related hearing loss. Oral treatment with selegiline, a neuroprotective antiparkinsonian drug, significantly alleviated hearing loss at higher frequencies in mice with moderate hearing loss, but not in mice with rapid progressive hearing loss (Szepesy et al., 2021). We believe that the drug treatment of presbycusis has broad prospects, but the current application is still concentrated in animal models and clinical trials, and its clinical application still needs to solve problems such as efficacy, safety, administration route and dosage. In addition, gene therapy and stem cell transplantation also have potential treatment for presbycusis, which still need further research (Chen et al., 2012; Davidsohn et al., 2019).

Conclusion

Presbycusis is a common chronic disease occurring in the process of aging, which is the result of the interaction of various extrinsic and intrinsic factors under the genetic background. With the aging of the body, the function or number of immune functions, antioxidant enzymes and self-repairing stem cells that are closely related to the body's self-protection decline, resulting in the accumulation of inflammatory cytokines, reactive oxygen species and tissue cell damage in the body. Therefore, under the action of chronic inflammation and oxidative stress, irreversible damage occurs to the cochlear stria vascularis, spiral ligament, sensory hair cells and auditory nerve fibers, and then lead to the occurrence of presbycusis.

Mitigating exposure to risk factors is essential for the prevention of presbycusis. The clinical treatment of presbycusis is still mainly based on wearing hearing aids and cochlear implants, but various drugs launched according to its pathogenesis are also undergoing clinical research. The in-depth exploration of the pathogenesis of presbycusis provides a reference for potential drug treatment and clinical intervention.

Author contributions

WY: Writing – original draft, Methodology. XZ: Writing – original draft, Methodology. RC: Visualization, Writing – review & editing. JF: Validation, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Altered static and dynamic intrinsic brain activity in unilateral sudden sensorineural hearing loss

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Introduction: Sudden sensorineural hearing loss (SSHL) is a critical otologic emergency characterized by a rapid decline of at least 30 dB across three consecutive frequencies in the pure-tone audiogram within a 72-hour period. This audiological condition has been associated with alterations in brain cortical and subcortical structures, as well as changes in brain functional activities involving multiple networks. However, the extent of cerebral intrinsic brain activity disruption in SSHL remains poorly understood. The aimed of this study is to investigate intrinsic brain activity alterations in SSHL using static and dynamic fractional amplitude of low-frequency fluctuation (fALFF) analysis.

Methods: Resting-state functional magnetic resonance imaging (fMRI) data were acquired from a cohort of SSHL patients (unilateral, n = 102) and healthy controls (n = 73). Static and dynamic fALFF methods were employed to analyze the acquired fMRI data, enabling a comprehensive examination of intrinsic brain activity changes in SSHL.

Results: Our analysis revealed significant differences in static fALFF patterns between SSHL patients and healthy controls. SSHL patients exhibited decreased fALFF in the left fusiform gyrus, left precentral gyrus, and right inferior frontal gyrus, alongside increased fALFF in the left inferior frontal gyrus, left superior frontal gyrus, and right middle temporal gyrus. Additionally, dynamic fALFF analysis demonstrated elevated fALFF in the right superior frontal gyrus and right middle frontal gyrus among SSHL patients. Intriguingly, we observed a positive correlation between static fALFF in the left fusiform gyrus and the duration of hearing loss, shedding light on potential temporal dynamics associated with intrinsic brain activity changes.

Discussion: The observed disruptions in intrinsic brain activity and temporal dynamics among SSHL patients provide valuable insights into the functional reorganization and potential compensatory mechanisms linked to hearing loss. These findings underscore the importance of understanding the underlying neural alterations in SSHL, which could pave the way for the development of targeted interventions and rehabilitation strategies aimed at optimizing SSHL management.

KEYWORDS

unilateral sudden sensorineural hearing loss, resting state functional magnetic resonance imaging, static fractional amplitude of low-frequency fluctuation, temporal variability, intrinsic brain activity

Introduction

Sudden sensorineural hearing loss (SSHL) is an otologic emergency and characterized by a rapid decline of at least 30 dB across three consecutive frequencies in the pure-tone audiogram within a 72-h period. It is usually unilateral, while bilateral involvement is observed in fewer than 5% of cases (Schreiber et al., 2010; Murray et al., 2022). Based on previous reports, the reported incidence of SSHL ranges from 5 to 27 per 100,000 individuals, and its annual incidence continues to rise (Alexander and Harris, 2013; Kim et al., 2017; Kuo et al., 2019). Prior studies have suggested various potential causes of SSHL, such as vascular disorders, viral or bacterial infections, inner ear membrane rupture, tumor, and autoimmune diseases (Cadoni et al., 2002; Schreiber et al., 2010; Kim et al., 2017; Chandrasekhar et al., 2019). The current treatment modalities for SSHL include intratympanic corticosteroid injections (Filipo et al., 2013; Lavigne et al., 2016; Li and Ding, 2020; Plontke et al., 2022), hyperbaric oxygen therapy (Attanasio et al., 2015; Lamm et al., 2016), and oral corticosteroids (Swachia et al., 2016). However, despite the administration of suitable high-quality therapy, patients with SSHL often experience limited improvement in their hearing function, leading to potential adverse impacts on economic indicators for society and resulting in diminished long-term quality of life (Kuhn et al., 2011). Nonetheless, the etiology of over two-thirds of SSHL cases remains uncertain and is classified as idiopathic. What is worse, SSHL not only impacts the structure and function of the inner ear, including cochlear hair cells, auditory nerve fibers, and membranous labyrinth, but it also gives rise to central system symptoms such as cognitive deficits, depression, and anxiety, which may significantly diminish the quality of life for affected individuals (Schreiber et al., 2010; Chandrasekhar et al., 2019). Hence, the evaluation of comprehensive brain functions may aid in uncovering the underlying mechanisms of SSHL.

The advancement of imaging technology has revealed that individuals with SSHL exhibit not only alterations in brain cortical and subcortical structures (Yang et al., 2014; Fan et al., 2015; Qu et al., 2020) but also associated with changes in brain functional activities involving various brain networks such as the default mode, auditory, executive control, and visual networks (Xu et al., 2016; Chen et al., 2020; Minosse et al., 2021; Wang et al., 2021; Hua et al., 2022). The investigation of brain functional activity has become increasingly important in understanding the underlying neural mechanisms associated with SSHL. Amplitude of Low-Frequency Fluctuations (ALFF) is a widely used measure that reflects the intensity of spontaneous neural activity within specific brain regions. Several studies have utilized ALFF to investigate alterations in spontaneous brain activity in healthy control (Newbold et al., 2020; Elias et al., 2022) and various neurological and psychiatric disorders, such as Alzheimer's disease (Liu et al., 2014), major depressive disorder (Gray et al., 2020), schizophrenia (Sui et al., 2018) and traumatic brain injury (Palacios et al., 2013). These studies have demonstrated the utility of ALFF in providing valuable insights into the functional changes that occur in the brain across different conditions. However, these studies have primarily focused on assessing the "static" temporal strength or inter-region relationship in the temporal pattern, thereby overlooking the investigation of dynamic brain alterations or the time-varying nature of the blood oxygen level dependent signal in resting-state functional MRI scanning. Measuring temporal variability offers valuable insights into the fluctuation patterns of brain activity amplitudes over time (Leonardi and Van De Ville, 2015; Yan et al., 2017).

Recent studies have increasingly proposed that the dynamic ALFF or dynamic fractional ALFF (fALFF) provides an approach to measure the alterations of temporal variability of intrinsic neural activity in many diseases (Wang et al., 2019; Ma et al., 2020a; Zhou et al., 2021; Dong et al., 2022). ALFF measures the amplitude of low-frequency fluctuations in the BOLD signal and provides information about the absolute intensity of neural activity within specific frequency bands, typically the low-frequency range (0.01–0.1 Hz). And ALFF has been extensively used to investigate regional spontaneous activity and has been associated with the baseline functional state of the brain. On the other hand, fALFF calculates the ratio of the power of low-frequency fluctuations to the total power across the entire frequency range, providing a measure of the relative contribution of low-frequency oscillations to the global brain signal. This normalization process is particularly useful in reducing the influence of physiological noise, making fALFF more robust and less sensitive to non-neural fluctuations (Zou et al., 2008). While ALFF provides localized information about absolute neural activity, fALFF offers a more global perspective on the brain's intrinsic activity.

By employing dynamic ALFF analysis, it has been observed that individuals with SSHL demonstrate decreased dynamic ALFF variability specifically in the bilateral inferior occipital gyrus, middle occipital gyrus, calcarine, right lingual gyrus, and right fusiform gyrus (Li et al., 2022). These findings indicate that SSHL patients may undergo cross-modal plasticity and visual compensation, which could be closely linked to the underlying pathophysiology of SSHL (Li et al., 2022). Therefore, studying these dynamic characteristics can potentially reveal additional pathological changes associated with SSHL and provide valuable guidance for more effective treatment strategies. Given the mentioned limitation of small sample size mentioned by the authors and the inherent heterogeneity of SSHL, conducting studies on dynamic intrinsic brain activity in SSHL with a larger sample size is indeed necessary, which would enhance the generalizability and statistical power of the findings, allowing for more robust conclusions to be drawn regarding the dynamic characteristics of intrinsic brain activity in individuals with SSHL.

In the current work, we utilized the fALFF combined with a sliding window approach to evaluate the temporal variability of intrinsic brain activity in individuals with SSHL. We hypothesized that SSHL patients would exhibit altered patterns of static and dynamic fALFF in comparison to healthy controls (HCs). By identifying regions with altered fALFF values, it may provide valuable biomarkers associated with SSHL. These biomarkers could serve as objective and quantifiable indicators of neural dysfunction in SSHL patients, aiding in early diagnosis and individualized treatment strategies. Moreover, we hypothesized that dynamic fALFF could reveal underlying abnormal intrinsic brain activity that static ALFF measures might not capture, thereby contributing to a deeper understanding of the physiological mechanisms underlying SSHL. It is hoped these results could offer valuable insights into the neural circuits and pathways involved in the pathophysiology of the SSHL. Additionally, we proposed that the dynamic indexes might be associated with clinical characteristics of SSHL. The identification of specific fALFF patterns associated with the severity and progression of SSHL may offer prognostic information for individual patients. By correlating fALFF findings with clinical outcomes, we can potentially identify patients at higher risk of developing complications or poorer outcomes, facilitating early intervention and personalized care.

Materials and methods

Subjects

A total of 175 subjects, which consisted of 102 participants with unilateral SSHL and 73 sex- and age-matched HC subjects, were recruited from Wuhan Union Hospital. All participants were right-handed. Demographic and clinical data for the subjects are shown in Table 1. No significant differences were found in gender, age, education level between SSHL patients and HC subjects. Pure-tone audiometry was performed using seven different octave frequencies (0.125, 0.25, 0.5, 1, 2, 4, and 8 kHz) to measure the pure tone average (PTA) and determine the hearing level. As described in our previous study (Leng et al., 2022), PTA was calculated as the simple arithmetic mean for these seven frequencies. The initial pure-tone audiometry curves were categorized into four types: (1) low-frequency hearing loss (the average threshold of 0.25 and 0.5 kHz is 20 dB higher than that of 4 and 8 kHz), (2) high-frequency hearing loss (the average threshold of 4 and 8 kHz is 20 dB higher than that of 0.25 and 0.5 kHz), (3) flat-type hearing loss (similar threshold is observed across the entire frequency range and the average threshold is less than 90 dB HL), and (4) profound hearing loss (the average threshold of 0.5, 1, 2, and 4 kHz exceeds 90 dB HL) (Leng et al., 2022).

The diagnosis of SSHL was established following the diagnostic guidelines of SSHL outlined by the Clinical Practice Guideline (Chandrasekhar et al., 2019). Exclusion criteria were as follows: (1) SSHL patients with bilateral hearing loss, (2) all subjects had neurological diseases (such as acoustic neuroma, brain tumors, and trauma), psychiatric diseases (such as depression and insomnia), and other otolaryngological diseases (such as otitis media, Meniere's disease, and pulsatile tinnitus), and (2) all subjects had claustrophobia and cardiac pacemakers. Due to the frequent occurrence of tinnitus and/or vertigo in SSHL, the severity of tinnitus was assessed using the Tinnitus Handicap Inventory (THI). The THI utilizes a scale ranging from 0 to 100 to measure the increasing level of handicap caused by tinnitus (Newman et al., 1996).

This study was approved by the Tongji Medical College of Huazhong University of Science and Technology medical ethics committee. All subjects were informed about the purpose of the study before giving their written consents in accordance with Chinese legislation.

TABLE 1 Demographic and clinical variables.

Subjects	SSHL	HC	*p* value
Number of subjects	102	73	N/A
Age (years)	38.89 ± 12.11	38.01 ± 16.52	0.685
Gender (male/female)	52/50	37/36	0.969
Education level (years)	12.67 ± 3.20	12.74 ± 2.93	0.878
Duration (days)	9.03 ± 4.27	N/A	N/A
Effected side (left/right)	56/46	N/A	N/A
PTA (dB)	73.66 ± 10.38	13.25 ± 5.08	<0.001
THI score	47.54 ± 26.37	N/A	N/A

A Pearson chi-squared test was used for gender comparison. Independent samples *t*-tests were used for age, education and PTA comparisons.
SSHL, sudden sensorineural hearing loss; HC, healthy control; PTA, pure tone average; THI, tinnitus handicap inventory.

Data acquisition

The study employed a 3 T MRI system (Siemens Trio Tim, Erlangen, Germany) with a 12-channel head coil to acquire anatomical and functional images. Prior to administering any drug treatment, all patients underwent imaging experiments during which they were instructed to remain still and with their eyes closed while wearing headphones to minimize noise. A foam cushion was used to reduce head movements and motion artifacts. Anatomical images were obtained using a 3-dimensional high-resolution T1-weighted magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence with the following parameters: repetition time (TR) = 2,250 ms, echo time (TE) = 2.26 ms, inversion time (TI) = 900 ms, flip-angle = 9°, voxel size = 1.0 × 1.0 × 1.0 mm^3, field of view (FOV) = 256 mm × 256 mm, slice thickness = 1.00 mm, and 176 sagittal slices covering the entire brain. Functional images were acquired using a gradient echo type echo planar imaging (EPI) sequence with the following parameters: TR = 2000 ms, TE = 30 ms, flip-angle = 90°, voxel size = 3.0 × 3.0 × 3.0 mm^3, and FOV = 200 mm × 200 mm, resulting in 240 images. Additionally, a T2-weighted sequence was acquired to assess the status of the peripheral auditory system using the following parameters: TR = 1,000 ms, TE = 132 ms, slice thickness = 0.5 mm, slice number = 64, flip-angle = 120°, FOV = 200 mm × 200 mm, and averages = 2. Two radiologists independently reviewed the MR images for abnormalities such as otitis media, acoustic neuroma, and brain tumors, and any subjects with such abnormalities were excluded from subsequent analysis.

Data preprocessing

The Brain Imaging Data Processing and Analysis (DPABI, v7.0) tool (Yan et al., 2016), based on the Statistical Parametric Mapping (SPM12) toolkits (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/), was used to preprocess the resting-state BOLD data (Yan et al., 2013). The first 10 volumes were discarded to eliminate T1 equilibration effects, resulting in 230 time points. Slice timing correction and realignment correction were then performed to address different slice acquisition times and head motion using six-parameter rigid-body transformation. Nuisance covariates, including linear trends, the white matter signal, cerebrospinal fluid signal, and Friston 24 head motion parameters, were regressed out from the functional signal. Normalization was carried out by coregistering functional images with corresponding structural images, which were segmented and normalized to the standard Montreal Neurological Institute (MNI) template using the Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) tool. Participants with maximum head motion larger than 1.5 mm in displacement or 1.5° in rotation, and mean frame-wise displacement (FD) calculated by Jenkinson method larger than 0.2 were excluded from the analysis.

Static and dynamic fALFF calculation

After normalization, the time series of functional imaging underwent a fast Fourier transform to transform them into the frequency domain. The power spectrum and square root

transformation were then used to calculate the ALFF. The ALFF in the low frequency band (0.01–0.1 Hz) was divided by the amplitude in the entire frequency band to obtain the fALFF (Zou et al., 2008). The fALFF data were then transformed into z maps using Fisher's r-to-z transformation to enhance normality for group-level analysis. Finally, a 6 mm full width at half-maximum (FWHM) Gaussian kernel was applied to smooth the functional images.

A sliding window approach via DPABI was used to compute the dynamic fALFF (Yan et al., 2017). The window length is a critical parameter when computing resting-state dynamics. Using a shorter window length can heighten the risk of introducing spurious fluctuations in the observed dynamic fALFF. On the other hand, opting for a longer window length may impede the accurate depiction of the temporal variability dynamics of fALFF (Leonardi and Van De Ville, 2015; Li et al., 2019). To avoid spurious fluctuations caused by a window length shorter than the minimum frequency of the time series (fmin), the window length was set to 50 TRs (100 s), which was longer than 1/fmin (Lawrence and Thevasagayam, 2015; Yan et al., 2017; Li et al., 2019). The window was shifted by 5 TRs (step size: 10 s) to capture temporal variability in fALFF. This approach generated 37 windows for each participant. The mean and standard deviation of each voxel were computed in each sliding window to assess variability in dynamic fALFF, which was expressed as the coefficient of variation (CV: standard deviation/mean). The CV maps were then Z-standardized and smoothed with a 6 mm full width at half-maximum (FWHM) Gaussian kernel for further statistical analysis (Zhou et al., 2021).

Statistical analysis

To investigate the spatial distribution, group means of static and dynamic fALFF were computed. Following that, a two-sample t-test analysis was conducted to assess the differences in static and dynamic fALFF between the SSHL and HC groups. Voxel-level significance was set at $p < 0.001$, with cluster-level correction using Gaussian random field (GRF) at $p < 0.01$. Age, sex, mean FD, education, and gray matter volume were included as covariates in the analysis. Furthermore, partial correlation analyses were conducted to examine the association between the altered functional index of static or dynamic fALFF and clinical assessments, with sex, age, mean FD, education, and gray matter volume included as covariates (Zhou et al., 2021). Besides, we also conducted a subgroup analysis to explore potential differences in fALFF patterns between patients with left-sided SSNHL and those with right-sided SSNHL, and the results were showed in Supplementary material (see Supplementary Figures S1-S5; Supplementary Table S1).

Results

Spatial distribution and group differences of static fALFF

Figure 1 shows the spatial distribution of static fALFF (Figures 1A,B) in the SSHL and HC groups. Compared with the HCs, the SSHL patients exhibited significantly (GRF correction; voxel-level $p < 0.001$, cluster-level $p < 0.01$) decreased static fALFF in the left fusiform gryus (lFG), left precentral gyrus (lPG) and right inferior frontal gyrus (rIFG), and increased static fALFF in the left inferior frontal gyrus (lIFG), left superior frontal gyrus (lSFG) and right middle temporal gyrus (rMTG) (Figure 1C; Table 2).

Spatial distribution and group differences of dynamic fALFF

Figure 2 shows the spatial distribution of dynamic fALFF (Figures 2A,B) in the SSHL and HC groups. Compared with the HCs, the SSHL patients only exhibited significantly (GRF correction; voxel-level $p < 0.001$, cluster-level $p < 0.01$) increased dynamic fALFF in the right superior frontal gyrus (rSFG) and right middle frontal gyrus (rMFG) (Figure 2C; Table 2).

Correlation analysis

Correlation analyses identified positive correlations between static fALFF in left fusiform gyrus and hearing loss duration ($R = 0.396$, $p < 0.001$) (Figure 3). No significant correlation was found between dynamic fALFF and clinical measurements.

Discussion

The results of the study revealed significant intrinsic brain activity differences in static and dynamic fALFF patterns in various brain regions between individuals with SSHL and HCs. Moreover, static fALFF in left fusiform gyrus was positively correlated with hearing loss duration. Our findings revealed static and dynamic changes of brain functional intrinsic activities in SSHL patients which provide the neurophysiological basis for SSHL.

In terms of static fALFF, we found SSHL patients exhibited significantly decreased static fALFF in the left fusiform gryus, left precentral gyrus and right inferior frontal gyrus, which suggests reduced spontaneous neural activity in these regions. The fusiform gyrus is known to be involved in high-order visual information processing, including face recognition and object perception (Weiner and Zilles, 2016; Palejwala et al., 2020). It is reported that unilateral hearing loss showed reduced grey matter volumes in fusiform gyrus (Heggdal et al., 2020). Indeed, alterations in the brain activity of the fusiform gyrus have been reported in various neurological conditions, including amyotrophic lateral sclerosis (Luo et al., 2012), Parkinson's disease (Pan et al., 2017), obsessive–compulsive personality disorder (Lei et al., 2020) and social anxiety disorder (Qiu et al., 2015). The precentral gyrus is a key region in the primary motor cortex, responsible for voluntary motor control (Silva et al., 2022; Wei et al., 2022). The inferior frontal gyrus has been implicated in various cognitive processes, including language production and executive functions (Hampshire et al., 2010; Ishkhanyan et al., 2020). Previous studies have reported similar intrinsic brain activity alterations in hearing loss patients (Zhou et al., 2019; Chen et al., 2020; Yang et al., 2021). For instance, Chen et al., (2020) found decreased static ALFF and fALFF in the auditory cortex and visual cortex in unilateral SSHL, and Zhou et al. (2019) found abnormal static ALFF in the inferior frontal gyrus in unilateral acute tinnitus patients with hearing loss. The observed decrease in static fALFF in these regions may reflect alterations in sensory and cognitive functions associated with SSHL.

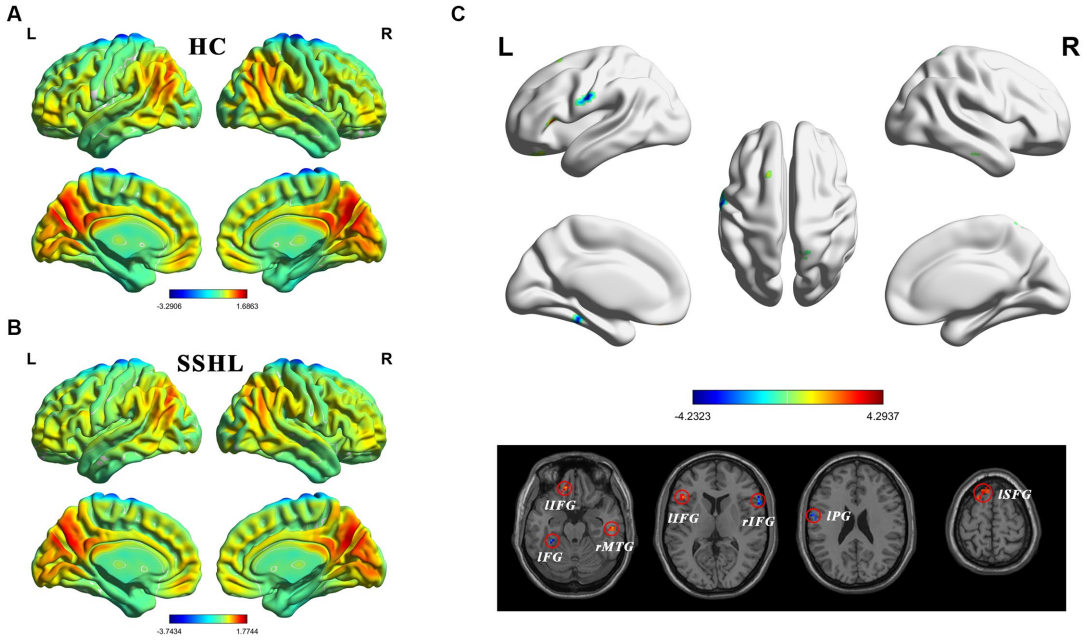

FIGURE 1
Altered static intrinsic brain activity in unilateral SSHL. **(A)** Within group mean static fALFF maps in the HC. **(B)** Within group mean static fALFF maps in the SSHL. **(C)** Group comparisons of static fALFF between the SSHL and HC groups (Gaussian random field correction; voxel-level $p < 0.001$, cluster-level $p < 0.01$). SSHL, sudden sensorineural hearing loss; HC, healthy controls; L, left; R, right; lFG, left fusiform gryus; lPG, left precentral gyrus; rIFG, right inferior frontal gyrus; lIFG, left inferior frontal gyrus; lSFG, left superior frontal gyrus; rMTG, right middle temporal gyrus.

TABLE 2 Comparison of static and dynamic fALFF between the SSHL and HC Groups (voxel-level $p < 0.001$ and GRF corrected at cluster-level $p < 0.01$).

Brain Regions	Voxels	MNI coordinates			*T* values
		X	Y	Z	
Static fALFF (SSHL vs. HC)					
Left fusiform gyrus (lFG)	37	−30	−36	−33	−4.6
Left inferior frontal gyrus (lIFG)	47	−12	39	−21	4.2
Right middle temporal gyrus (rMTG)	21	51	−24	−18	4.6
Left inferior frontal gyrus (lIFG)	45	−45	24	9	3.8
Right inferior frontal gyrus (rIFG)	44	60	18	12	−4.1
Left precentral gyrus (lPG)	14	−57	−9	27	−4.4
Left superior frontal gyrus (lSFG)	16	−21	18	63	3.7
Dynamic fALFF (SSHL vs. HC)					
Right superior frontal gyrus (rSFG)	14	18	45	30	4.0
Right middle frontal gyrus (rMFG)	16	15	9	63	3.8

MNI, montreal neurological institute; SSHL, sudden sensorineural hearing loss; HC, healthy control.

Conversely, the increased static fALFF in the left inferior frontal gyrus, left superior frontal gyrus, and right middle temporal gyrus suggests enhanced neural activity in these areas. The superior frontal gyrus is implicated in cognitive control, working memory, and attentional processes (du Boisgueheneuc et al., 2006; Briggs et al., 2020). Indeed, alterations in the intrinsic brain activity of the superior frontal gyrus have been reported in various conditions, including single-sided deafness (Qiao et al., 2022), Alzheimer's disease with depression (Mu et al., 2020) and left-sided long-term hearing impairment (Xie et al., 2019). The middle temporal gyrus is involved in auditory processing and language comprehension (Yu et al., 2022; Sugimoto et al., 2023). The increased fALFF in the superior frontal gyrus observed in individuals with SSHL is consistent with previous findings reported in the literature (Chen et al., 2020). The increased static fALFF in these regions may indicate compensatory mechanisms or heightened cognitive engagement in response to the hearing loss experienced in SSHL. The observed differences in static fALFF patterns suggest that individuals with SSHL may exhibit both hypoactive and hyperactive neural responses compared to HCs (Liu et al., 2021; Yao et al., 2021). These alterations could be associated with compensatory mechanisms or functional changes in response to the hearing loss.

Interestingly, when examining dynamic fALFF, SSHL patients only displayed significantly increased dynamic fALFF in the right superior frontal gyrus and right middle frontal gyrus compared to HCs. The middle frontal gyrus plays a role in working memory and attention (Briggs et al., 2021; Martin-Luengo et al., 2023). Static fALFF represents the fractional amplitude of low-frequency fluctuations calculated over the entire resting-state fMRI scan duration, providing a snapshot of regional spontaneous brain activity. On the other hand, dynamic fALFF captures the temporal variability of fALFF values

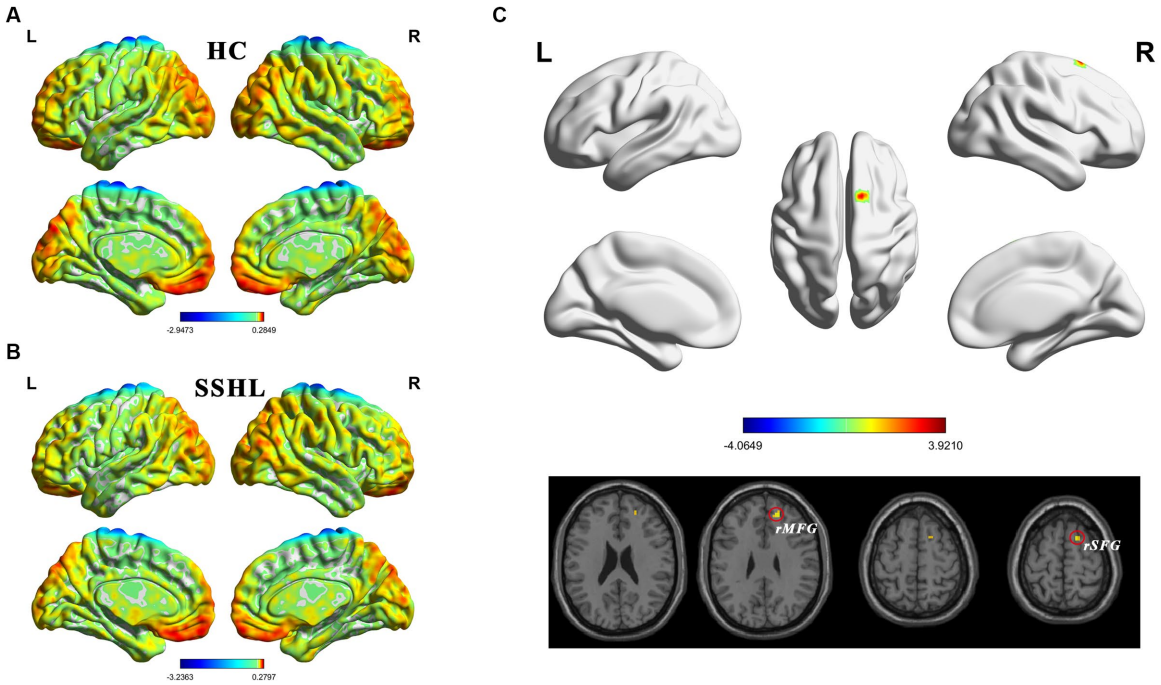

FIGURE 2
Altered dynamic intrinsic brain activity in unilateral SSHL. **(A)** Within group mean dynamic fALFF maps in the HC. **(B)** Within group mean dynamic fALFF maps in the SSHL. **(C)** Group comparisons of dynamic fALFF between the SSHL and HC groups (Gaussian random field correction; voxel-level $p < 0.001$, cluster-level $p < 0.01$). SSHL, sudden sensorineural hearing loss; HC, healthy controls; L, left; R, right; rSFG, right superior frontal gyrus; rMFG, right middle frontal gyrus.

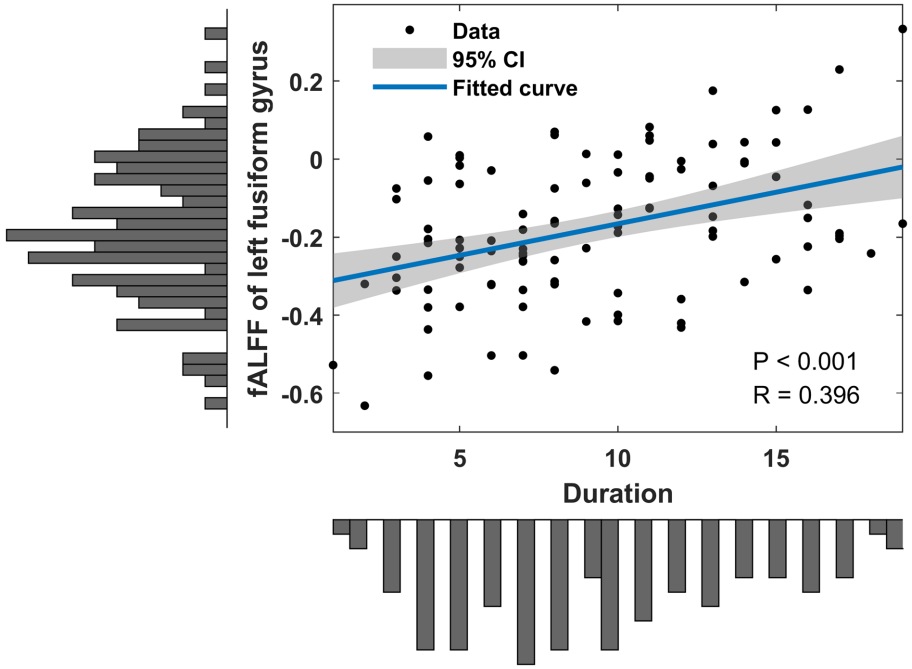

FIGURE 3
Clinical associations of altered static fALFF in SSHL patients. SSHL, sudden sensorineural hearing loss; fALFF, fractional amplitude of low-frequency fluctuation.

throughout the entire resting-state scan, offering insights into how brain activity dynamically changes over time. These findings indicate that SSHL patients exhibit altered temporal variability in neural activity specifically within these regions. Increased dynamic fALFF in

these regions has also been reported in individuals with Alzheimer's disease (Li et al., 2021), major depressive disorder with suicidal ideation (Yang et al., 2022), and amyotrophic lateral sclerosis (Ma et al., 2020b). These results suggest that dynamic fALFF analysis provides unique insights into the time-varying nature of intrinsic brain activity in SSHL. These dynamic alterations may reflect adaptive or compensatory mechanisms to cope with the hearing loss or could be related to the underlying pathophysiology of SSHL.

These findings collectively highlight the involvement of various brain regions in SSHL, indicating potential disruptions in sensory, cognitive, and attentional processes. The alterations in both static and dynamic fALFF provide valuable insights into the complex neural changes associated with SSHL, contributing to a deeper understanding of the condition. Overall, the study highlights the presence of both static and dynamic fALFF alterations in SSHL patients compared to HCs. These measures provide complementary information, allowing us to gain insights into both stable alterations in brain activity and dynamic fluctuations that may not be apparent in static measures alone. The findings suggest that SSHL is associated with disrupted intrinsic brain activity in multiple regions, involving both increased and decreased neural activity. The differential patterns observed in static and dynamic fALFF emphasize the importance of investigating both static and dynamic aspects of intrinsic brain activity to gain a more comprehensive understanding of the neural changes associated with SSHL.

Additionally, in our study, we observed positive correlations between static fALFF in the left fusiform gyrus and the duration of hearing loss in individuals with SSHL. This finding suggests that the severity or duration of hearing loss may be associated with altered neural activity in the fusiform gyrus, which implies a potential compensatory mechanism or adaptive changes in auditory processing associated with prolonged hearing loss. It is important to note that the positive correlation observed in our study does not imply a causative relationship, and further research is needed to elucidate the underlying mechanisms. Nevertheless, these findings provide preliminary evidence of the potential link between the duration of hearing loss and altered neural activity in the fusiform gyrus.

Despite the significant findings of our study, several limitations should be acknowledged. Firstly, the cross-sectional design of the study prevented us from establishing causal relationships between the observed brain activity alterations and SSHL symptom duration. Longitudinal studies are warranted to elucidate the temporal dynamics and progression of these changes over time. It would be essential to explore changes in brain activity over multiple time points during the course of SSHL to better understand their persistence or potential resolution over time. Secondly, our study focused specifically on investigating fALFF as an indicator of intrinsic brain activity alterations in SSHL. While fALFF is a valuable metric, we acknowledge that other functional connectivity metrics or brain imaging modalities, such as seed-based functional connectivity analysis or diffusion tensor imaging, could provide additional insights into the neural changes associated with SSHL. Further studies incorporating a broader range of imaging modalities may offer a more comprehensive understanding of the underlying pathophysiological mechanisms of SSHL. Thirdly, it is important to acknowledge that all patients included in our study exhibited unilateral SSHL. As SSHL is typically unilateral, with bilateral involvement accounting for less than 5% of the cases, we were unable to perform a subgroup analysis based on the bilateral side of SSHL. In future studies, if a sufficient number of bilateral SSHL cases are available, a specific analysis to investigate these aspects may gain deeper insights into the neural mechanisms associated with bilateral SSHL. Finally, the heterogeneity of SSHL, including variations in the etiology, duration, and severity of hearing loss, should be considered when interpreting the results. Future studies should consider these factors and incorporate more comprehensive assessments to further elucidate the complex mechanisms underlying SSHL.

Conclusion

In conclusion, our study on SSHL revealed significant alterations in both static and dynamic fractional amplitude of low-frequency fluctuation (fALFF) patterns, providing valuable insights into the intrinsic brain activity changes associated with the condition. SSHL patients exhibited decreased static fALFF in the left fusiform gyrus, left precentral gyrus, and right inferior frontal gyrus, indicating reduced spontaneous neural activity, while increased static fALFF was observed in the left inferior frontal gyrus, left superior frontal gyrus, and right middle temporal gyrus, suggesting heightened neural activity and potential compensatory mechanisms. Additionally, SSHL patients showed increased dynamic fALFF in the right superior frontal gyrus and right middle frontal gyrus, indicating altered temporal variability of neural activity within these frontal regions. Furthermore, a positive correlation was found between static fALFF in the left fusiform gyrus and the duration of hearing loss in SSHL, suggesting a potential association between hearing loss severity and altered neural activity in this region. These findings emphasize the complex nature of intrinsic brain activity changes in SSHL and provide insights into the functional reorganization and compensatory mechanisms that occur in response to hearing loss. Further research is needed to explore the functional significance of these alterations, develop targeted interventions, and optimize rehabilitation approaches for SSHL management.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Tongji Medical College of Huazhong University of Science and Technology medical ethics committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JL: conceptualization, data curation, formal analysis, methodology, visualization, writing – original draft. XY: methodology, data curation, formal analysis, software, validation, writing – original draft. YZ: data curation, investigation, methodology. YL: data curation, funding acquisition, resources, validation, writing – review and editing. FY: resources, supervision, validation, writing – review and editing. BL: conceptualization, funding acquisition, investigation, methodology, supervision, writing – review and editing. WF: conceptualization, funding acquisition, investigation, methodology, project administration.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1257729/full#supplementary-material

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Stem cells as potential therapeutics for hearing loss

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Hearing impairment is a global health problem. Stem cell therapy has become a cutting-edge approach to tissue regeneration. In this review, the recent advances in stem cell therapy for hearing loss have been discussed. Nanomaterials can modulate the stem cell microenvironment to augment the therapeutic effects further. The potential of combining nanomaterials with stem cells for repairing and regenerating damaged inner ear hair cells (HCs) and spiral ganglion neurons (SGNs) has also been discussed. Stem cell-derived exosomes can contribute to the repair and regeneration of damaged tissue, and the research progress on exosome-based hearing loss treatment has been summarized as well. Despite stem cell therapy's technical and practical limitations, the findings reported so far are promising and warrant further investigation for eventual clinical translation.

KEYWORDS

hearing loss, stem cells, exosomes, nanomaterials, clinical trial

Introduction

Hearing impairment is one of the most prevalent sensory disorders worldwide, affecting millions. Hearing aids and cochlear implants cannot restore normal hearing, warranting new therapeutic approaches (Lieu et al., 2020). Stem cell therapy has gained considerable attention over the years due to its substantial regenerative potential.

Depending on the location of the damage in the auditory system, deafness is divided into conductive and sensorineural types (Seddon et al., 2012). Conductive deafness occurs due to lesions in the tympanic membrane and the auditory tuberosity, which impede sound transmission to the inner ear (Lauer et al., 2019). On the other hand, sensorineural deafness is mainly the result of lesions in the auditory center, including the inner ear and the auditory nerve. HCs and SGNs are crucial in transmitting peripheral acoustic signals (Nayagam et al., 2011; Moser and Starr, 2016). However, mammalian cochlear HCs do not regenerate spontaneously after injury (Swan et al., 2008; Omichi et al., 2019). Causes of sensorineural deafness include noise, aging, drug cause hearing loss, genetics, bacterial and viral infections, immunological diseases, and endolymph fluid (Meniere's disease) (Wang and Puel, 2018; Plontke et al., 2022). Currently, induction of stem cell differentiation and replacement of damaged HCs and SGNs are increasingly considered feasible treatment options for auditory regeneration.

Historical overview of stem cell research

The origin of stem cell therapy dates back to 1888. when German zoologists Theodor Heinrich Boveri and Valentin Haecker introduced the concept of stem cells, they identified various cell populations in the embryo that could differentiate into specific cell types (Ramalho-Santos and Willenbring, 2007). In 1961, Till and Mc (1961) discovered that stem cells obtained from mouse bone marrow cells, which could differentiate into various cell types, and termed pluripotent stem cells (PSCs). Reynolds and Weiss (1992) isolated pluripotent neural stem cells (NSCs) from the forebrain of adult mammals in 1992. Thomson et al. (1998) first isolated human embryonic stem cells (hESCs) from embryos in 1998. In 1999, Pittenger et al. showed that bone marrow-derived human adult mesenchymal stem cells (BM-MSCs) can differentiate into multiple cell types, thus demonstrating the pluripotency of adult stem cells (ASCs) *in vitro*. BM-MSCs exist in almost all tissues and are crucial for maintaining tissue homeostasis through their self-renewal capacity (Pittenger et al., 1999; Tuan et al., 2003). Huawei et al. identified PSCs in the inner ear of adult mice and found that these cells could self-renew and differentiate into HC-like cells (HCLs) when cultured *in vitro* 16 (Li et al., 2003a). Takahashi and Yamanaka used the four transcription factors Oct3/4, Sox2, c-Myc, and KLF4 to transform mouse fibroblasts into induced pluripotent stem cells (iPSCs) for the first time (Takahashi and Yamanaka, 2006; Takahashi et al., 2007). This groundbreaking 2006 study paved the way for reprogramming mature somatic cells into a pluripotent state and opened new avenues for stem cell research. For this discovery, Shinya Yamanaka and John Gurdon received the Nobel Prize in Physiology or Medicine in 2012 (Figure 1) (Johnson and Cohen, 2012). Over the past decade, stem cell-based therapies have garnered considerable attention in hearing loss treatment.

The diversity of stem cells in regenerative medicine

Stem cells are a group of undifferentiated cells that can self-renew and differentiate into one or more cell types at different times of life (Ho et al., 2012; Hao et al., 2020). Based on their origin, stem cells can be categorized into various types, such as embryonic stem cells (ESCs), iPSCs, adult or somatic stem cells, and NSCs (Bongso and Richards, 2004; Ilic and Polak, 2011; Bond et al., 2015).

ESCs are pluripotent stem cells derived from the inner cell mass of blastocysts formed 5–6 days after fertilization (Evans and Kaufman, 1981). All three ectoderm, mesoderm, and endoderm germ layers can be differentiated from ESCs (Yao et al., 2006). ESCs can be obtained by culturing inner cell masses isolated from trophoblasts under specific conditions (Bongso, 2006).

IPSCs are produced by reprogramming mature somatic cells into ESC-like cells through genetic or chemical intervention (Yamanaka, 2012; Hockemeyer and Jaenisch, 2016). IPSCs are suitable models for investigating disease treatment, drug discovery, and regenerative medicine because they can self-renew and differentiate into various cell types (Ohnuki and Takahashi, 2015). Somatic cells can be reprogrammed to iPSCs by transducing them with the Oct4, Sox2, Klf4, and c-Myc transcription factors (Takahashi et al., 2007). In addition, certain chemicals or microenvironmental factors have also been used to stimulate the generation of iPSCs.

Adult or somatic stem cells are undifferentiated cells derived from various adult tissues with pluripotency, self-renewal, and limited differentiation potential (Zakrzewski et al., 2019). MSCs are the most common adult or somatic stem cells. Among them, BM-MSCs have limited differentiation capacity for osteocytes, chondrocytes, and adipocytes (Caplan, 2010). Although their differentiation capacity is limited, they exhibit anti-inflammatory properties and augment tissue regeneration (Ilancheran et al., 2009; Moodley et al., 2010).

NSCs have remarkable self-renewal and differentiation capabilities and continuously generate new neurons and glial cells (Xing et al., 2021). They play crucial roles in embryonic development and post-natal growth, particularly in the brain and spinal cord, wherein they help maintain neural tissue homeostasis and regenerative capacity (Shao et al., 2019). NSCs are also the seed cells for neural stem cell therapy and can promote nerve regeneration and restore function when implanted into damaged nerve tissue (Wang et al., 2019). The clinical applicability of NSCs is constantly being explored for treating neurological diseases.

Stem cell therapy for hearing impairment

Due to their capacity to differentiate into numerous cell types and repair tissues that have been damaged, stem cells may offer a promising treatment option for hearing loss (Bacakova et al., 2018; Camp et al., 2018). The ESCs, iPSCs, and ASCs have been tested for

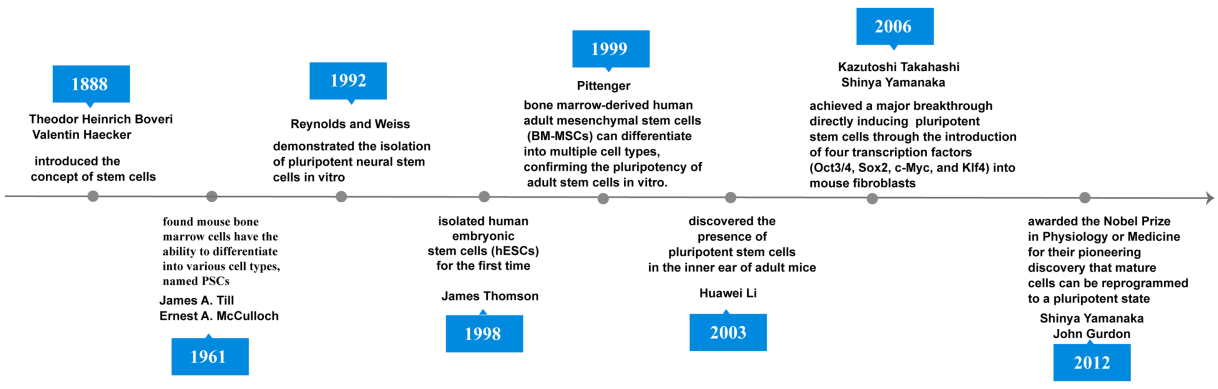

FIGURE 1
The timeline of major discoveries and breakthroughs in stem cell research.

treating hearing impairment (Boer et al., 2009; Stojkovic et al., 2021; Zine et al., 2021). Nevertheless, each variety has advantages and disadvantages concerning differentiated future potential applicability and immunogenicity (Figure 2) (He et al., 2021). In hearing loss research, stem cells have successfully generated HCLs *in vitro* (Li et al., 2003b; Takeda et al., 2018).

ESCs for treating hearing loss

Recent studies have shown that hESCs can be differentiated *in vitro* into cochlear sensory epithelial cells containing HCs using a three-dimensional culture system (Koehler et al., 2013). In addition, hESCs have also been differentiated into purified ear nerve precursor cells and spiral ganglion-like cells, which can survive for extended periods *in vitro* (Matsuoka et al., 2017). hESC-derived precursor cells transplanted into the cochlear region of Pou4f3DTR/+ mice with selective diphtheria toxin-induced HC ablation were viable and differentiated into HC-like and SC-like cells (Takeda et al., 2021). These findings suggest hESCs may be a potential treatment for hearing impairment and warrant further investigation.

IPSCs for the treatment of deafness

Recently, iPSCs have become known as a potential biological treatment for deafness. IPSCs derived from human urinary cells obtained from donors in good health were differentiated into HCLs with the morphological and electrophysiological characteristics of inner ear HCs. These HCLs established synaptic connections with the SGNs that were co-cultured. In addition, the transplanted iPSCs migrated to the organ of Corti site of resident HCs, differentiated into HCLs, and established synaptic links with the native SGNs (Chen J. et al., 2018). Somatic cells from patients with *myosin7a* and *myosin15a* mutations were also reprogrammed into iPSCs, and the mutations were corrected using gene editing techniques. Restoring gene function in iPSCs enabled differentiated HCLs to regain morphology and functioning (Chen et al., 2016; Tang et al., 2016). Similarly, iPSCs derived from patients with A8344G and *trmu* mutations in mitochondrial DNA were differentiated into inner ear HCLs. These cells exhibited normal electrophysiological properties after gene restoration (Chen Y. C. et al., 2018; Chen and Guan, 2022). These cellular models can elucidate the functional connection between inner ear HCs development and mitochondrial DNA. Additionally, human iPSCs derived from skin cells of patients with connexin 26 mutations, encoded by GJB2, were differentiated into auditory neural progenitor and hair cell precursor cells (Fukunaga et al., 2021). Connexin 26 mutations are a common cause of hereditary deafness. Overall, these findings provide novel insights and highlight potential therapeutic uses of iPSCs for treating hearing loss.

The therapeutic potential of MSCs for hearing loss

Although ESCs and iPSCs can differentiate into inner ear HCs, their application in medicine is limited due to the risk of tumorigenicity. Direct reprogramming of fibroblasts into HCLs of the inner ear could be a viable

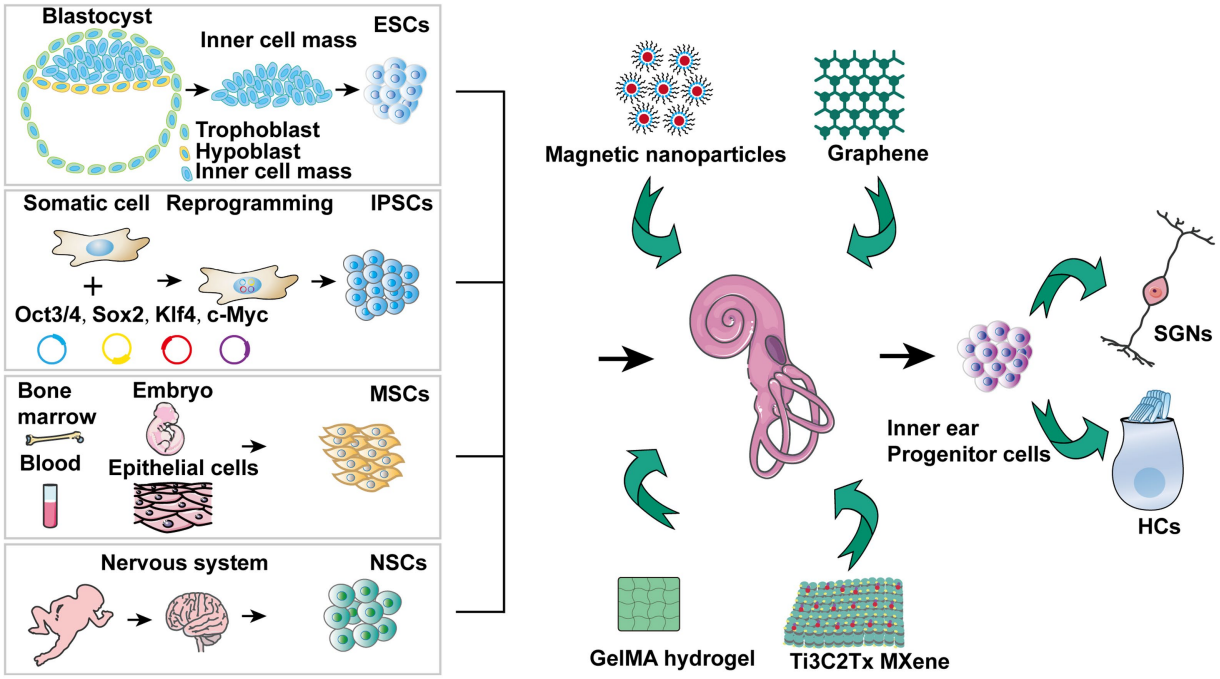

FIGURE 2
Stem cell treatment for hearing impairment mechanisms. 1) ESCs are able to differentiate into that resemble SGNs, SCs, and HCs, offering potential replacement strategies. 2) A particular strategy can be realized by stimulating iPSCs from patients who have hearing loss for developing into HCLs. 3) A range of growth factors and cytokines are secreted by MSCs, which may help prevent hearing loss. 4) NSCs together with nanomaterials hold promise for protecting against hearing loss. 5) The inner ear precursor cells are capable of being stimulated to develop into HCs and SGNs, offering another avenue for regeneration. 6) Exosomes derived from stem cells demonstrate potential in preventing sensorineural hearing loss.

alternative. Mouse Embryonic Fibroblasts can be differentiated into HCLs *via* mesenchymal-to-epithelial transition, followed by increased the expression of three important transcription factors, Sox2, Eya1 and Six1, to induce ear-sensory epithelial cell characteristics (Yang et al., 2021). Conductive hearing loss is commonly caused by cerumen embolism and chronic otitis media, leading to perforation of the tympanic membrane and erosion of the auditory ossicles due to recurrent infections. While the tympanic membrane can be regenerated using fascia or perichondrium, stem cells are essential for effectively enhancing hearing (Goncalves et al., 2017; Maharajan et al., 2020). In the rat model of subacute tympanic membrane perforation, bioprinted polycaprolactone/collagen/alginate-mesenchymal stem cell scaffolds have demonstrated efficacy and feasibility for subacute tympanic membrane regeneration (Jang et al., 2017). BM-MSCs have also been shown to promote healing in a chronic tympanic membrane perforation rat model (Shahal et al., 2022). Other ossicles or cartilage may be utilized to surgery restore hearing in cases of bone erosion. Additionally, MSCs have demonstrated promise in the therapy of conductive hearing loss (Maharajan et al., 2020). The resident MSCs protect the cochlear epithelium and prevent noise-induced hearing damage by secreting various growth factors and cytokines (Warnecke et al., 2021a). Moreover, pre-treatment of MSCs with deferoxamine can enhance their homing ability, which refers to the migration ability to damaged sites, through activation of the PI3K/AKT signaling pathway (Peyvandi et al., 2018).

NSCs for hearing loss

There have been considerable efforts in recent years to treat sensorineural hearing loss by inducing the regeneration of damaged auditory HCs and SGNs (Wang and Puel, 2018; Shu et al., 2019). The combination of nanomaterials and stem cells is a promising new therapeutic approach against hearing loss that combines the proliferation capacity of the stem cells with the tissue-targeting ability of the nanocarriers (Chang et al., 2020; Zhang et al., 2022). Several studies have demonstrated that stem cells and nanomaterials can support auditory regeneration by accelerating the repairing of damaged tissues (Zhong et al., 2016; Hu et al., 2021).

Graphene, a single layer of carbon atoms arranged in a hexagonal lattice, has been shown to play a critical role in tissue reconstruction (Kim et al., 2013; Akhavan, 2016; Guo et al., 2016). Autologous tissue grafts of perforated tympanic membranes can restore low-frequency hearing but often impair high-frequency hearing. In a rat deafness model, thin multilayer graphene membranes restored broadband hearing by inducing tympanic membrane repair (Li C. et al., 2022). In addition, the electrical stimulation device was developed by the combination of a cochlear implant and NSCs cultured on a graphene substrate. The machine was biocompatible and induced regeneration of NSCs in response to high-frequency, high-amplitude electroacoustic stimulation (Guo et al., 2021).

Magnetic nanoparticles are widely used in biomedical applications such as magnetic labeling, magnetic imaging, tumor treatment, and drug delivery due to their good biocompatibility (Lin et al., 2021; de Vincentiis et al., 2023). Superparamagnetic iron oxide nanoparticles can promote the proliferation of NSCs in a static magnetic field by enhancing cell cycle progression (Li et al., 2021). Furthermore, the directed growth of cochlear spiral neurons can be regulated by magnetic field-induced self-assembly of magnetic nanoparticles into multi-directional nanowires (Xia et al., 2022).

GelMA hydrogel is synthesized from methacrylic anhydride (MA) and gelatin. It is an ideal scaffold for 3D cell culture, tissue engineering, and biological 3D printing due to its excellent biocompatibility and visible light-curing properties (Fan et al., 2018; Cai C. et al., 2022). Composite scaffolds of super-aligned carbon nanotubes and GelMA promote the SGNs growth and orientation (Hu et al., 2022). Grooved GelMA-MXene enhanced the adhesion, differentiation, and directed proliferation of NSCs *in vitro* (Cai J. et al., 2022). Ti3C2Tx MXene, composed of transition metals, carbides, nitrides, or carbonitrides, exhibits a large surface area, adjustable surface functional groups, and good electrical conductivity (Rasool et al., 2016; Wu et al., 2021; Serles et al., 2022). It can enhance the proliferation and neural differentiation of NSCs, and promote the development of SGN growth cones and neurite growth by delivering electrical stimuli (Guo et al., 2022; Liao et al., 2022; Li Y. et al., 2022).

Inner ear progenitors for auditory regeneration

HCs and supporting cells (SCs) are critical inner ear components that arise from a common sensory progenitor. Inner ear progenitor cells are pluripotent cells with self-renewal ability that can differentiate into HCs under suitable induction conditions. During embryonic development, signaling pathway regulation plays vital roles in the formation of the organ of Corti. Activation of the Wnt pathway and inhibition of the Notch pathway promote partial regeneration of HCs (Mizutari et al., 2013; Shi et al., 2014; Li et al., 2015). Lgr5, a receptor of the Wnt pathway, is also a marker of cochlear stem cells (Chai et al., 2012; Shi et al., 2013; Bramhall et al., 2014). Under specific conditions, Lgr5-expressing Sertoli cells can transdifferentiate into HCs postnatally (McLean et al., 2017). Additionally, Sox2 is crucial for cell division and differentiation during development. Inner ear epithelial cells of Sox2 haploinsufficient mice showed increased differentiation and proliferation, resulting in expanded HCs and SCs and eventual regeneration of cochlear function. Sox2 haploinsufficiency also activates the cochlear Wnt pathway, further enhancing regeneration (Atkinson et al., 2018; Steevens et al., 2019).

The let-7 microRNA is a conserved activator that promotes proliferative quiescence and terminal differentiation by repressing CHD7, which controls progenitor cell behavior during cochlear development. Inhibition of let-7 in chicken auditory organ slices prolonged pre-sensory cell differentiation and proliferation (Evsen et al., 2020; Nie et al., 2022). In mice, the RNA-binding protein LIN28B promotes HC generation from auditory SCs *via* the mTOR pathway during embryonic development (Li and Doetzlhofer, 2020). The Yap-Lin28a axis can also activate Wnt signaling and promote inner ear cell regeneration by inhibiting let-7 expression (Kempfle et al., 2020; Ye et al., 2020). Knockdown of Foxg1 in neonatal mouse SCs promoted their transdifferentiation into HCs (Zhang et al., 2020). Furthermore, the Yap/Tead complex regulates a proliferation gene network in cochlear progenitors. Tead transcription factors directly bind regulatory elements of stem cell and cell cycle genes. In Sox2-positive cells, Yap as a Tead activator is rapidly degraded (Gnedeva et al., 2020; Currey et al., 2021). The transcriptional repressors TBX2 and TBX3 play essential roles in cochlear morphogenesis (García-Añoveros et al., 2022; Kaiser et al., 2022). Loss of Tbx2 causes cochlear hypoplasia, while Tbx3 mutants exhibit inner ear morphogenesis defects (Vitelli et al., 2003; Kaiser et al., 2021; Bi et al., 2022; Kaiser

et al., 2022). The transcription factor ATOH1 promotes HC differentiation by upregulating Pou4f3, which facilitates ATOH1 binding and activation of other target genes (Yu et al., 2021; Costa et al., 2022). As a transcriptional activator of Sonic hedgehog (Shh), Gli2 is negatively regulated by Suppressor of Fused Homolog (Sufu). Controlling Gli2 is critical for regulating cochlear HC differentiation, as Sufu inhibition can disrupt Atoh1 expression and delay differentiation (Yin et al., 2019; Qin et al., 2022). Overexpression of Rps14 in the mouse cochlea promotes SC proliferation by activating Wnt signaling and inducing HC regeneration (Xu et al., 2023). These studies show that co-regulation of the Wnt, Notch and Shh pathways promotes HCs regeneration, and provides a new insight for the potential application of HC regeneration.

Stem cell-derived exosomes have broad therapeutic prospects in hearing impairment

A class of small extracellular vesicles called exosomes that diameters ranging between 30 and 150 nm (Kalluri and LeBleu, 2020). Many cells, such as immune cells, cancer cells, and stem cells, can secrete exosomes (Yang et al., 2019; Cully, 2021). Exosomes derived from various cell types are highly heterogeneous. However, stem cell-derived exosomes have multiple mechanisms for repairing tissue damage, including promotion of cell proliferation and survival, enhancement of angiogenesis, and inhibition of inflammation and oxidation. For example, exosomes secreted by adipose-derived mesenchymal stem cells that are enriched in miR-25-3p induced neuroprotection through activation of autophagic flux (Kuang et al., 2020). The formation of exosomes through the endocytic pathway includes the following process: cytoplasmic membrane invagination, encapsulating some extracellular components and cell membrane proteins to form early endosomes (ESEs), followed by fusion between different ESEs to form late endosomes (LSEs), and further formation of multivesicular bodies (MVBs) (Chang et al., 2021). MVBs contain many intraluminal vesicles (ILVs) that may be released into exosomes (Han et al., 2022). MVBs are degraded by fusion with lysosomes or by fusing with the plasma membrane, releasing their substances, including ILVs, which are the final exosomes (Figure 3) (Kumar et al., 2020).

Exosomes deliver the vesicle's load, such as lipids, proteins, and other molecules, to the destination cells (Sun et al., 2020). Studies show that these exosomes can promote the regeneration of damaged tissues, modulate cellular immune responses, and reduce cellular inflammatory responses by activating specific signaling pathways (Dai et al., 2020; Ocansey et al., 2020; Xu et al., 2020; Cao et al., 2021; Isaac et al., 2021). Exosomes derived from stem cells can help regenerate neurons and synapses, alleviating the symptoms of neurodegenerative disorders (Vogel et al., 2018; Riazifar et al., 2019; Guo et al., 2020; Fayazi et al., 2021). Moreover, exosomes play an important role in cochlear sensory HCs protection. After stress stimulation, the cochlear SCs can release exosomes containing heat shock protein 70 (HSP70). To prevent the death of HCs, HSP70 takes a paracrine method to act on toll-like receptor 4 (TLR4) (Breglio et al., 2020; Muller, 2020). Another study showed that extracellular vesicles from human vestibular schwannomas are able to damage cochlear HCs and SGNs, leading to hearing loss (Soares et al., 2016).

Exosomes have also been demonstrated to protect against drug-induced hearing loss. For example, in response to cisplatin and other drugs *via* the HSP70 pathway, exosomes secreted by BM-MSCs reduced the apoptosis of mouse cochlear HCs (Park et al., 2021). Furthermore, human MSCs were able to regenerate SGNs and restore hearing in mice with autoimmune sensorineural deafness induced by β-tubulin through paracrine activity (Tsai et al., 2021). Furthermore, human MSCs were able to regenerate SGNs and restore hearing in mice with autoimmune sensorineural deafness induced by β-tubulin through paracrine activity (Yoo et al., 2015). Human MSC-derived extracellular vesicles also protect against noise-induced deafness in mice (Warnecke et al., 2020; Huang et al., 2023).

Clinical trials of stem cell therapy for deafness

Although multiple cellular and animal studies have demonstrated the security and feasibility of stem cell treatment for deafness, stem cell-based clinical trials for deafness treatment are still scarce. Alpha mannosidase deficiency is a rare genetic disorder that can lead to multi-organ dysfunction and cognitive deficits. One clinical study showed that five patients with α-mannosidase deficiency significantly improved their symptoms after transplantation of the allogeneic hematopoietic stem cells (Grewal et al., 2004). Blood cells, nerve cells, and cardiomyocytes can differentiate from umbilical cord stem cells. Studies have shown that after transplanting stem cells from autologous cord blood, auditory function is restored in children with acquired sensorineural hearing loss (Baumgartner et al., 2018; Sun and Yang, 2020). miR-22-3p, a microRNA relatively highly expressed in mesenchymal stem cell-derived exosomes, reduces inflammation by inhibiting expression of NLRP3. Additionally, mesenchymal stem cell-derived exosomes significantly inhibit expression of the pro-inflammatory factors TNF-α, IL-1β, and iNOS while promoting expression of the anti-inflammatory factor IL-10, thereby suppressing inflammation (Liu et al., 2020; Wang et al., 2023). In a clinical trial concluded in 2021, human umbilical cord MSCs-derived extracellular capsules were transplanted into the inner ear, reducing the inflammatory side effects caused by cochlear implantation (Warnecke et al., 2021b).

Conclusion

This review discusses the present status of the use of MSCs, ESCs, iPSCs, inner ear progenitor cells, and NSCs in the repair and regeneration of auditory impairment. MSCs are easily accessible and expandable and are, therefore, the most commonly used stem cell type. ESCs and iPSCs have strong differentiation potential, but their clinical application is limited due to ethical and safety concerns. Cells that can differentiate into cochlear HC and spiral neurons are inner ear progenitor cells, a type of ASCs. Although several preclinical and clinical studies have proved the therapeutic potential of stem cells in auditory impairment, Stem cell therapy also has some significant limitations, such as safety and feasibility. Specifically speaking, stem cell transplantation carries risk of tumourigenesis and immune rejection after transplantation, and existing delivery methods for stem cells can affect their therapeutic efficiency. In addition, the ethical issues also need to be addressed. In the future, the source of stem cells and the time and cell dosage for treatment will be optimized, More and more superior

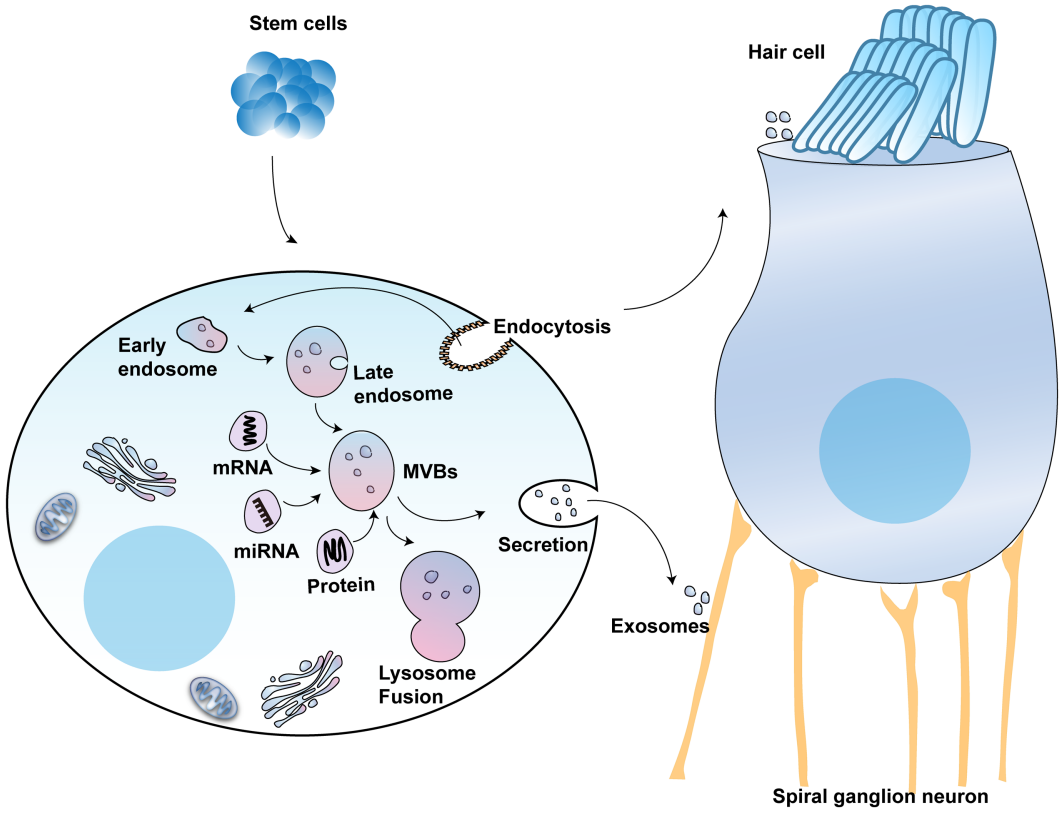

FIGURE 3
The potential use of exosomes in hearing protection: The schematic model shows the molecular composition of exosomes formed from stem cells, which include a wide range of cargo molecules, such as DNAs, RNAs, and proteins. Exosomes develop when the lipid bilayer membrane of MVBs undergoes an inward budding process. These MVBs can either be degraded by lysosomes or combined with the plasma membrane, releasing exosomes.

biomaterials and targeted delivery modalities will be developed. Overall, stem cell therapy is a brilliant way to restore hearing loss.

Author contributions

QF: Conceptualization, Writing – original draft. YW: Data curation, Writing – original draft. YuZ: Formal analysis, Writing – original draft, Data curation. WC: Resources, Writing – original draft, Formal analysis. LY: Writing – original draft, Funding acquisition. MK: Writing – original draft, Investigation. YoZ: Formal analysis, Writing – original draft, Methodology. YX: Writing – original draft, Project administration. LG: Writing – original draft, Resources. LZ: Project administration, Resources, Writing – original draft, Software. WW: Writing – original draft, Supervision. YY: Funding acquisition, Supervision, Writing – review & editing. JS: Funding acquisition, Validation, Writing – review & editing, Supervision. JY: Funding acquisition, Supervision, Writing – review & editing, Writing – original draft, Validation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Recent advances in genetic etiology of non-syndromic deafness in children

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Congenital auditory impairment is a prevalent anomaly observed in approximately 2–3 per 1,000 infants. The consequences associated with hearing loss among children encompass the decline of verbal communication, linguistic skills, educational progress, social integration, cognitive aptitude, and overall well-being. Approaches to reversing or preventing genetic hearing loss are limited. Patients with mild and moderate hearing loss can only use hearing aids, while those with severe hearing loss can only acquire speech and language through cochlear implants. Both environmental and genetic factors contribute to the occurrence of congenital hearing loss, and advancements in our understanding of the pathophysiology and molecular mechanisms underlying hearing loss, coupled with recent progress in genetic testing techniques, will facilitate the development of innovative approaches for treatment and screening. In this paper, the latest research progress in genetic etiology of non-syndromic deafness in children with the highest incidence is summarized in order to provide help for personalized diagnosis and treatment of deafness in children.

KEYWORDS

genetic hearing loss, deafness genes, non-syndromic, genetic etiology, children

Introduction

Congenital deafness is a common birth defect. It is estimated that there are 2–3 clinically deaf babies for every 1,000 births (Gaffney et al., 2010; Korver et al., 2017). Congenital deafness can be categorized into different levels of severity, namely mild (26–40 decibels), moderate (41–55 decibels), moderately severe (56–71 decibels), profound (71–90 decibels), and severe (greater than 91 decibels). In the case of children, approximately 29% experience unilateral hearing loss while bilateral hearing loss is present in about 71% (van Beeck Calkoen et al., 2019). The incidence rate of congenital permanent bilateral hearing loss is even lower, affecting only 1.2 per 1,000 births (Morton and Nance, 2006). Congenital deafness has multifactorial etiology and regrettably lacks a definitive cure. However, cochlear implants and hearing aids serve as the primary modalities employed to ameliorate the quality of life for children with auditory impairment, but these do not fundamentally restore hearing. Even with a cochlear implant, it produces hearing that mimics only a small part of the sound that can be perceived by a healthy ear. Congenital deafness contributes in part to children's speech impairments and social and cognitive impairments (Lieu et al., 2020). Early prevention and diagnosis of congenital hearing loss is critical, making universal newborn hearing screening of great value (Morton and Nance, 2006; Katbamna et al., 2008). In addition to conventional audiometry in screening, molecular genetic screening is evolving (Smith, 2004), making it particularly important to explore the molecular pathogenesis of congenital hearing loss.

Hearing loss can be caused by a variety of environmental and genetic factors, statistically equal parts of each (Gaffney et al., 2010). Among the environmental factors are infections, drugs or trauma. Vertical mother-to-child transmission is a common neonatal infection factor. Infectious viruses commonly transmitted to the fetus through teratogenic effects include TORCH (Toxoplasmosis-Other-Rubella-Cytomegalovirus-Herpes Simplex) (Nance, 2003). Among these, congenital hearing loss can be attributed to cytomegalovirus, rubella, herpes simplex, lymphocytic choroidal meningitis, and Zika virus infections (Macedo-da-Silva et al., 2020). Other environmental factors occurring at or after birth that can lead to hearing loss include hypoxia, prematurity, complications related to Rh factors in the blood, and bacterial meningitis. They usually result in a degree of sensorineural hearing loss that ranges from mild to severe. The hearing process involves at least 1% of human genes (about 300 genes), and alterations in any single gene or regulatory element can lead to hearing loss (Friedman and Griffith, 2003). The hereditary causes of congenital deafness are mainly categorized into syndromes and non-syndromes. The former accounts for 30% of cases with hereditary causes and is accompanied by other symptoms in addition to deafness, while the latter accounts for 70% of cases where deafness is the only symptom (Smith et al., 2005; Lammens et al., 2013).

The majority of cases involving hereditary auditory impairment are not accompanied by additional symptoms. Approximately 80% of non-syndromic cases can be attributed to autosomal recessive hearing loss (ARNSHL), while autosomal dominant hearing loss (ADNSHL) accounts for 20%, and X-linked or mitochondrial inheritance contributes to approximately 1–2% of the total number of cases (Hilgert et al., 2009). To date, 124 genes have been reported to be associated with nonsyndromic hearing loss.[1] ARNSHL is highly genetically heterogeneous, with approximately 89 genetic loci and 76 mutations associated with the etiology of ARNSHL (Imtiaz, 2022). The primary etiology of ARNSHL cases is attributed to genetic mutations in GJB2, while the gene SLC26A4 ranks as the second most prevalent factor associated with ARNSHL. Other ARNSHL-related genes include MYO15A, OTOF, CDH23 and TMC1 (Hilgert et al., 2009). Currently, approximately 70 loci and 51 genes are associated with ADNSHL, mainly including TECTA, WFS1, KCNQ4, COCH and GJB2 (Hildebrand et al., 2011). Five genes and six loci have been identified as being associated with X-linked nonsyndromic hearing loss, including PRPS1, POU3F4, SMPX, AIFM1and COL4A6(de Kok et al., 1995; Liu et al., 2010; Huebner et al., 2011; Schraders et al., 2011; Zong et al., 2015).

However, the primary pathologic mechanisms by which these genes contribute to hearing loss are still unclear. Therefore, we review the genes associated with nonsyndromic hearing loss in recent years. We explore the causes of congenital deafness and clarify their pathologic mechanisms, aiming to provide assistance in the clinical diagnosis and treatment of congenital deafness.

Genetic causes of deafness

Over 180 genes associated with deafness have been identified and investigated in past studies (Ma et al., 2023). Hereditary deafness can be categorized into four main groups based on their pathomechanisms: ciliopathy, neuropathy, synaptopathy, neuropathies, or homeostasis disorders.

Cochlear ciliopathy refers to a condition resulting from the disruption of normal ciliary function. These disorders, known as cochlear ciliopathies, affect the growth and maintenance of bundles of mechanosensory stereocilia on the apical surface of sensory hair cells that are crucial for auditory perception. Although stereocilia themselves are not classified as cilia, their proper development and alignment depend on primary cilia present in hair cells (Pollock and McDermott Jr., 2015); Auditory neuropathy is a condition characterized by impaired transmission of auditory signals from the cochlea to the brain, resulting from disrupted nerve impulses between the spiral ganglion and the central auditory pathway. Consequently, this disruption can lead to hearing loss. It is worth noting that disturbances in inner ear equilibrium may also contribute to auditory impairments. Cellular connections, ion channels, and their regulators are essential for the development of endolymphatic structures critical to auditory function. Moreover, neuropathy is characterized by aberrations in neural crest cell differentiation, which significantly impact the formation of various normal ear components including cartilage, auditory ossicles, cochlear glial cells, and interneurons involved in hearing (Ritter and Martin, 2019). All of these disorders may be recessively or dominantly inherited and result in hearing loss.

Individuals with nonsyndromic deafness have only symptoms of deafness. In most cases, the onset of non-syndromic deafness in patients with autosomal dominant inheritance occurs in the first to fourth decade of life (Van Laer et al., 1999). Autosomal recessive nonsyndromic deafness, on the other hand, is congenital or prelingual, and most of the time it leads to severe hearing loss (Sundstrom et al., 1999). Nonsyndromic deafness disorders can be classified by their locus names, with autosomal dominant abbreviations DFNA and autosomal recessive abbreviations DFNB, followed by a number representing the order in which they are found. Some DFNAs are located in the same spot on the chromosome as DNFBs because certain genes may carry both recessive and dominant variants. To date, there have been more than 180 loci associated with hearing loss reported, but only 121 genes associated with deafness are known. Several common genes associated with nonsyndromic deafness will be presented below (Table 1).

GJB2, *GJB3*, and *GJB6*

The connexin gene family is the most common gene contributing to hearing loss, with mutations in Connexin 26 being the major protein responsible for non-characteristic hearing loss and the most abundantly expressed connexin in the inner ear (Cohn and Kelley, 1999; Chai et al., 2022; Zong et al., 2023). Connexin 26 can bind to itself or to other connexins to form channels that connect exons. Two homologous or heterologous connexins on neighboring cells can form gap junctions through which ions and small molecules can pass for intercellular communication. The presence of connexin 26 proteins was demonstrated in Sertoli cells, as well as in sulcus and border cells within the organ of Corti, and also in the lateral wall of the cochlea, based on a rat study. Therefore, it can be inferred that both the epithelial gap junction system and connective tissue gap junction system exist within the cochlea (Kikuchi et al., 1995).

1 https://hereditaryhearingloss.org/

TABLE 1 Several common genes associated with nonsyndromic hearing loss.

Function	Gene	Location	Pubmed
Electrolyte recycling	*GJB2*	13q11-q12	8136828;37373495;37333892
	GJB3	1p35.1	9843210;37373495
	GJB6	13q12	10471490;37373495
	Connexin 26		9139825;37373495
Changes in ATP release and Ca + signaling	*SLC26A4*	7q31	8541853;36362242
	KCNQ1		36140355
	KCNA4	1p34	8035838;36140355
Auditory neuropathy	*OTOF*	2p22-p23	8789454;37189200
	DFNB59	2q31.1-q31.3	16804542
Cytoskeletal alterations of inner ear hair cells	*MYO7A*	11q12.3-q21	9171832;34979615
	MYO15A	17p11.2	7704031;37189200
	CLDN14	21q22	31781163
	DIAPH1	5q31	1350680;35060117

GJB2 encodes connexin 26 protein (Kiang et al., 1997). *GJB2* gene mutations can cause hearing loss, both recessive and dominant, mainly due to mutations in the *DFNB1A* or *DFNA3* motifs (Kelsell et al., 1997). Mutations in the *GJB2* gene lead to 50% of autosomal recessive non-syndromic hearing disorders in Europe (Al Mutery et al., 2022). Global research findings estimate the occurrence rate of autosomal recessive hearing loss caused by *GJB2* mutations to be approximately 16.9% (Chan and Chang, 2014), with Europe exhibiting the highest prevalence (27.1%) and sub-Saharan Africa displaying the lowest prevalence (5.6%). Distinct causative mutations have been extensively investigated in various populations, including 35delG in Europe, 235delC in East Asia, and W24X in India. These documented mutations could potentially be regarded as founding variants (Buonfiglio et al., 2020). One recent study showed the presence of *GJB2* or *GJB6* mutations in 38% of patients with non-syndromic hearing loss in Argentina (Rabionet et al., 2000). Many genetic alterations in *GJB2* causing deafness have been described so far (Figure 1).

In an animal laboratory study, it was observed that mice conditioned to interfere with *GJB2* exhibited significant hearing loss and displayed signs of hair cell apoptosis and degeneration in Sertoli cells. The process of apoptosis initiated shortly after the onset of auditory function, suggesting that exposure to sound stimulation may trigger this apoptotic response. A significant decrease in cochlear potential and endolymphatic potassium concentration was observed within the cochlea. It is hypothesized that the absence of connexin 26 disrupts the movement of K+ ions, impeding glutamate absorption due to elevated extracellular K+ levels, ultimately leading to apoptosis of hair cells (Teubner et al., 2003). Connexin 26-based gap junction channels facilitate the transfer of small molecules, such as glucose, ions, second messengers, and other substances. Consequently, *GJB2*-deficient mice exhibit prenatal mortality during the early to mid-pregnancy stage due to significantly compromised nutrient absorption capacity for essential substances like glucose and others, ultimately resulting in fetal demise (Gabriel et al., 1998). Deletion of Connexin 26, which is abundant in inner ear cells, also leads to impaired nutrient delivery to inner ear cells. All of these contribute to the development of hearing loss (Wang et al., 2023).

Connexin 31 and connexin 30 are encoded by the *GJB3* and *GJB6* genes, respectively. These genes have been implicated in hereditary hearing loss. The etiology of autosomal dominant deafness, known as *DFNA2B*, associated with mutations in the *GJB3* gene remains unknown. Conversely, mutations in the *GJB6* gene can result in both autosomal recessive deafness (*DFNB1B*) and autosomal dominant deafness (*DFNA3B*) (Soleimani et al., 2001). Mice lacking *GJB6* exhibit significant structural hearing damage, while their cochlear and vestibular end-organs develop normally. These mice experience a lack of cochlear potential from the onset of hearing, which is crucial for high sensitivity in mammalian auditory systems. After 18 generations, initiation of apoptosis was observed in sensory epithelial cells within the cochlea, suggesting that *GJB6* plays a critical role in generating intracochlear potentials and maintaining survival of auditory hair cells once hearing has commenced (Bidart et al., 2000).

However, the underlying mechanism of how mutations in the *GJB* gene lead to deafness remains unclear, and several current findings contradict previous hypotheses, which remain to be clarified by further studies.

SLC26A4, *KCNQ1*, and *KCNQ4*

The gene *SLC26A4* is responsible for encoding pendrin, a transmembrane transporter that facilitates the transport of anions (such as chloride ions, iodide ions, and bicarbonate ions) across cellular membranes (Soleimani et al., 2001). This gene exhibits elevated expression levels in specific organs including the cochlea, thyroid gland, and renal tubules, Its mutations can lead to autosomal recessive inheritance (Bidart et al., 2000). Pituitary adrenocorticotropic hormone dysfunction leads to impaired hearing in patients with Pender syndrome (PDS) and non-syndrome associated with dilated vestibular aqueduct (*EVA*) (*DFNB4*) (Campbell et al., 2001). *SLC26A4*-associated hearing loss is associated with inner ear malformations, hearing loss, vestibular dysfunction, and thyroid abnormalities (Honda and Griffith, 2022). *SLC26A4* deficient mice exhibited profound hearing loss and impairment in their vestibular system (Everett et al., 2001). The underlying molecular mechanism responsible for the enlargement of endolymphatic fluid may be attributed to the compromised ability to absorb ions and water from the inner cavity during the development of endolymphatic sacs (Choi et al., 2011). The study revealed that the *SLC26A4* knockout mouse model exhibited a more pronounced auditory and inner ear phenotype compared to individuals with hearing loss associated with *SLC26A4* (Lu et al., 2011; Wen et al., 2019).

KCNQ, a cluster of potassium channels, is associated with various medical conditions such as auditory impairment and cardiac arrhythmia (Robbins, 2001). The *KCNQ* protein is a membrane potential-dependent voltage-gated channel that can be activated upon

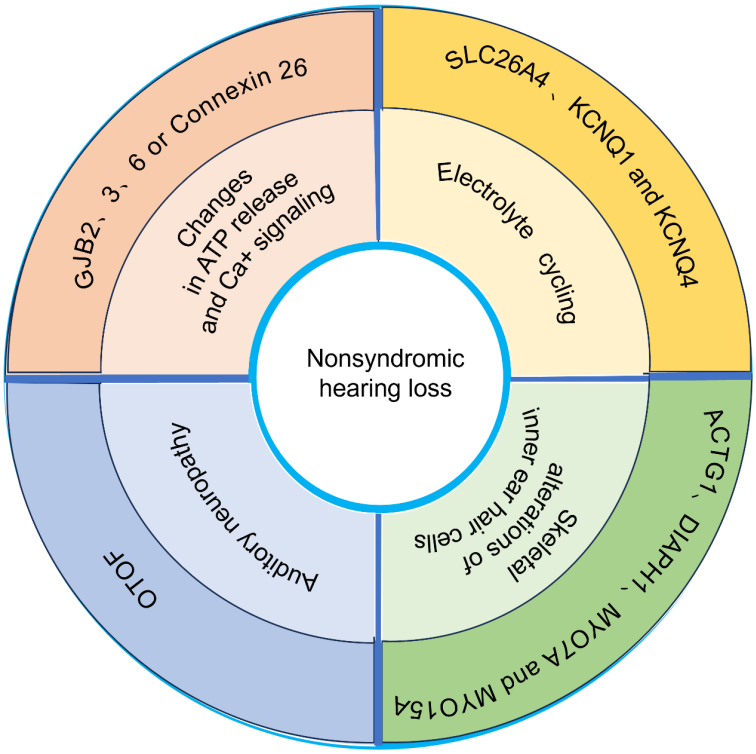

FIGURE 1
Schematic representation of several common genes associated with nonsyndromic hearing loss.

cell membrane depolarization. The normal functioning of auditory perception relies on the presence of *KCNQ1* and *KCNQ4*, two pivotal members within the *KCNQ* family, they can cause autosomal dominant deafness. This underscores the critical role of potassium in maintaining fluid homeostasis and its dynamic nature within the inner ear (Reardon et al., 1993; Nie, 2008). Kv7.1 and Kv7.4 are potassium channels that undergo voltage-gating, which are encoded by *KCNQ1* and *KCNQ4* genes, respectively. While *KCNQ1* has been associated with both cardiovascular diseases and hearing loss, *KCNQ4* is specifically linked to auditory impairment (Maljevic et al., 2010). Interference with KCNQ1 gene expression in mice results in deafness and severe inner ear morphologic abnormalities (Rivas and Francis, 2005). *KCNQ4* is expressed in sensory outer hair cells, and mutations in its gene lead to *DFNA2* (Kharkovets et al., 2000). It allows potassium to flow out of the extracellular space to restore the cell to an excited state. In mice, disruption of *KCNQ4* channels leads to damage in the outer hair cells. The pathomechanism of hearing loss due to *KCNQ* is usually explained by dominant-negative inhibition or haploinsufficiency (Wang and Li, 2016; Homma, 2022).

OTOF and *DFNB59*

Another pivotal factor contributing to recessive hearing loss is the presence of mutations in the *OTOF* gene, commonly referred to as *DFNB9*. Multiple studies conducted across diverse regions have indicated that alterations in the pathogenic *OTOF* gene account for approximately 2.3 to 7.3% of cases involving recessive hearing impairment (Duman et al., 2011; Iwasa et al., 2019). The *OTOF* gene comprises 28 exons that encode diverse long and short isotypes (Yasunaga et al., 1999). Studies in affected families have shown that long isotypes are necessary for hearing function (Yasunaga et al., 2000). Pathogenic mutations in genes display a wide distribution, with the majority being unique to individual families. Furthermore, there have been instances of recurring variants identified across diverse racial populations. *Q829X* was found in the Spanish population and *p.R1939Q* in the Japanese population among the most common mutations (Iwasa et al., 2013). The *OTOF* gene encodes otoferlin, which is a key protein for synapses in auditory sensory cells (Yasunaga et al., 1999). Deficiency of otoferlin leads to impaired release of synaptic vesicles at synapses in inner hair cells, which manifests as auditory synucleinopathy (Pangrsic et al., 2010).

Cochlear cell abnormalities are the most common cause of hereditary sensorineural hearing loss. However, lesions other than those of the cochlea account for a sizable part of cases, up to 10% of which result in permanent hearing loss in youngsters. Otoacoustic emission (OAE), which is low-level sound produced in the cochlea as a result of mechanical activity of OHCs, and recording auditory brainstem response (ABR), which measures the electrophysiological response evoked by acoustic stimulation of the auditory nerve and brainstem, are clinical tests for sensorineural hearing impairment. With auditory neuropathy, the outer hair cells of the cochlea are still functional but the ABR is absent or badly distorted while the OAE is preserved. Auditory neuropathy affects the neurotransmission of auditory signals. The first gene associated with cochlear cell disease in humans is called DFNB59, and it is found on chromosome 2q31.1–q31.3. This gene was first identified as the source of four congenic families' autosomal recessive hereditary auditory neuropathy, and it

has since been linked to autosomal recessive inheritance in numerous other families (Delmaghani et al., 2006). Pejvakin, a DFNB59 product, plays a significant function in the physiology of auditory neurons and is expressed in all relays of the afferent auditory route from the cochlea to the midbrain (Delmaghani et al., 2006).

Pejvakin, a 352-residue protein, has been linked to the oxidative stress-induced proliferation of this organelle, according to studies. Peroxisomes play a vital role in homeostasis and defense against noise-induced hearing loss in the auditory system, as shown by pejvakin defective mice (Delmaghani et al., 2015). It is thought that the following factors play a major part in hearing loss. (1) Pejvakin deficiency results in peroxisome anomalies in cochlear hydroxy carbon after hearing onset, impairing the cochlear antioxidant defense and causing damage to auditory hair cells as a result of reactive oxygen species (ROS) (Delmaghani et al., 2015; Defourny et al., 2019); (2) A significant amount of phenotypic heterogeneity in hearing is caused by pejvakin loss in mice (Delmaghani et al., 2015); (3) The Pejvakin deficiency makes one more susceptible to low energy (Delmaghani et al., 2015); (4) Loss of pejvakin impairs the propagation of action potentials in the auditory pathway following controlled electrical and acoustic exposures, as shown by decreased E II wave amplitude and increased E II-E IV and ABRI-IV wave intervals in PIVK-mice. (5) Loss of pejvakin renders auditory pathway neurons extremely susceptible to exposure to mild, brief stimuli (Delmaghani et al., 2015). In conclusion, DFNB59 mutations harm sensory hair cells in addition to causing neurological abnormalities.

ACTG1, DIAPH1, MYO7A, MYO15A, CLDN14, and *GRHL2*

The cytoskeleton is a complex network of interconnected filaments and tubules that extend from the nucleus to the plasma membrane (Hoyt et al., 1997), consisting of intermediate filaments, microtubules, and actin filaments. Numerous genes associated with hereditary hearing loss have been linked to the cytoskeleton, including *ACTG1* (*DFNA20/26*), which encodes gamma actin; *DIAPH1* (*DFNA1*), which regulates actin filament polymerization; *ESPN* (*DFNB36*), involved in producing actin bundles; *RDX (DFNB24)*, facilitating connections between actin filaments and stereocilia. Additionally, several unconventional myosin-encoding genes such as *MYO7A* (*DFNA11, DFNB2, Usher syndrome 1B*), *MYO6* (*DFNA22* and *DFNB37*) and *MYO15A* (*DFNB3*) are also implicated.

The gene *ACTG1* is responsible for the production of gamma actin, predominantly found in the hair cells of the inner ear. Mutations in this gene have been associated with autosomal dominant inherited hearing loss (known as *DFNA20/DFNA26*) (van Wijk et al., 2003). Exposure to noise or aging can cause damage to the cochlear structure and disrupt various processes such as bundling, gelation, polymerization, or myosin movement within hair cells (Morin et al., 2009). Consequently, this impedes their self-repair capacity and leads to a gradual decline in auditory function over time. Recent findings from a DNA sequencing study suggest that defective gamma actin may impair proper formation of F-actin, thereby contributing to the pathogenesis of *ACTG1* mutations (Miyajima et al., 2020).

DIAPH1 plays a crucial role in the regulation of actin polymerization within the hair cells of the inner ear. The expression of DIAPH1 is predominantly observed in the inner pillar cells, as well as the basal and outer pillar cells of outer hair cells (Neuhaus et al., 2017). Mutations in *DIAPH1* are responsible for autosomal dominant hearing loss (*DFNA1*) (Lynch et al., 1997).

The unconventional myosin proteins encoded by *MYO7A* and *MYO15A* are implicated in Usher syndrome, accounting for approximately 50% of cases (Millan et al., 2011). Furthermore, mutations in the *MYO7A* gene can result in autosomal recessive nonsyndromic hearing impairment. Animal models such as Shaker-1 and headband mice carrying the *MYO7A* mutation have exhibited symptoms indicative of vestibular dysfunction, including hyperactivity, head shaking, and head twisting. These models also demonstrate progressive degeneration of the organ of Corti (Gibson et al., 1995). In hair mice with cephalic abnormalities, outer hair cells exhibit O-shaped stereocilia instead of V-shaped ones, while inner hair cells present giant stereocilia (Rhodes et al., 2004). These findings suggest that defects in stereocilia morphogenesis contribute to both vestibular dysfunction and deafness.

Autosomal recessive hearing impairment, known as *DFNB3*, is caused by mutations in the *MYO15A* gene. *MYO15A* plays a crucial role in the elongation and development of stereocilia and actin filaments. The interaction between retention factors and *MYO15A* is essential for maintaining the cohesion of stereocilia structures (Belyantseva et al., 2005). Mutations in the *MYO7A* gene were initially identified in Indonesian families, resulting in shortened stereocilia associated with cochlear and vestibular dysfunction (Anderson et al., 2000). These findings suggest that alterations caused by *MY015* may disrupt both the structural integrity and functional capabilities within the sensory epithelium.

Mutations in the *CLDN14* gene are responsible for *DFNB29*, an autosomal recessive nonsyndromic hearing loss disorder (Ben-Yosef et al., 2003). The expression of *CLDN14* is primarily observed in the cochlea, liver, and kidney. This gene encodes claudin-14, a tight junction protein that enhances trans-epithelial resistance by reducing cation permeability, particularly potassium ions. Insufficient production of claudin 14 leads to inner hair cell deterioration and rapid outer hair cell death (Lee et al., 2012).

GRHL2 exhibits widespread expression across various human tissues, including the prostate, thymus, kidney, lung, salivary gland, mammary gland, and digestive tract. Mutations in *GRHL2* have been associated with *DFNA 28* (Autosomal dominant nonsyndromic tone neuropathy hearing loss) (Peters et al., 2002; Petrof et al., 2014), resulting in notable phenotypic changes such as enlarged ear sacs, smaller or absent otoliths, malformed semicircular canals, insensitivity to acoustic stimuli, and impaired swimming maneuvers. In embryonic ear epithelial cells affected by *GRHL2* mutations, there is a significant reduction or elimination of claudin-b and EpCAM expression while exhibiting aberrant formation of the apical junction complex (Han et al., 2011).

Summary and outlook

In summary, the main pathological mechanisms of hearing loss may focus on the following: (1) Electrolyte cycling, including imbalance and impaired uptake of K^+, Na^+, and Cl^+, etc.(2) Changes in ATP release and Ca^+ signaling, which may lead to impaired development of the columnar cytoskeleton as well as cochlea development (Bobbin and Thompson, 1978; Kujawa et al., 1994;

Munoz et al., 1995) (3) Auditory neuropathy (mainly caused by mutations in the OTOF gene). (4) Cytoskeletal alterations of inner ear hair cells. Different genetic alterations lead to different pathologic phenotypes. The occurrence of hearing loss due to GJB2 mutations has been found to precede hair cell degeneration, as indicated by recent studies. This finding suggests that K+-cycling is unlikely to be the underlying mechanism responsible for GJB2-related hearing impairment (Liang et al., 2012).

Congenital hearing loss is the most prevalent congenital defect, affecting two to three out of every 1,000 newborns. The etiology of congenital deafness is multifactorial and can be attributed to both genetic and environmental factors. In recent decades, genetic studies on hearing loss have yielded valuable insights into the molecular basis, development, and function of the auditory system. Furthermore, recent technological advancements have significantly improved our ability to accurately diagnose various forms of hereditary deafness at a molecular level. At present, we can use Sanger sequencing, Gene chip and other hot spot mutation screening technology, Deafness gene targeted capture sequencing (Panel sequencing), Whole exome sequencing, Whole genome sequencing, Whole genome scanning, and other genetic testing methods (such as Multiplex Ligation-dependent Probe Amplification, MLPA) and other technologies to improve our early identification and diagnosis of hereditary deafness.

However, despite the numerous advancements made in this field, certain limitations exist. The timely detection and intervention of congenital hearing impairment in newborns can significantly enhance their linguistic and verbal development while also improving their motor, cognitive, and social capabilities. Therefore, it is imperative to prioritize active screening for hearing loss in infants as well as molecular genetic screening procedures. Identifying the etiology of congenital deafness in some cases will help to achieve the best therapeutic effect and personalize each etiology in the future.

Author contributions

YF: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. SH: Data curation, Formal analysis, Methodology, Software, Writing – original draft. SZ: Conceptualization, Project administration, Supervision, Validation, Visualization, Writing – review & editing. MC: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Potential application value of pigment epithelium-derived factor in sensorineural hearing loss

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The inner ear is a complex and precise auditory perception system responsible for receiving and converting sound signals into neural signals, enabling us to perceive and understand sound. However, the occurrence and development of inner ear diseases and auditory disorders, such as sensorineural hearing loss, remain a global problem. In recent years, there has been increasing research on the treatment of inner ear diseases and auditory regeneration. Among these treatments, pigment epithelium-derived factor (PEDF), as a multifunctional secretory protein, exhibits diverse biological activities and functions through various mechanisms, and has shown potential applications in the inner ear. This minireview comprehensively evaluates the performance of PEDF in sensorineural hearing loss in inner ear and its potential targets and therapeutic prospects.

KEYWORDS

pigment epithelium-derived factor (PEDF), therapeutic prospects, potential, auditory function, signaling pathway, sensorineural hearing loss

1 Introduction

Inner ear diseases, such as sensorineural hearing loss, inner ear ischemia and anoxia, and deafness can all involve damage to auditory function. Pigment epithelium-derived factor (PEDF), as a protein with cellular protection and anti-angiogenic properties, might offer new avenues for the treatment of inner ear diseases. The promising application of PEDF in the treatment of inner ear diseases has received much research attention. Researchers have attempted to apply PEDF to treat inner ear diseases through approaches such as gene therapy, protein delivery, and drug development. Experimental studies have allowed researchers to speculate that the application of PEDF could reduce the damage to inner ear cells, promote cell survival and regeneration, and improve auditory function. However, the application of PEDF in the treatment of inner ear diseases is still in its early stages, and further research and clinical trials are needed to verify its safety and effectiveness.

PEDF is a member of the serine protease inhibitor (SERPIN) superfamily and exhibits diverse biological activities; although it does not possess protein inhibitory function, hence its rich biological functionality. Initially, PEDF was thought to be a neurotrophic factor present in retinal pigment epithelial cells (Tombran-Tink et al., 1991). However, it was gradually recognized that PEDF is a potent anti-angiogenic factor that can effectively inhibit angiogenesis without affecting the structure and function of normal blood vessels. Additionally, PEDF possesses neurotrophic properties, exhibits anti-tumor effects, reduces oxidative stress, and improves immune function (Bernard et al., 2009).

PEDF is expressed not only in the eyes and brain, but also in many other tissues, such as the blood, liver, kidney, heart, and testis, playing an important role in maintaining and regulating microvascular homeostasis (Brook et al., 2019). Previous study indicated that PEDF is expressed in the inner ear of rats, suggesting its potential expression in the mammalian inner ear (Gleich and Piña, 2008). However, its expression level and pattern in the inner ear have not been confirmed.

The specific effects of PEDF on cochlear hair cells and spiral ganglion cells and its mechanisms are still unclear. Further exploration is needed to uncover the anti-inflammatory and neurotrophic effects of PEDF in the inner ear, as well as its underlying mechanisms.

2 The structure of PEDF and its diverse biological activities and functions

PEDF is a 50 kDa endogenous glycoprotein composed of 418 amino acids, encoded by the *SERPINF1* gene, located on human chromosome 17p13 and comprising 12 exons and 34 introns (Becerra et al., 1995). PEDF possesses two important domains: an anti-angiogenic domain composed of 34 amino acids (Asp44-Asn77) and a neurotrophic domain composed of 44 amino acids (Val78-Thr121; Becerra, 2006).

PEDF exhibits anti-angiogenic properties, thereby mitigating inflammatory reactions. Studies have shown that PEDF maintains vascular homeostasis and alleviates inflammation by inhibiting the proliferation and migration of endothelial cells, suppressing angiogenesis, and reducing vascular permeability (Yamagishi et al., 2007; Zhang et al., 2015; Ju et al., 2020). This is particularly important for the inner ear, because normal hearing requires appropriate blood supply and angiogenesis. The anti-angiogenic properties of PEDF help to maintain the balance of blood perfusion in the inner ear, (Gleich et al., 2008; Sheikpranbabu et al., 2009; Eslani et al., 2018) preventing excessive angiogenesis and inflammatory responses that could lead to vascular abnormalities and auditory impairment.

PEDF also exhibits anti-apoptotic and anti-oxidative stress properties. Inner ear cell damage and apoptosis are one of the main causes of hearing loss and inner ear diseases. Studies have found that PEDF can protect inner ear cells from damage and apoptosis through various mechanisms. It regulates cell apoptosis-related signaling pathways, for example, by inhibiting the nuclear factor kappa B (NF-κB) signaling pathway and modulating the expression of B-cell CLL/lymphoma 2 (Bcl-2) family proteins (Zhang et al., 2019; Zhao et al., 2022), thereby alleviating cellular stress responses and promoting cell survival and regeneration.

PEDF is also involved in regulating the differentiation and functional maturation of inner ear cells. Sensory cells and neurons in the inner ear are crucial for normal auditory function. Research has shown that PEDF can promote the survival and development of sensory cells and protect auditory neurons from damage. PEDF plays an important regulatory role in maintaining auditory function in the inner ear through mechanisms such as regulating cell signaling pathways and promoting cell differentiation.

In summary, PEDF inhibits oxidative stress, suppresses inflammatory reactions, has anti-apoptotic effects, promotes cell differentiation, and exerts neuroprotection in the inner ear, making it a potential candidate for the treatment of inner ear diseases and the restoration of auditory function. Researchers have begun to explore the applications of PEDF in the treatment of inner ear diseases and auditory regeneration, with some encouraging results.

3 Mechanisms of PEDF's biological functions

3.1 PEDF activates the AKT and Wnt signaling pathways thereby inhibiting oxidative stress reactions

Noise, aging, and ototoxic drugs can cause sensorineural hearing loss via cellular damage caused by oxidative stress reactions (Fujimoto and Yamasoba, 2014; Wu et al., 2022; Baek et al., 2023). Activation of the protein kinase B (AKT) signaling pathway is crucial for the survival of auditory hair cells during ototoxic damage (Jiang et al., 2006) and aging (Sha et al., 2010). In addition, AKT activation leads to activation of downstream mammalian target of rapamycin (mTOR). Studies have shown that upregulation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT signaling pathway contributes to increased hair cell survival rates in ototoxic hearing loss (Bu et al., 2022). Conversely, in noise-exposed mice with hearing loss, levels of PI3K-AKT were reduced (Fan et al., 2023). PEDF plays an important role in protecting retinal pigment epithelial cells from oxidative stress (Kim et al., 2021). In another study, PEDF in olfactory mesenchymal stem cells promoted phosphorylation of the PI3K/AKT/mTOR pathway members to minimize stress reactions after brain injury (He et al., 2021). Therefore, we speculated that sensorineural hearing loss might be reduced through PEDF-mediated activation of the PI3K/AKT/mTOR pathway, thereby decreasing oxidative stress reactions.

Activation of the Wnt (a portmanteau of int. and Wg, standing for "Wingless-related integration site") pathway has been shown to effectively restore cochlear hair cell-like cell regeneration in mice (Quan et al., 2023; Weng et al., 2023). Conversely, inhibition of the Wnt signaling pathway leads to increased apoptosis, heightened ototoxic damage to spiral ganglion neurons, and worsened hearing loss. The mechanism involves decreased expression of the apoptosis regulator TP53 induced glycolysis regulatory phosphatase (TIGAR) and increased levels of reactive oxygen species (ROS) after inhibition of Wnt/beta-catenin (Liu et al., 2019). Thus, activation of the Wnt pathway plays a crucial role in cochlear hair cell regeneration and spiral ganglion neuron repair. A study has shown that PEDF might reduce oxidative stress through the Wnt signaling pathway (Ma et al., 2017). Therefore, we speculated that PEDF might regulate oxidative stress status through the Wnt signaling pathway in cochlear hair cells or spiral ganglion neurons.

3.2 PEDF participates in downregulating inflammatory mediators through the MAPK and NF-κB signaling pathways, and suppresses inflammation

PEDF can improve retinal diseases, such as age-related macular degeneration, by effectively downregulating the mRNA expression of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, inducible nitric oxide synthase (iNOS), and IL-17a (Wang et al., 2013). In another study, PEDF reduced the expression of inflammatory cytokines, including monocyte chemoattractant protein-1 (MCP-1) and TNF-α, in retinal extracts, serum, in the culture medium of retinal Müller cells (Filleur et al., 2009).

In vivo and *in vitro* evaluation of the anti-inflammatory activity of PEDF in ApoE mice (mice lacking the apolipoprotein E gene),

revealed that PEDF significantly decreased the expression of phosphorylated extracellular regulated kinase (ERK)-mitogen-activated kinase (MAPK), p38-MAPK, and JUN N-terminal kinase (JNK)-MAPK. Additionally, overexpression of PEDF resulted in a significant reduction in the expression of inflammatory factors such as IL-1β, IL-6, TNF-α, MCP-1, and matrix metalloprotein-9 (MMP-9; Wen et al., 2017). Thus, there is hope that PEDF might have the potential to reduce inner ear inflammation by lowering the levels of inflammatory mediators.

Researchers have studied the role of the NF-κB signaling pathway in the rat cochlea, particularly its inhibitory effects on inflammation induced by cisplatin toxicity (Kaur et al., 2011). Overexpression of PEDF could restore the activity of the NF-κB pathway and NLR family pyrin domain containing 1 (NLRP1) inflammasomes (Zhao et al., 2022). These observations suggested that PEDF might inhibit inflammatory responses and reduce ototoxic damage in the cochlea.

3.3 PEDF might exert anti-apoptotic effects and promote cell survival and differentiation through the MAPK/ERK pathway

The MAPK/ERK pathway plays a vital role in maintaining cell survival and protecting spiral ganglion neurons from apoptosis, demonstrating neuroprotective effects (Lallemend et al., 2003). *In vitro*, PEDF has been shown to activate the MAPK/ERK pathway, thereby promoting the migration and invasion of tumor cells (Chen et al., 2021). Another study reported that the MAPK signaling pathway is involved in the activation of JNK, which can inhibit the cell apoptosis caused by noise-induced hearing loss (Hu et al., 2009). This suggested that PEDF might play a role in protecting hearing through the MAPK/ERK and JNK pathways.

3.4 PEDF has the potential to activate anti-apoptotic pathways and achieve neuroprotective effects

PEDF has the potential to promote neuronal differentiation, regeneration, and the survival and differentiation of stem cells, making it a promising candidate for regenerative therapies (Ramírez-Castillejo et al., 2006; Brook et al., 2020). In a study on traumatic brain injury, elevated levels of PEDF were observed, indicating a potential activation of its neuroprotective functions through the inflammatory response and neural proliferation after injury (Terzi et al., 2015). Emerging evidence suggests that PEDF might play a role in protecting neurons in neurodegenerative diseases. A recent analysis identified PEDF as a promising therapeutic agent for multiple sclerosis, based on its ability to enhance remyelination by increasing the number of oligodendrocyte precursor cells and mature oligodendrocytes (Hooijmans et al., 2019).

PEDF appears to mediate the neuroprotective effect on retinal progenitor cells by binding to its receptor, PEDF-R, and activating anti-apoptotic pathways, such as Bcl-2, and blocking the translocation of apoptosis-inducing factor (AIF) and photoreceptor cell death (Subramanian et al., 2013). The biological functions of PEDF might not operate independently, but rather through the interplay and intersection of multiple functions. Moreover, the neuroprotective effect might be based on its anti-apoptotic function.

Overall, PEDF exhibits various mechanisms in achieving its biological functions, including reducing oxidative stress reactions, suppressing inflammation, promoting cell survival and differentiation, and achieving neuroprotective functions through signaling pathways such as AKT, Wnt, MAPK/ERK, and NF-κB.

4 Application prospect and conclusions

In summary, PEDF has received widespread attention for its potential applications in the inner ear. PEDF is considered a promising therapeutic agent to treat inner ear diseases. Although inner ear diseases often result in hearing loss, PEDF's ability to alleviate cellular stress and inflammatory responses, as well as promote cell survival and regeneration, make it a beneficial option for treatment. PEDF's potential as an intervention target for oxidative stress regulation and neurotrophic intervention in some inner ear diseases has theoretical support in clinical applications. Experimental results have shown that the application of PEDF can alleviate cellular damage in the inner ear, and promote cell survival and regeneration, thereby improving auditory function. However, the specific mechanisms by which PEDF activates auditory hair cells and spiral ganglion cells in the treatment of inner ear diseases are still mostly unknown, and further research and clinical trials are needed to verify its safety and efficacy.

Author contributions

ZS: Writing – original draft, Conceptualization, Formal analysis, Resources. XL: Data curation, Investigation, Writing – original draft. GL: Investigation, Methodology, Supervision, Writing – original draft. YX: Formal analysis, Project administration, Writing – original draft. JM: Supervision, Validation, Writing – review & editing. WM: Funding acquisition, Project administration, Writing – review & editing. SH: Writing – review & editing, Supervision, Validation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Macrophage-related immune responses in inner ear: a potential therapeutic target for sensorineural hearing loss

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Hearing loss is the most common sensory disorder in human beings. Cochlear sensory cells are the basis of hearing. Cochlear sensory cells suffer from various acute or chronic injuries, such as excessive sound stimulation, ototoxic drugs, and age-related degeneration. In response to these stresses, the cochlea develops an immune response. In recent years, studies have shown that the immune response of the inner ear has been regarded as one of the important pathological mechanisms of inner ear injury. Therapeutic interventions for inflammatory responses can effectively alleviate different types of inner ear injury. As the main immune cells in the inner ear, macrophages are involved in the process of inner ear injury caused by various exogenous factors. However, its specific role in the immune response of the inner ear is still unclear. This review focuses on discusses the dynamic changes of macrophages during different types of inner ear injury, and clarifies the potential role of macrophage-related immune response in inner ear injury.

KEYWORDS

sensorineural hearing loss, hair cell, gene therapy, immune response, macrophage

1 Introduction

Hearing loss is one of the most common sensory functional diseases in humans. At present, as many as 1.5 billion people worldwide suffer from hearing loss. Sensorineural hearing loss (SNHL), an important type of hearing loss, resulting from dysfunction of the inner ear, auditory nerve, or the auditory central nervous system. The loss or dysfunction of cochlear sensory cells and spiral ganglion is the main cause of SNHL. Currently, the molecular mechanisms of SNHL has not been fully clarified. Recently, there is increasing evidence that the cochlear immune responses are directly involved in the process of inner ear injury and immune-mediated SNHL has been widely accepted. The inner ear has been recognized as an immune privileged organ due to the blood-labyrinth barrier that separates the cochlear microenvironment from circulation. Harris et al. (1997) observed direct contact between lymphocytes and macrophages in the endolymphatic sac. Fredelius and Rask-Andersen (1990) subsequently reported infiltration of immune cells into the cochlea following noise exposure. In addition, studies have shown the presence of resident

immune cells in the cochlea under normal conditions, this phenomenon has attracted renewed interest in inner ear immunity (Sato et al., 2008). These results suggest that the cochlea, like other organs, interacts with the systemic immune system through lymphatic drainage and vascular circulation to fight infection or exogenous injury. In recent years, a variety of inner ear immune cells have been identified, among which macrophages are the most abundant immune cells in the cochlea. Studies have shown that macrophages exist at different stages of cochlear development, and the presence of macrophages in the otic capsule was observed on embryonic day 10 (E10), suggesting that cochlear macrophages may originate in the embryo. In addition, macrophage precursor cells were detected in the mouse cochlea early after birth, and these precursor cells may be an important source of tissue macrophages (Dong et al., 2018). Although the origin and precise functions of cochlear macrophages are not clear, the presence of cochlear macrophages throughout life suggests that they are involved in maintaining cochlear tissue homeostasis. More specifically, cochlear macrophages not only participate in the development of the cochlea and maintain homeostasis, but also play an important role in the local immunity of the cochlea. Macrophage-mediated immune response is involved in a variety of acute and chronic inner ear injury processes, including noise, ototoxic drugs, viral infections, cochlear implant surgery, or age-related hearing loss. After cochlear injury, the number of cochlear macrophages increased significantly, and the morphology and phenotype of macrophages also changed significantly. Studies have shown that cochlear macrophages exhibit different phenotypes in different types of injury. In the acute injury of the cochlea, the increase of cochlear macrophages is mainly derived from monocytes in peripheral blood. However, in the chronic injury of the cochlea, the cochlea is mainly characterized by the differentiation and activation of resident macrophages. The activation of both infiltrating macrophages and resident macrophages can lead to changes in the local internal environment of the cochlea. Infiltrating macrophages can aggravate the occurrence of cochlear inflammation by directly contacting damaged cells or secreting cytokines. However, the exact role of macrophage-mediated immune response in the cochlea injury remains unclear.

In recent years, the immune response of the inner ear has been regarded as one of the important pathological mechanisms of inner ear injury. Therapeutic interventions for inflammatory responses can effectively alleviate different types of inner ear injury. In this review, we will discuss the dynamic changes of macrophages in the process of different types of inner ear injury, and try to clarify the potential role of macrophage-related immune response in inner ear injury.

2 Origin of cochlear macrophages

Traditionally, the inner ear has been considered to be an immune privileged organ. However, in recent years, a variety of inner ear immune cells have been identified, among which macrophages are the most abundant immune cells in the cochlea (Hirose et al., 2005; Okano et al., 2008). Macrophages are present at different stages of cochlear development. The presence of macrophages in the otic capsule was observed on embryonic day 10, suggesting that cochlear macrophages may originate from embryos. In the early postnatal period, macrophage precursor cells were detected in the cochlea, which may be an important source of tissue macrophages (Dong et al., 2018). In adult mice, the origin of macrophages in the cochlea is not fully understood. In peripheral tissues, bone marrow-derived precursor cells are the main source of tissue macrophages (Ginhoux and Jung, 2014). Shi (2010) have reported that the cochlear macrophages were differentiated from bone marrow-derived monocytes under steady-state conditions through bone marrow transplantation experiments in irradiated mice. In addition to macrophages, the cochlear immune cell population also includes granulocytes (3.1%), T cells (0.8%), B cells (0.4%), and natural killer cells (3.4%) (Matern et al., 2017). The cochlea of newborn mice has undergone remodeling during development, and the composition of its immune cells has also changed. Identifying the origin of macrophages in different developmental stages of the cochlea is crucial for understanding their role in cochlear homeostasis and disease formation.

3 Distribution of cochlear macrophages

Under steady-state conditions, macrophages are mainly distributed in the spiral ligament and stria vascularis of the lateral wall of the cochlea (Sato et al., 2008). In the spiral ligament, the macrophages were irregularly branched and mainly distributed in the lower region of the spiral ligament. This region is rich in type II and type IV fibroblasts, which are prone to pathological changes (Hirose and Liberman, 2003). During acute injury, the number of macrophages in the area adjacent to the scala tympani of the spiral ligament increases sharply (Miyao et al., 2008; Du et al., 2011). In addition, there are also abundant macrophages in the stria vascularis. In the stria vascularis, macrophages are mainly distributed around the capillaries, and the dendritic branches are consistent with the blood vessels, which together with the endothelial cells of the capillaries constitute the blood membrane labyrinth barrier (Zhang et al., 2012). Shi et al. showed that lipopolysaccharide-induced inflammatory response significantly increased vascular permeability and leakage, and destroyed the integrity of the blood membrane labyrinth (Jiang et al., 2019). In addition, macrophages are widely distributed in the cochlear nerve tissue. Near the nerve fibers of the cochlear axis, the dendritic protrusions of macrophages are the same as the nerve fibers. Studies have shown that macrophages in these regions are involved in the development of auditory nerves in addition to the inflammatory response in the cochlea (Brown et al., 2017). In the spiral limbus, macrophages and nerve fibers can pass through the habenula, but do not contact with the inner hair cells (Hirose et al., 2017). In the Rosenthal duct, macrophages are mainly distributed around the spiral ganglion. Dong et al. (2018) analyzed the number of macrophages in this region at different stages of cochlear development. The results showed that the number of macrophages region decreased with age in the spiral ganglion. Macrophages increase significantly when spiral ganglion cells are damaged, and aggregated macrophages can alleviate the damage of spiral ganglion to a certain extent. Under physiological conditions, there are abundant macrophages on the tympanic side under the basilar

membrane of the mature cochlea, which are distributed along the basilar membrane from top to bottom. Macrophages are immersed in the tympanic perilymph and in direct contact with basement membrane mesothelial cells. This unique distribution allows it to monitor changes in the basilar membrane and perilymph microenvironment. The number and morphology of macrophages in different turns of the basilar membrane are different (Hu et al., 2018). The macrophages in the apical of the basilar membrane were dendritic, with small cell bodies and long branches (**Figure 1A, a**). The macrophages near the middle turns were amoeba-like, the cell body became larger, and the branches became shorter and thicker (**Figure 1B, b**). The macrophages in the basal turns of the basilar membrane were round, with almost no branched protrusions (**Figure 1C, c**). In addition, there are also some differences in the expression patterns of macrophage immune molecules in different sections of the basilar membrane, suggesting that they play different roles in the immune response (Hashimoto et al., 2005; Yang et al., 2015). Basilar membrane macrophages are closest to sensory cells, so they are more likely to feel the damage of sensory cells. Studies have shown that these macrophages monitor changes in the basilar membrane microenvironment (Zhang et al., 2017).

4 Macrophage phenotype in acute cochlear injury

In recent years, there are increasing evidence that the inner ear immune response were involved in the acute injury of sensory epithelial cells caused by a variety of exogenous factors, including noise, ototoxic drugs, viral infection, cochlear implantation, and other injuries (Fujioka et al., 2006; Warchol et al., 2012; Verschuur et al., 2015; Tan et al., 2016). The immune response of the cochlea is a sterile inflammatory response without the participation of pathogens, death of hair cells and secretion of pro-inflammatory factors are one of the main factors that trigger the recruitment and activation of cochlear immune cells. The inflammatory response of the inner ear caused by acute cochlear injury is characterized by the infiltration of monocytes. Peripheral circulating monocytes are the main source of immune cells in the cochlea (Hirose et al., 2005). After cochlear injury, peripheral circulating monocytes enter the cochlea and differentiate into functional macrophages. Yang et al. (2015) observed the infiltration monocytes in the lateral wall of the cochlea after acoustic injury, and the infiltrated monocytes were transformed into macrophages. The activated monocyte-derived macrophages amplify the cochlear inflammatory response through antigen presentation and secretion of inflammatory factors, and participate in the process of sensory epithelial injury (Yang et al., 2015). Excessive noise stimulation can cause damage to hair cells, and the cytokines secreted by disintegrated hair cells and resident cells promote the recruitment of cochlear macrophages to the damaged area for local inflammatory response. In addition, after noise exposure, the expression of cochlear inflammation-related cytokines increased significantly, including chemokines, adhesion molecules, and macrophage migration inhibitory factors (Frye et al., 2019; Landegger et al., 2019). Chemokines are powerful inducers of macrophage activation and migration, and adhesion molecules can mediate macrophage recognition and adhesion. Studies have shown that the activated cochlear macrophages can secrete a variety of cytokines. In the acute phase of the inflammatory response of the inner ear after noise exposure, the expression of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) increased sharply, which played an important role in respond to tissue damage (Ladrech et al., 2007; Wakabayashi et al., 2010).

In addition, the recruitment and activation of macrophages were also observed in the ototoxicity model. In the mouse cochlear culture model, the number of macrophages in the sensory epithelial injury area was significantly increased after kanamycin treatment. The activated macrophages can quickly migrate to the damaged cells and protrude to engulf the damaged sensory cells (Hirose et al., 2017). Sato et al. (2010) also observed monocyte infiltration and macrophage activation in the basilar membrane and spiral ganglion cell injury area in an mouse ototoxic model. Lenoir et al. showed that macrophages are involved in amikacin-induced

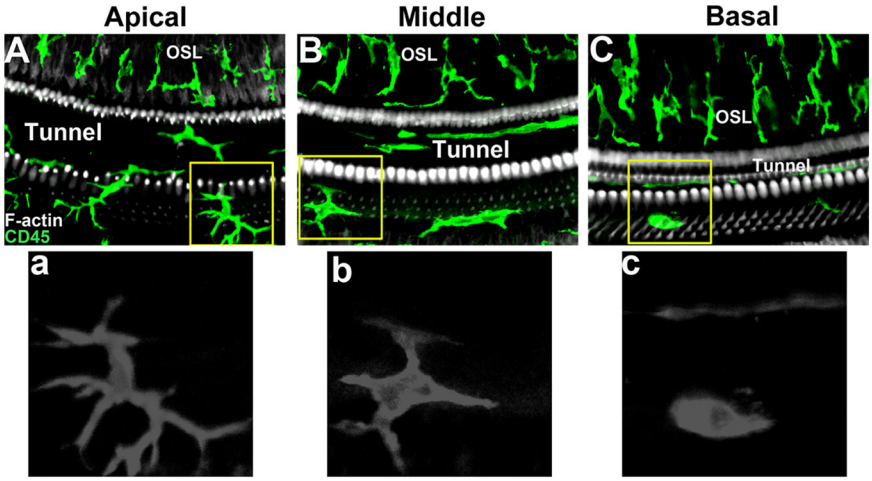

FIGURE 1
Macrophage morphology in different regions of mouse basilar membrane under steady-state conditions. **(A, a)** Apical turn of basilar membrane. **(B, b)** Middle turn of basilar membrane. **(C, c)** Basal turn of basilar membrane.

sensory epithelial injury in rats, and early anti-inflammatory treatment may promote the survival of supporting cells (Ladrech et al., 2007). Kaur et al. (2015b) constructed a diphtheria toxin receptor knock-in mouse model, and observed a large number of hair cells loss and the supporting cells were not damaged after injection of diphtheria toxin, accompany with the number of basement membrane macrophages increased significantly. It indicates that the pro-inflammatory signal released by the damage of hair cells is sufficient to activate the basement membrane macrophages, even if the macrophages cannot directly contact with the damaged hair cells. However, many macrophages appeared to actively phagocytose hair cell debris in the damaged area of sensory cells in the utricle of mice, suggesting that macrophages may be directly involved in the corpse clearance process of mammalian vestibular organs (Kaur et al., 2015a). In addition, cochlear implantation can cause transient damage to the cochlea and nerves. The electrode implantation was simulated on the animal model, and the inflammatory reaction of macrophages in the electrode injury area was involved in the repair and healing of the wound (Okayasu et al., 2019, 2020). In general, macrophage related immune response of inner ear has been regarded as one of the important pathological mechanisms of acute inner ear injury.

5 Macrophage phenotype in Chronic cochlear injury

At present, the understanding of macrophage-related inflammatory response mainly comes from the study of acute inner ear injury. The infiltration and activation of cochlear macrophages play a crucial role in the process of acute inner ear injury. In recent years, researchers have also observed the participation of macrophages in chronic cochlear injury models. There are many differences in the pathological mechanisms of acute and chronic cochlear injury. The degeneration of sensory epithelial cells progresses rapidly during acute injury, followed by monocyte infiltration and macrophage activation. On the contrary, the damage of cochlear sensory epithelium in chronic injury progresses slowly, and the cochlea shows chronic inflammatory response. In this process, the activation of cochlea resident macrophages was mainly observed, and macrophages differentiated from infiltrating monocytes into the cochlea are rarely observed. Hu et al. have reported the dynamic activation of basilar membrane resident macrophages response to chronic hair cell degeneration, and tissue-resident macrophages, not infiltrated monocytes, are the main executors of immune response (Frye et al., 2017). Notably, activation of basilar membrane resident macrophage precedes hair cell degeneration, and the activity of macrophages is maintained until sensory cell are completely degraded. In the cochlear specimens of the elderly, the number of activated macrophages around the auditory nerve was increased, and macrophages surrounded the myelinated axon fibers, suggesting that macrophages were involved in the degeneration of the auditory nerve (Fischer et al., 2020). In addition, after low-intensity noise stimulation, mice showed temporary hearing impairment and no death of sensory epithelial cells was observed. The number of macrophages in the basilar membrane and spiral limbus was still significantly increased, suggesting that macrophages could monitor the changes of cochlear microenvironment in the early stage of sensory epithelial injury or before death (Frye et al., 2018). Our recent study found that macrophage activation is involved in the pathological process of sensory cell and spiral ganglion injury induced by GJB2 gene knockout in mice, and CX3CL1-CX3CR1 signaling axis is involved in regulating the recruitment of macrophages in the inner ear (Xu et al., 2020). Previous studies have reported that acoustic injury causes the infiltration of circulating leukocytes into the cochlea. Zhang et al. (2021) revealed that CX3CR1 is involved in regulating the infiltration of neutrophils in the cochlea, lack of CX3CR1 results in the augmentation of neutrophil infiltration into cochlear tissues after acoustic trauma. Sato et al. (2010) pointed out that CX3CR1-deficient cochlear macrophages exacerbate kanamycin induced damage, suggest that CX3CR1 plays a role in modulating the cochlear macrophages after kanamycin ototoxicity. In addition, disruption of CX3CL1-CX3CR1 signaling reduced macrophage infiltration into both the sensory epithelium and spiral ganglion, and also resulted in diminished survival of spiral ganglion neurons (Kaur et al., 2015b). Jabba et al. (2006) also observed macrophage infiltration in the stria vascularis of the Pendred syndrome mouse model. Chronic inflammation is a crucial contributor to various age-related disease and natural processes in aging tissue, including the cochlear and nervous system. Verschuur et al. (2012) reported that the hearing threshold in the population was directly related to the key serum biomarkers of low-grade inflammation. Inflammatory cytokines such as IL-1α, IL-2, TNF-α and NF-κB play an important role in the initiation and regulation of chronic inflammation in the inner ear (Satoh et al., 2003, 2006). These data support that the chronic inflammation plays a role in mechanisms of age-related hearing loss, and there is still little known about inflammation in the ageing cochlea.

6 Function of macrophages in cochlear injury

6.1 Phagocytosis

Phagocytosis is one of the important functions of macrophages. Like macrophages in other organs, cochlear macrophages also have phagocytic function. They continuously monitor the dynamic changes of the cochlear microenvironment. Frye et al. observed macrophage phagocytosis of red blood cells on the surface of the mouse cochlea (Hu et al., 2018). Sensory cells of the cochlea are susceptible to external injury factors, resulting in apoptosis, necrosis, or mixed-mode death (Ylikoski et al., 2002; Bohne et al., 2007). The phagocytosis of macrophages is more obvious in the cochlear injury area. *In vitro* experiments by Hirose et al. (2017) observed that in the kanamycin-induced cochlear basement membrane injury model, activated macrophages can quickly migrate to the vicinity of damaged hair cells and protrude to phagocytose damaged cells. Kaur et al. (2015a) reported that macrophages were observed to be involved in the phagocytosis and clearance of damaged cell debris in a mouse utricle sensory cell injury model induced by diphtheria toxin targeting. However,

the Corti organ of the mature cochlea lacks macrophages under steady-state conditions. There is no evidence that macrophages can directly enter the Corti organ damage area and directly participate in the cleaning of damaged cells in the *in vivo* model. Studies have reported that Deiter cells are involved in the phagocytosis of damaged sensory cells and the filling of missing sensory epithelial cells (Abrashkin et al., 2006). As immune cells adjacent to the organ of Corti, the recruitment of macrophages on the tympanic side of the basilar membrane in the damaged area of the organ of Corti may play a role by secreting cytokines.

6.2 Secretion of inflammation-related cytokines

In recent years, a series of studies have shown that increased expression of inflammation-related cytokines in the cochlea has been detected in many different injury models (Cho et al., 2004; Gratton et al., 2011; Cai et al., 2014). In the cochlea, the interaction between inflammatory factors and macrophages is an important part of the cochlear immune response. Macrophages are involved in the mobilization and amplification of inflammatory response. When stimulated by exogenous stress, cytokines in the cochlea recruit macrophages to damaged tissues, and adhesion molecules promote macrophages to move slowly to the place where resident cells secrete cytokines. After acoustic injury, the expression of intercellular adhesion molecular-1 (ICAM-1) in the cochlea increased, and blocking ICAM-1 can inhibit the recruitment of macrophages and reduce noise-induced cochlear injury (Seidman et al., 2009). In the acute injury phase of noise stimulation, monocytes enter the cochlea and differentiate into macrophages, which can engulf and secrete more molecules, thereby mobilizing further immune response. After noise stimulation, the expression of inflammation-related cytokines in the cochlea, including intercellular adhesion molecule-1, TNF-α, chemokine CCL2, interleukin-6, and interleukin-1β, increased sharply after noise exposure and played an important role in the inflammatory response (Fujioka et al., 2006; Tan et al., 2016; Landegger et al., 2019). The application of RNA sequencing technology to the overall analysis of the transcriptional level of the cochlea under various pathological conditions has basically clarified the participation of a large number of immune-related molecules and multiple immune-related pathways (Patel et al., 2013; Yang et al., 2016). In addition, chemokine-chemokine receptor interactions, complement cascades, NOD-like receptor signaling, and TLR signaling are all involved in the immune response of the cochlea (Sato et al., 2008; Vethanayagam et al., 2016). Immune cells are the main source of immune-related cytokines. Pressure stimulates endothelial cells to present cell adhesion molecules and recruit macrophages to damaged tissues. Macrophages can engulf and secrete more immune factors, thereby mobilizing further immune response. Toll-like receptor 4 (TLR4) is a member of the pattern recognition receptor (PRR) family. Tlr4 deficiency inhibits the expression of major histocompatibility complex class II (MHC-II) in macrophages and reduces the antigen presentation activity of macrophages. However, the precise contribution of immune cells during inner ear injury remains unclear.

6.3 Antigen presenting

Under steady-state conditions, there are abundant immune cell populations in the cochlea, including macrophages, granulocytes, T cells, B cells and natural killer cells (Matern et al., 2017). Antigen presentation is an important part of immune activity, which occurs in the initial stage of immune response. It refers to the process of antigen presenting cells ingesting antigens to be processed, presenting on the surface of presenting cells in the form of immune peptides after a series of processing, and being recognized by immune cells to activate immune active cells (Steinman, 1991; Unanue, 1984). The main antigen-presenting cells include dendritic cells, macrophages, B lymphocytes and monocytes. Under steady-state conditions, macrophages in the cochlea express antigen-presenting molecules such as MHC II and CIITA. After excessive noise stimulation, a significant increase in activated macrophages was detected in the damaged area of cochlear sensory cells, and the expression of MHC II and CIITA in activated macrophages was significantly increased (Yang et al., 2015). In addition, the number of T cells is also increasing, and the increase of T cells indicates the activation of adaptive immune response. Our recent study found that in the model of sensory epithelial injury induced by GJB2 gene in targeted knockout mice, activated macrophages expressed MHC II molecules, and the number of MHC II positive cells increased significantly, suggesting that activated macrophages have potential antigen presentation function (Xu et al., 2020).

7 Application of anti-inflammatory therapy in the inner ear

In recent years, studies have shown that the treatment of inflammatory response can effectively alleviate different types of inner ear injury. Okano et al. reported that blocking IL-6 with anti-IL-6 antibody can improve the hearing of noise-exposed mice at a specific frequency. Histological analysis showed that the number of activated macrophages in the spiral ganglion region was significantly reduced and the damage of the spiral ganglion was significantly improved (Wakabayashi et al., 2010). Hu et al. showed that knockout of TLR4 gene can inhibit the function of macrophages in the inner ear. After noise stimulation, the hearing loss and hair cell death of TLR4 knockout mice were significantly reduced compared with wild-type mice (Vethanayagam et al., 2016). Mizushima et al. (2017) used the macrophage scavenger clodronate liposomes. The intervention of clodronate liposomes can significantly ameliorated noise-induced hearing loss in mice and protect the loss of outer hair cells (Mizushima et al., 2017). CCL2 and its main receptor CCR2 are important chemokines of monocytes. Hirose et al. reported that after noise stimulation, the hearing loss and hair cell death of CCR2 knockout mice were significantly reduced compared with wild-type mice. However, there was no significant changes in macrophage migration were observed, suggesting that CCR2 receptor is not necessary for situational cell migration (Sautter et al., 2006). However, the study of Kaur et al. (2015b) showed that knockout of CX3CR1 could significantly inhibit the recruitment of macrophages, and the damage of spiral ganglion

in CX3CR1 knockout group was aggravated, suggesting that the recruited macrophages could participate in promoting the survival of spiral ganglion. The chemokine CX3CL1 acts on the CX3CR1 receptor on the surface of macrophages to regulate macrophages, which has been reported in adipose tissue and skeletal system (Han et al., 2014; Shen et al., 2018). Sun et al. (2015) reported that inhibition of macrophage activation can reduce neomycin-induced hair cell loss and improve the hearing function of neomycin-treated mice. Benseler et al. used IL-1 blockers to significantly improve hearing in patients with Muckle-Wells syndrome (MWS), and the degree of hearing improvement was related to the time of treatment initiation (Kuemmerle-Deschner et al., 2015). Glucocorticoids have many effects such as anti-inflammatory, antitoxin and immune regulation. Systemic or local application of glucocorticoids is the main method for the treatment of SNHL, including sudden sensorineural hearing loss, noise-induced hearing loss, and autoimmune inner ear disease. Intratympanic injection of glucocorticoids can partially improve noise-induced hearing impairment and reduce hair cell apoptosis (Ozdogan et al., 2012; Müller et al., 2017). In recent years, steroids combined with other treatments is also a new treatment strategy. Han et al. (2020) showed that for some patients with refractory sudden deafness, nimodipine combined with steroids had better hearing improvement than single steroid therapy (Han et al., 2020). Prostaglandin E1 combined with glucocorticoid is recommended for the treatment of sudden sensorineural hearing loss patients with severe hearing loss in Japan (Kitoh et al., 2020). Eastwood et al. (2010) showed that injection of glucocorticoids through the round window can improve the high-frequency hearing loss caused by cochlear implantation injury. Ahmadi et al. (2019) reported that hydrogel-loaded dexamethasone can significantly protect the auditory nerve injury caused by cochlear implantation, but the specific mechanism is still unclear. The application of targeted gene therapy has opened up a new way for the treatment of inner ear diseases, through a variety of ways to achieve the expression of exogenous genes in the inner ear. Gene therapy focuses on the reconstruction of genetic materials through repair or gene modification to treat diseases, which is currently the most promising treatment strategy for genetic diseases (Jiang et al., 2023). Therefore, gene therapy based on immune response may be an important potential therapeutic target for sensorineural hearing loss.

8 Summary

In this review, we discuss the immune response and inflammatory activity after cochlear injury. Under steady-state conditions, the cochlea contains a variety of immune cells, of which tissue macrophages are the most abundant. The specific function of resident macrophages in the cochlea and the difference from circulating infiltrating macrophages need to be further explored. In different types of cochlear injury models, tissue macrophages exhibit a variety of morphological and gene expression patterns, indicating that they are in different functional states. The cochlea recruits circulating monocytes when it encounters external pressure, and these immune cells participate in the cochlear immune response together with cochlear resident macrophages (**Figure 2**). In addition, macrophage-related immune response not only plays a role in acute cochlear injury, but also studies have proposed the concept of inflammation as a mechanism of aging and age-related hearing loss. Macrophage-related immune response in the inner ear has been regarded as one of the important pathological mechanisms of inner ear injury. The role of macrophages in maintaining cochlear homeostasis and participating in inflammatory regulation is becoming more and more clear. In recent years, some progress has been made in the treatment of multiple models of inflammatory response. Macrophage-related inflammation will be used as a target for the treatment of inner ear injury. In the future, we look forward to the discovery of more targeted drugs based on inner ear immune-related molecules.

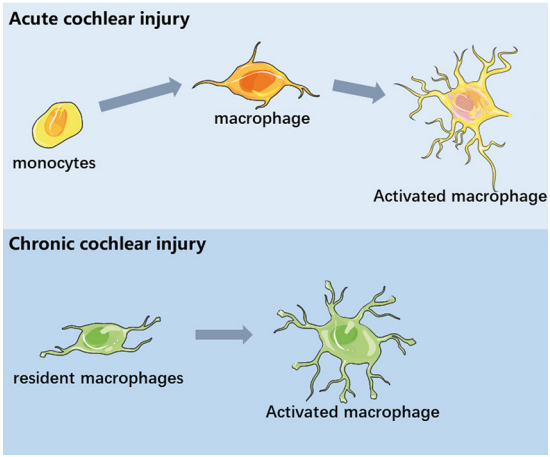

FIGURE 2
Schematic diagram of macrophages in different inner ear injuries. In acute cochlear injury, circulating monocytes infiltrated the cochlea and differentiate into functional macrophages. In chronic cochlear injury, the activation of basilar membrane resident macrophages is involved in the process of sensory epithelial injury.

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Conflict of interest

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Current advances in biomaterials for inner ear cell regeneration

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Inner ear cell regeneration from stem/progenitor cells provides potential therapeutic strategies for the restoration of sensorineural hearing loss (SNHL), however, the efficiency of regeneration is low and the functions of differentiated cells are not yet mature. Biomaterials have been used in inner ear cell regeneration to construct a more physiologically relevant 3D culture system which mimics the stem cell microenvironment and facilitates cellular interactions. Currently, these biomaterials include hydrogel, conductive materials, magneto-responsive materials, photo-responsive materials, etc. We analyzed the characteristics and described the advantages and limitations of these materials. Furthermore, we reviewed the mechanisms by which biomaterials with different physicochemical properties act on the inner ear cell regeneration and depicted the current status of the material selection based on their characteristics to achieve the reconstruction of the auditory circuits. The application of biomaterials in inner ear cell regeneration offers promising opportunities for the reconstruction of the auditory circuits and the restoration of hearing, yet biomaterials should be strategically explored and combined according to the obstacles to be solved in the inner ear cell regeneration research.

KEYWORDS

biomaterials, inner ear cell regeneration, hearing loss, hydrogel, conductive materials

1 Introduction

The inner ear is important in the management of hearing and balance. The cochlea, an intricate organ of hearing, is a delicate membranous labyrinth surrounded by dense temporal bone. Anatomically, the cochlea contains at least four functional domains including sensory epithelium, neuronal compartment, lateral wall, and immune cells (Milon et al., 2021). Among these compartments, hair cells (HCs) located on the sensory epithelium are the first-level receptors for auditory conduction, responsible for converting acoustic signals into electrical signals and transmitting acoustic information to the neuronal compartment (Sun et al., 2018). Spiral ganglion neurons (SGNs) located in the neuronal compartment receive the acoustic signals and transmit them upward to the central auditory system (Shrestha et al., 2018). The lateral wall consists of the stria vascularis and spiral ligament. They maintain blood flow to the cochlea and generate the endocochlear potential necessary for sensory HC transduction by secreting potassium ions into the endolymph (Zhang Y. et al., 2023). Immune cells maintain the stability of the inner ear environment and respond to inflammation in the inner ear (Zhang D.-G. et al., 2023). Multiple functional domains and various cell types in the cochlea interact to sustain hearing formation. These intricate and delicate structures make the cochlea a challenging target for both basic research and therapeutic intervention.

Hearing loss is a common sensory disorder in people and has a significant impact on reducing quality of life (Chadha et al., 2021). Sensorineural hearing loss (SNHL), one of the most common types of hearing loss, is caused by damage to inner ear HCs and SGNs (Wong and Ryan, 2015; Wang and Puel, 2018). HCs and SGNs in the inner ear are the core components involved in auditory information transfer (Coate and Kelley, 2013) and are susceptible to various external stimuli, such as noise (Chen et al., 2022), aging (Garg et al., 2021), ototoxic drugs (Wang et al., 2019), etc. Damage to mammalian HCs and SGNs could lead to permanent hearing loss, as they are terminally differentiated cells and cannot be self-regenerated (Daudet et al., 1998; Géléoc and Holt, 2014). Cochlear implantation (CI) helps to restore hearing in most patients with severe SNHL caused by HC damage (Naples and Ruckenstein, 2020). Nevertheless, the degeneration of SGNs may have a poor effect on CI (Webster and Webster, 1981) and there is currently no effective treatment for SNHL caused by SGN damage at present (Wang et al., 2022). Over the past few decades, important discoveries, including pharmacological therapies, genetic cell-based therapies, and biotherapies have led to the development of promising treatments for SNHL. However, there have been no approved medications or clinical therapies capable of rescuing or regenerating damaged HCs or SGNs.

Currently, stem/progenitor cell-based inner ear cell regeneration including inner ear organoids *in vitro* (Pouraghaei et al., 2020; Hocevar et al., 2021), elimination of the electrode-neuron gap after CI (Mattotti et al., 2015; Cai et al., 2016; Nella et al., 2022), and attempts at inner ear regeneration *in vivo* (Zhong et al., 2016; Chang H. T. et al., 2020) are some of the primary directions for inner ear regeneration research. Among these studies, the application of biomaterials and developments in bioengineering have also provided strategies to overcome the obstacles in inner ear cell regeneration (Brant et al., 2021). Biomaterials are defined as any natural or synthetic substance or a combination of substances that can interact with biological systems and can be used to improve biological function or life quality at any point of time (Marin et al., 2020). In recent years, biomaterial applications in the field of regeneration have been greatly developed owing to their excellent chemical versatility and biocompatibility (Bao et al., 2018; Liu et al., 2018). Biophysical signals transmitted by biomaterials, such as substrate stiffness, topography, mechanical forces and electric stimuli, have been proven to influence cell activity as well as cell fate determination (Shao et al., 2015; Yue et al., 2015; Ravikumar et al., 2017). For instance, some topical gels have been assessed to promote tissue regeneration and enhance diabetic foot ulcers (DFUs) wound healing in patients with diabetes (Bardill et al., 2022). Furthermore, Regranex gel has been approved by the Food and Drug Administration (FDA) as a growth factor mixture for clinical treatment of DFUs (Piascik, 1996). Biomaterials have also contributed significantly to the rapid advancement of both bone (Lee et al., 2018) and skin tissue regeneration technologies (Wu, 2021). Developments in bioengineering have also provided strategies to overcome obstacles in the process of inner ear cell regeneration (Brant et al., 2021). Several studies have reported that biomaterials play important roles in establishing inner ear organoids, promoting HCs and SGNs survival and regeneration, as well as enhancing the maturation and function of newly-generated inner ear cells.

In this review, we describe the recent advances in biomaterial applications for inner ear cell regeneration. We summarize the biomaterials that have been used for inner ear regeneration thus far, describe the advantages and limitations of these materials, analyze the mechanisms through which materials with different physicochemical properties act on inner ear cell regeneration, and depict the current status of material selection based on their characteristics to achieve the reconstruction of auditory circuits.

2 Biomaterials applied to inner ear cell regeneration

2.1 Hydrogels

Hydrogels have been widely used in stem cell bioengineering research (Liu et al., 2018), and according to our statistics, hydrogels may also be the most widely used biomaterial for inner ear cell regeneration. As hydrogels have excellent biocompatibility, bioactivity, and tunable mechanical properties (Liu et al., 2020), they can simulate the three-dimensional (3D) environment of the extracellular matrix (ECM) in which cells survive *in vivo* and always act as scaffolds to support cell growth in *in vitro* cultures (Evans et al., 2006). Matrigel is a kind of hydrogel extracted from the basement membrane of Engelbreth-Holm-Swarm mouse sarcoma cells (Arnaoutova et al., 2012). In addition to the shared advantages of hydrogels, Matrigel contains ECM components such as collagen, laminin, and nestin, as well as chemical cues for the maintenance of cell survival, such as growth factors, which provide complex tissue microenvironments that are more similar to those *in vivo* (Arnaoutova et al., 2012). Sarah et al. reported that matrigel was essential for the establishment of mouse embryonic stem cell (ESC)-derived inner ear organoids (Hocevar et al., 2021). Matrigel has been shown to promote neurite extension, maintain the electrophysiological function of purified SGN (Yan et al., 2018), and preserve the structure of SGN explant (Sun et al., 2016) *in vitro*. Matrigel has also been used to promote HC regeneration from Lgr5+ progenitor cells (Xia et al., 2023). However, batch-to-batch differences in the composition of Matrigel should be considered (Broguiere et al., 2018), which would inevitably lead to differences in cell culture results. Moreover, the elasticity modulus provided by Matrigel is insufficient to provide adequate support for cells (Soofi et al., 2009). To address these problems, hydrogels with defined components and improved mechanical properties have been investigated for their biological effects on inner ear regeneration (Rajasingh et al., 2017; Pouraghaei et al., 2020; Shi et al., 2023). One study demonstrated that Matrigel mixed with a certain ratio of alginate induced better differentiation of human gingival mesenchymal stem cells into auditory progenitor cells than Matrigel alone (Pouraghaei et al., 2020). Moreover, alginate modified by the Arg-Gly-Asp (RGD) sequence, a common cell adhesion peptide mainly derived from fibronectin, could also provide bioactive sites for cell-hydrogel interaction (Chikar et al., 2012). A single-component hydrogel crosslinked by self-assembling peptide amphiphiles with an Ile-Lys-Val-Ala-Val (IKVAV) epitope, another signal peptide epitope from laminin, has also been shown to promote the differentiation of hESCs into otic neural progenitors, as well as cell survival and localization after transplantation (Rajasingh et al., 2017). Interestingly, bacterial cellulose can also be fabricated into hydrogels for the differentiation and functional maintenance of SGNs (Shi et al., 2023). However, even if bioactive sites can be added or modified artificially, these single-component hydrogels are difficult to provide a complex

and anisotropic biological environment similar to that of basement membrane extracts, which is especially required for the establishment of organoids (Shi et al., 2023). Hydrogels could also be used as carriers for inner ear delivery (Zhong et al., 2016; Chang H. T. et al., 2020) and as coatings for other synthetic materials and implants to reduce cytotoxicity owing to their exceptional biocompatibility (Chen et al., 2017; Hu et al., 2022). Another advantage of hydrogels for *in vivo* inner ear regeneration is that they provide a stem cell niche for transplanted stem cells and limit their dispersion (Nayagam et al., 2012).

At present, commonly used hydrogels are temperature-sensitive, such as Matrigel (Arnaoutova et al., 2012), or are formed through a cross-linking agent, such as alginate (Pouraghaei et al., 2020). GelMA, a photopolymerized hydrogel, is often used as a micropatterned topographical hydrogel prepared by combining gelatin and methacrylic anhydride (MA; Yue et al., 2015). It can be crosslinked and solidified into a gel form using ultraviolet (UV) or visible light in the presence of photoinitiators, enabling the design and adjustment of micron-scale morphological features using photomask to guide the directional growth of axons of SGNs (Clarke et al., 2011; Tuft et al., 2014a,b; Xu et al., 2018). A previous study demonstrated that the combination of topological GelMA and chemical cues could better guide and promote the neurite extension of SGNs by coating laminin on the grooves to facilitate neurite growth while coating EphA4-Fc on the ridges to repel it (Truong et al., 2021). Study of neurite-directed regrowth is necessary to guide regenerated neurons to connect with HCs in an orderly manner so as to establish auditory circuits.

2.2 Conductive nanomaterials

As afferent auditory signals are transducted mechanoelectrically by cochlear HCs and then transmitted into the cochlear nucleus by SGNs, maturation of the electrophysiological functions of regenerated HCs and SGNs is paramount (Sun et al., 2018). The electroactivity of biomaterials has been applied to the regulation of cell fate determination, especially in regenerated excitable cells such as cardiomyocytes and neurons (Ning et al., 2018). The known conductive material graphene and its surface modification products have been reported to have broad applications in the behavioral regulation of neurons (Tu et al., 2013) and neural stem cells (Guo et al., 2021). Recent studies have shown that the combination of graphene oxide and hydrogels can promote the expansion of SGN growth cones and the outgrowth of neurites (Shi et al., 2023). Graphene substrates have also been revealed to promote the proliferation and HC differentiation of inner ear Lgr5+ progenitor cells, despite having no effect on cell viability (Ding et al., 2022). MXene, an emerging two-dimensional layered material with a structure similar to that of graphene, has been used in a wide range of applications since its discovery (Naguib et al., 2011) due to its excellent hydrophilicity and electrical conductivity (Naguib et al., 2021). MXene has recently been applied in the *in vitro* study of SGNs and HCs, and was shown to promote the elongation of SGN neurites while maintaining their electrophysiological properties (Zhang et al., 2022). Carbon nanotubes (CNTs), classical conductive material with high mechanical strength and high electrical conductivity, are generally categorized as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs; Mattson et al., 2000). CNTs have been shown to lead to the acquisition of longer and more aligned neurites in SGNs *in vitro* (Hu et al., 2022). In an interesting study combining CNTs onto butterfly wings with surface topologies, SGNs were not only able to grow directionally but also gained more mature synaptic functions (Wei et al., 2021). Nevertheless, in a biocompatibility study of CNTs, SGNs did not grow better when platinum electrodes were coated with CNTs even though the number of SGNs decreased in the SWCNTs group (Giugliano et al., 2016). This proves that CNTs are not well biocompatible; therefore, most of the conductive materials mentioned above have been mixed with hydrogels to improve their biocompatibility when applied. However, all of the above studies were conducted *in vitro*, and the roles of these materials in *in vivo* applications requires further investigation.

Conductive nanomaterials have also been studied as coatings for CI electrodes to explore the effect of appropriate electrostimulation on the neurite regrowth of damaged or degenerated SGNs, thus eliminating the electrode-neuron gap. Polypyrrole (Ppy), another conductive material, has been used as an electrode coating to facilitate electrical stimulation of SGNs (Richardson et al., 2009). Ppy is an electroactive polymer that composed of pyrrole monomers linked by negatively-charged dopants and exhibits electrical conduction based on its redox state without reducing the resistivity (Wallace and Kane-Maguire, 2002). Furthermore, Ppy is biocompatible and can be coupled with other bioactive materials; therefore, it can be used as an excellent substrate for cell adhesion (Richardson et al., 2007).

2.3 Superparamagnetic iron oxide

Superparamagnetic iron oxide (SPIO) nanoparticles (NPs) have been extensively studied as nanoscale drug carriers for nerve growth factors (Yuan et al., 2019) and have been reported to promote neuronal regeneration themselves (Yuan et al., 2018). Recent studies have demonstrated that SPIO NPs could be endocytosed by SGNs to promote the development of growth cones and neurite extension of SGNs with or without a magnetic field (Hu et al., 2021). In a study by Ye et al., SPIO NPs, after being endocytosed by mesenchyme stem cells (MSCs), induced the migration and homing of MSCs toward mouse cochleae under an external magnetic field, either by intratympanic or tail vein administration (Ahn et al., 2021). The movement of SPIO NPs relies on the magnetic field, a relatively less traumatic operation, which undoubtedly provides novel options for the application of stem cells in the inner ear as well as the neurite growth direction of regenerated SGNs.

2.4 Photomodulation and photo-responsive materials

Compared to biomechanical cues, electrical stimulation and magnetic fields, the application of light is more attractive because of its easier operation, minimal risk of trauma, and low toxic side effects (Yuan et al., 2019). Photomodulation initially applied to the inner ear demonstrated that 808 nm wavelength light emitting diode (LED) irradiation on the tympanic membrane of mice was effective in reducing ouabain-induced SGN degeneration (Lee et al., 2016). Following a study of HC differentiation from mouse ESC-developed organoids, 630 nm LED irradiation facilitated the differentiation of

HC-like cells (Chang S.-Y. et al., 2020). Additionally, some common biomaterials respond to light and contribute to neuroregeneration (Yuan et al., 2019; Zheng et al., 2022). For example, gold (Au) NPs altered the level of cellular permeability and ion transport through photothermal effects from localized surface plasmon resonance, which resulted in inducing neuronal proliferation and differentiation (Yuan et al., 2019). CNTs could also respond to light and generate ultrasound through the photoacoustic effect to stimulate neuronal growth (Zheng et al., 2022). Unfortunately, these photo-responsive materials have not yet been used for inner ear cell regeneration.

3 Mechanisms of biomaterials acting on inner ear cell regeneration

Almost all cells *in vivo* are housed in the ECM, which forms a complicated 3D microenvironment of fibers and interstitial spaces that provide abundant physical and biochemical cues and play an essential role in determining stem or progenitor cell fate and function (Arnaoutova et al., 2012). Therefore, it is crucial to select biomaterials with different properties to provide the most appropriate signals for the regeneration of cochlear cells (Figure 1). We analyzed the potential underlying mechanisms through which biomaterials may act on the inner ear regeneration process.

3.1 3D culture environment provided by biomaterial-based scaffolds

Scaffold-based 3D cell culture has now been applied in a broad range of fields, such as tissue engineering and regenerative medicine, and can simulate biophysical signals and biochemical cues provided by the ECM of the native microenvironment (Liu et al., 2018). Mechanical support, adhesion, and chemical gradients are key properties that are optimized in scaffold-based 3D culture systems and interact with each other to affect gene expression, cell signaling, and regulation of cell behavior. 3D culture systems are commonly created using hydrogels and are used for organoid establishment (Ishikawa et al., 2017). Composites of hydrogels and other scaffold materials have been reported to provide better mechanical support for the 3D culture of stem cells *in vitro* (Shi et al., 2023). The mechanical properties of the culture system, including bulk stiffness, local stiffness, and strain, have been considered extremely important for cell fate determination because mechanical stimuli can be converted into chemical signals that regulate gene expression (Bao et al., 2018; Jeon et al., 2021). The anisotropy of the structure and force direction provided by the scaffolds could influence cell morphology and even the expression of genes and proteins (Heo et al., 2011). For instance, when cultured in hydrogel-encapsulated scaffolds, nuclear localization of Yes-associated protein (YAP) was facilitated and the proliferation of the inner ear Lgr5+ progenitor cells was enhanced (Xia et al., 2020). In contrast, exposure to a conventional 2D culture plate, such as a stiff substrate of mouse embryonic fibroblasts, led to direct force transmission between the cytoskeleton and nucleus and disrupted the transport balance of the transcriptional regulator YAP in cells with flattened morphology (Elosegui-Artola et al., 2017).

The spatial distribution of adhesions around the cells, which link the intracellular cytoskeleton to the ECM, is also a prominent feature in the scaffold-based 3D culture system (Baker and Chen, 2012) and optimizes mechanotransduction from the ECM (Wang et al., 2009). Multidirectional ECM-cytoskeleton binding has been verified to be conducive to the formation of cellular actin-rich extension (Langevin et al., 2005), which may contribute to neurite growth. Hydrogels with modified cell adhesion-related signaling peptide epitopes, such as RGD (Chikar et al., 2012) or IKVAV (Frick et al., 2017; Rajasingh et al., 2017), have been reported to promote cell-hydrogel interactions and neuronal differentiation, which further demonstrated the importance of cell adhesion for inner ear cell regeneration.

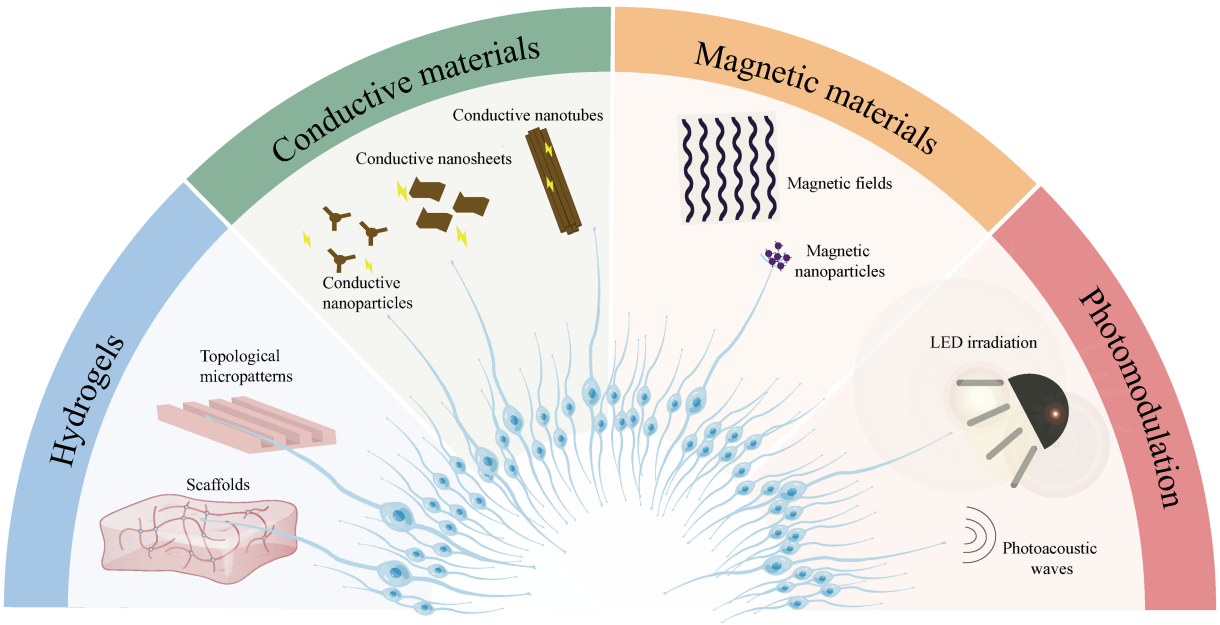

FIGURE 1
Schematic drawing of biomaterials used in the study of inner ear regeneration currently.

A gradient of biochemical cues, such as exogenous or autocrine cytokines and growth factors, was formed in scaffold-based 3D cultures because the presence of scaffolds slowed down the diffusion of biochemical cues (Baker and Chen, 2012). Chemical cues with spatial gradients act effectively on target cells rather than those with a homogeneous distribution, which is conducive to the differentiation process of stem cells and critical for the maintenance of differentiated tissues (Liu et al., 2015). Furthermore, organoids cultured with scaffolds could receive anisotropic signaling cues relying on their various spatial locations within the aggregates, which often leads to the asynchronous differentiation of cells located at different depths and contributes to organoid establishment *in vitro* (Koehler and Hashino, 2014).

3D cultures with various scaffolds allow cells to self-organize freely and develop more naturally (Koehler and Hashino, 2014). Stem cells or inner ear progenitor cells cultured in 3D scaffolds can be induced to generate otic organoids, resulting in SGNs and HCs similar to those in native cochleae (Hocevar et al., 2021; Sun et al., 2023). Electrophysiological evaluations confirmed the identity and maturation of the newly generated HCs as well as the co-cultured SGNs (Xia et al., 2023). Moreover, functional synapses, which are remarkable biological structures that allow communication between neurons and their targets, were formed between SGNs and HCs (Xia et al., 2023). These studies suggested that the otic organoids formed *in vitro* could mimic the developmental pattern of cochleae *in vivo*. The establishment of functional inner ear organoids is of great significance to further investigate the molecular mechanisms involved in the development of the inner ear as well as facilitate studies on hearing disorders and drug screening (Koehler and Hashino, 2014; Munnamalai and Fekete, 2017).

3.2 Biomaterials-based coating for CI interfaces

CI technology has restored partial hearing in patients with SNHL over the past few decades; however, challenges still remain (Naples and Ruckenstein, 2020). Research on coating materials for CI electrodes has never stopped, and these coating materials, especially topologically structured (Clarke et al., 2011; Tuft et al., 2014a; Mattotti et al., 2015; Cai et al., 2016; Leigh et al., 2017; Xu et al., 2018; Truong et al., 2021) or conductive materials (Wei et al., 2021; Ding et al., 2022; Hu et al., 2022; Liao et al., 2022; Zhang et al., 2022), can be used as interfaces to promote axonal regrowth of degenerated SGNs.

The surface of GelMA can be designed by photomasking to form topological micropatterns with grooves and ridges, which guide the neurites to track the patterns closely and align strongly (Clarke et al., 2011). A study on neural stem cell found that GelMA with microgrooves may promote the directed extension of neurites by enhancing the expression of cell adhesion factors (Cai et al., 2022). Many other photopolymerized materials have been fabricated in the same way to control the spatial alignment of regenerated SGN neurites through surface topological patterns, which were expected to realize the precise connection of regenerated neurons to their targets (Tuft et al., 2014a; Leigh et al., 2017; Xu et al., 2018; Truong et al., 2021). Similarly, the topological microstructure of silicon micro-pillar substrates (Mattotti et al., 2015) and topographically modified nanocrystalline diamond (Cai et al., 2016) were also confirmed to play roles in guiding the orderly arrangement of SGN neurites while promoting their adhesion.

Electrical stimulation combined with conductive materials has also been shown to facilitate the extension of SGNs and promote the neuroelectric activity (Liao et al., 2022). Although the exact mechanisms through which electrical stimulation promotes neuronal regeneration are still unclear, the role of ion exchange, signaling pathways, and cytoskeletal rearrangements have been observed (Liu et al., 2021). First, ion exchange occurred in response to electrical stimulation modulates the membrane potential and neuronal electrical activity, such as calcium (Ca^{2+}) transients (Cho et al., 1999; Wan et al., 2021). It is generally assumed that high-amplitude Ca^{2+} transients are generated by Ca^{2+} influx after the activation of voltage-gated Ca^{2+} channels (Murillo et al., 2017), and whereas low-amplitude Ca^{2+} transients are owing to the phospholipase C-mediated release of intracellular Ca^{2+} from endoplasmic reticulum (Khatib et al., 2004; Marino et al., 2015). In addition, the application of conductive materials could help transmit electrical signals more quickly and effectively, and the electrical signals could also be converted into biochemical cues to control cell behaviors (Thrivikraman et al., 2018). For example, the increase in intracellular Ca^{2+} concentration induced by electrical stimulation activates multiple signaling pathways, such as Ras/MAPK and PI3K/AKT, which regulate cell proliferation and differentiation (Zhao et al., 2020; Cheng et al., 2021). Membrane depolarization could also phosphorylate tyrosine in growth factor-like receptors and thus activate the cascade response of MAPK/ERK pathways (Sheikh et al., 2013). Furthermore, activated MAPK could trigger the c-Fos synthesis through the phosphorylation of cyclic AMP/Ca^{2+}-responsive element-binding protein (Tan et al., 2007), and then regulate neuronal activity (Joo et al., 2015). For another reason, external electric fields may also affect cytoskeletal rearrangement by the reorganization of microtubes and actin filaments (Li and Kolega, 2002; Han et al., 2009; Thrivikraman et al., 2014), which are the main component of growth cones to direct the neurite outgrowth (Hur et al., 2011). Some mixtures of topological and conductive materials can simultaneously promote the functional recovery of SGNs through topological characterization and electrical stimulation (Wei et al., 2021).

As SGN degeneration occurs inevitably after HC damage (Kanzaki et al., 2002), an "electrode-neuron gap," which exists between CI electrodes and the membranes at the terminal of SGNs neurites (Frick et al., 2017), and is usually produced after CI operation and considered to be the most remarkable obstacle in improving CI performance (Nella et al., 2022). The application of biomaterials can eliminate such "electrode-neuron gap" to a certain extent. For example, a resist pattern on the surface of nanocrystalline diamond has been designed to arrange specific neural interfaces, thereby creating independent electrical stimulation signals for individual neuron on the CI electrode array (Cai et al., 2016). Another research group managed to eliminate the "electrodes-neuron gap" by establishing a "neuro-regenerative nexus," a concept that was first proposed in 2022 by Kevin et al. The "neuro-regenerative nexus" refers to the biological interface provided by transplanted hPSC-derived ONPs, the two neurites of which are expected to connect to native SGNs and CI electrodes, respectively (Nella et al., 2022). To provide more precise connections between the SGNs and the electrodes, researchers used PODS® crystals for continuous delivery of growth factors and hydrogel to provide a stem cell niche for ONPs after transplantation into the inner ear (Chang H. T. et al., 2020), and a device with microgrooves to control the gradient of growth factors (Nella et al., 2022). which research has contributed to the development of "biohybrid" CI.

3.3 Superparamagnetic iron oxide and magnetic fields

Magnetic NPs have been introduced to clinical applications for a long time, such as magnetic resonance imaging and their effects on biochemical processes have recently been studied. SPIO NPs, the only magnetic NPs used in inner ear regeneration studies, were found to be taken up by SGNs to promote their neurite extension (Hu et al., 2021). Two cellular uptake pathways of SPIO NPs have been identified, one of which was diffusion occurred at the NPs concentration of 10 μg/mL (Mustafa et al., 2011) and the other was endocytosis that occurred when nanoparticles were functionalized by NGF (Nam et al., 2009). SPIO NPs without MFs have also been reported to promote nerve growth, possibly through the release of Fe ions from SPIO NPs and their involvement in cell adhesion-mediated biological processes (Kim et al., 2011). The tensile force generated by endocytosed NPs driven by an external static MFs could stretch the ends of neurites and align them toward the direction of MFs (Riggio et al., 2014). SPIO NPs with dynamic MFs have also proved to promote neuronal differentiation and neurite orientation through mechanisms of the cytoskeletal forces (Yuan et al., 2018) and the increased intracellular Ca^{2+} concentration (Georgas et al., 2023).

3.4 Biochemical cues loaded-materials for inner ear regeneration *in vivo*

Neurotrophins have been shown to be essential for maintaining survival and promoting differentiation during regeneration of inner ear cells (Gillespie et al., 2003; Pettingill et al., 2008; Xia et al., 2023). However, owing to the unstable biochemical properties of neurotrophic proteins, their half-life is extremely short, leading to difficulties in the long-term regeneration studied *in vivo* after inner ear transplantation of stem cells (Glueckert et al., 2018). Therefore, growth factor-loaded biomaterials have been used in *in vivo* inner ear cell regeneration studies. For example, the aforementioned brain-derived neurotrophic factor (BDNF) loaded-PODS® crystals exhibited a slow, sustained, and stable release of BDNF after inner ear transplantation, maintaining a stable concentration of growth factors in the differentiation environment for stem cells *in vivo* (Chang H. T. et al., 2020). Ultra-high viscious alginate encapsulated with BDNF-overexpressing MSCs was reported to enable the continuous BDNF release and prevent the unpredictable migration of MSCs, which promotes the survival of rat SGNs and their neurite outgrowth (Schwieger et al., 2020). In another study, an anti-EGF antibody was loaded onto alginate microcapsules and implanted in the inner ear of guinea pigs, and EGF was consequently highly aggregated around the microcapsules and promoted microcapsule differentiation (Zhong et al., 2016). SPIO is also commonly used as a nanoscale drug carrier (Yuan et al., 2019), MSCs endocytosed with SPIO NPs could be homed into the inner ear by tympanic ventricular administration or caudal vein injection (Ahn et al., 2021), which illustrating the promising applications of that SPIO.

4 Conclusions and perspectives

Current studies on biomaterials with different properties applied to inner ear regeneration have focused on several broad directions such as serving as scaffolds to establish inner ear organoids, providing topological micropatterns to guide cell regrowth, enhancing electrical signals to facilitate the electrical activity of inner ear cells and the formation of neural networks, as well as loading with chemical cues to maintain the stable regeneration of stem cells *in vivo*. However, the present multiple approaches have not allowed for the functional maturity of regenerated cells or the establishment of auditory circuits. In order to achieve these ultimate goals, several issues must be addressed. First, the details of the application of biomaterials for inner ear regeneration need to be further confirmed, such as the mechanical properties of the scaffolds and the parameters of the electrical signals, because the extracellular environment should be tissue-specific. Second, most of the current studies have only includes the exploration of cellular behavior, with little research on the underlying mechanisms, which would be the foundation for the further development of material-based inner ear regeneration. Furthermore, due to the intricate extracellular environment and complicated process of cell fate determination, biomaterials should be strategically selected and combined in view of the obstacles to be solved in inner ear cell regeneration research.

Author contributions

JL: Conceptualization, Writing – original draft, Data curation, Investigation. MW: Conceptualization, Writing – original draft, Data curation, Methodology. YM: Writing – original draft, Investigation. WA: Data curation, Writing – original draft. XW: Writing – original draft, Supervision. GS: Conceptualization, Investigation, Writing – original draft. HW: Funding acquisition, Writing – review & editing. WL: Conceptualization, Funding acquisition, Writing – review & editing, Supervision.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

SNHL	Sensorineural hearing loss
HCs	Hair cells
SGNs	Spiral ganglion neurons
CI	Cochlear implantation
DFAs	Diabetic foot ulcers
3D	Three-dimensional
ECM	Extracellular matrix
ESC	Embryonic stem cell
ONPs	Otic neural progenitors
MA	Methacrylic anhydride
CNTs	Carbon nanotubes
SWCNTs	Single-walled carbon nanotubes
MWCNTs	Multi-walled carbon nanotubes
Ppy	Polypyrrole
SPIO	Superparamagnetic iron oxide
NPs	Nanoparticles
NGFs	Nerve growth factors
MSCs	Mesenchyme stem cells
LED	Light emitting diode
LSPR	Localized surface plasmon resonance
YAP	Yes-associated protein
BDNF	Brain-derived neurotrophic factor

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NcRNA: key and potential in hearing loss

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Hearing loss has an extremely high prevalence worldwide and brings incredible economic and social burdens. Mechanisms such as epigenetics are profoundly involved in the initiation and progression of hearing loss and potentially yield definite strategies for hearing loss treatment. Non-coding genes occupy 97% of the human genome, and their transcripts, non-coding RNAs (ncRNAs), are widely participated in regulating various physiological and pathological situations. NcRNAs, mainly including micro-RNAs (miRNAs), long-stranded non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), are involved in the regulation of cell metabolism and cell death by modulating gene expression and protein-protein interactions, thus impacting the occurrence and prognosis of hearing loss. This review provides a detailed overview of ncRNAs, especially miRNAs and lncRNAs, in the pathogenesis of hearing loss. We also discuss the shortcomings and issues that need to be addressed in the study of hearing loss ncRNAs in the hope of providing viable therapeutic strategies for the precise treatment of hearing loss.

KEYWORDS

hearing loss, non-coding RNA, micro-RNA, long-stranded non-coding RNA, sensorineural hearing loss, age-related hearing loss

1 Introduction

Based on the Global Burden of Disease (GBD) study, deafness is the third major cause of disability in the world, which affects more than 5% of the population overworld (Tyrovolas et al., 2022). Meanwhile, more people are suffering from mild, undiagnosed hearing loss. About 1.57 billion of the world population were impacted by hearing loss, a number that could grow to 2.45 billion by 2050 (Haile et al., 2021). Thus, hearing loss is an emerging serious problem. The World Health Organization (WHO) classifies hearing loss as mild,

Abbreviations: ncRNA, non-coding RNAs; GBD, Global Burden of Disease; WHO, World Health Organization; SNHL, sensorineural hearing loss; AHL, age-related hearing loss; NIHL, noise-induced hearing loss; miRNAs, micro-RNAs; lncRNAs, long-stranded non-coding RNAs; circRNAs, circular RNAs; SAG, static auditory ganglion; BMSCs, bone marrow stem cells; FOXO3a, forkhead box O3a; PI3K, phosphatidylinositol-3 kinase; TMPRSS3, transmembrane protease serine 3; ISSNHL, idiopathic sudden sensorineural hearing loss; SIRT1, sirtuin-1; ONIHL, occupational noise-induced hearing loss; FOXG1, forkhead box G1; ROS, reactive oxygen species; SSNHL, sudden sensorineural hearing loss; ABR, auditory brainstem response; SGNs, spiral ganglion neurons.

moderate, severe, and more severe, corresponding to levels of 26–40, 41–60, 61–80, and 81 dB HL or higher (Nieman and Oh, 2020). Long-term hearing loss may negatively impact life qualities. Communication barriers, social isolation, and complex mental health issues, are potentially some of the serious consequences of hearing loss (Lin et al., 2013; Mener et al., 2013; Bainbridge and Wallhagen, 2014; Mick et al., 2014; Kamil and Lin, 2015).

According to the location of the lesion, hearing loss could be categorized as conductive hearing loss, sensorineural hearing loss (SNHL), and mixed hearing loss (Shapiro et al., 2021). Obstructions and diseases in the outer or middle ear could prevent the normal transmission of sound from the environment to the eardrum and middle ear, leading to conductive hearing loss (Hill-Feltham et al., 2021). For adults, earwax impaction, otosclerosis, otitis media, and secretory otitis media are the most common causes of conductive hearing loss. In children, the most frequent causes are middle ear effusion and congenital malformations of the ear canal (Michels et al., 2019). Sensorineural hearing loss is caused by dysfunction in the cochlea and spiral ganglion or lesions in the auditory center. Age-related hearing loss (AHL), chronic noise-induced hearing loss (NIHL), and ototoxic drug-related hearing loss, are the major types of sensorineural hearing loss (Wang et al., 2019; Prince and Stucken, 2021). AHL is a bilateral, symmetrical, progressive pathologic process in which the extent of hearing loss increases with age (Pacala and Yueh, 2012; Cunningham and Tucci, 2017). NIHL often occurs with AHL and is difficult to distinguish, the patients usually have a significant history of noise exposure (Sliwinska-Kowalska and Davis, 2012; Chen K. et al., 2020). However, if the ears are exposed to asymmetrical noise, hearing loss could also occur asymmetrically. Hearing loss induced by ototoxic drugs usually develops with injury of hair cells, vascular striae, or the auditory nerve. Typical ototoxic drugs include salicylic acid preparations, cisplatin, and aminoglycoside antibiotics, as well as some diuretics (Li and Chai, 2019; Dillard et al., 2021). Although many factors participate in the development and progression of hearing loss, genetics may be the most common causative factor (Cai et al., 2017; Mittal et al., 2017; Azadegan-Dehkordi et al., 2018).

The results of genome sequencing have proven that only 2–3% of the human genome is responsible for protein coding (Djebali et al., 2012). The coding genes, occupying a quite small portion of the genome, could be transcribed into mRNA and then translated and modified in three dimensions to form functional proteins. Non-coding RNAs (ncRNAs) are the major component of RNA, though not involved in coding proteins in general, these transcription products make up more than 90% of all RNA (Kopp and Mendell, 2018; Ransohoff et al., 2018). At first, ncRNAs are considered to be just massive junk sequences without protein-coding ability. The importance of ncRNAs in normal life regulation processes and disease processes has been gradually recognized, and the possibility of clinical applications has been explored (Adams et al., 2017; Anastasiadou et al., 2018). Micro-RNAs (miRNAs), long-stranded non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) were the major components of regulatory ncRNAs (Adams et al., 2017; Zhu et al., 2018; **Figure 1**). These ncRNAs are widely engaged in the regulation of various life processes, including cell metabolism, development, proliferation, transcription, and post-transcriptional modifications (Deveson et al., 2017; Esposito et al., 2019).

Human cochlear tissue that could be used for studies is difficult to obtain, which limits the progress in the hearing loss area. The construction of applicable animal models and the development of new analytical techniques have improved this situation in recent years. Although ncRNA research is still lagging behind other fields, much relevant literature suggests that ncRNAs are equally relevant in the field of hearing loss. The study of these regulators could reveal the pathologic mechanisms of the relevant diseases, provide potential medicinal targets, and guide the development of therapeutic agents (van Zandwijk et al., 2017; Seto et al., 2018; Kimura, 2020).

In this review, we detailed the role of ncRNAs in hearing loss and their involvement in potential treatment options. We also discuss the current limitations and perspectives of this field, with the hope that more valuable discoveries will be made in the future of hearing loss research.

2 MiR-183 family in hearing loss

The MiR-183 family is a group of three miRNAs with conserved expression, including miR-183, miR-182, and miR-96 (Dambal et al., 2015; Mahmoodian Sani et al., 2016). They were first found to be abundantly expressed in hair cells and ear neurons in mice and zebrafish, and shared nearly identical sequences in humans. The specific expression and conserved sequences indicated their importance in auditory development and function, and they were also the most studied miRNAs in hearing loss.

Aberrant expression of the miR-183 cluster could induce hearing loss. Weston et al. (2006) confirmed that the miR-183 cluster was abundantly expressed in inner ear sensory neurons and hair cells of adult mice. In contrast, the expression of miR-181 family and miR-183 family was significantly down-regulated in the Corti organ of aging individuals, and these changes could be detected even before the onset of organ morphological changes and hearing loss (Zhang et al., 2013). Emphasizing their importance in hair cell differentiation and maturation. On the other hand, different kinds of aberrant expression of miR-183 clusters might lead to abnormal development and auditory functions of the inner ear. Overexpression of the miR-183 cluster could lead to progressive sensorineural hearing loss in mice (Weston et al., 2018). Li et al. (2010) found that duplicated ear cysts, ectopic or dilated sensory patches, extra hair cells, and morphologic abnormalities of the static auditory ganglion (SAG) occurred in a miR-96- or miR-182-overexpressed zebrafish model, while knocking down miR-183 gene cluster led to reduced inner ear hair cell numbers, smaller SAGs, hemianopsia defects, and posterior lateral line neuroma abnormalities. In mice, the inactivation of miR-183 gene cluster significantly affected the development, morphology, and function of cochlear hair cells, leading to severe hearing loss (Geng et al., 2018).

MiR-96 was the first miRNA to be identified concerning deafness. Mencia et al. first described two different point mutations in the seed region of miR-96, and the mutation found in two Spanish families caused non-syndromic progressive sensorineural hearing loss (Lewis et al., 2009; Mencía et al., 2009). The involvement of miR-96 in the pathogenesis of human deafness was further demonstrated by Soldà et al. (2012) who performed

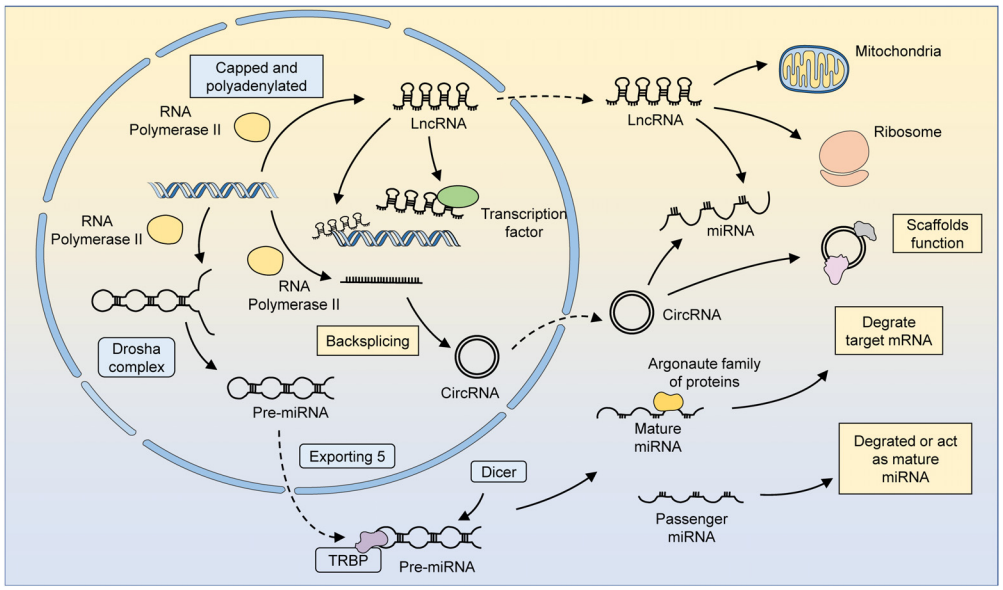

FIGURE 1
The biogenesis and function of several major ncRNAs. MiRNAs are transcribed by RNA polymerase 2/3 to form primary miRNAs (pri-miRNAs). Pri-miRNAs have specific hairpin loops that can be recognized and cleaved by the Drosha complex along the RNA-binding protein DGCR8. The cleaved pri-miRNA forms a precursor miRNA, which then translocates from the nucleus to the cytoplasm via exportin 5. Precursor miRNAs can be processed by Dicer in an RNA-binding protein TRBP-dependent manner to form double-stranded somatic RNAs. One strand of the double-stranded somatic RNA is loaded into the RNA-induced silencing complex (RISC) as a mature miRNA, leading to the degradation of target mRNAs. The other strand, known as a transient miRNA, is usually degraded. However, in some cases, these miRNAs can also function as mature miRNAs. LncRNAs could be transcription products of different DNA elements, such as promoters, enhancers, and exons. Most lncRNAs are transcribed by RNA polymerase 2 and have similar characteristics as mRNAs, but lncRNAs do not possess translatable ORFs. The regulatory mechanism of lncRNAs can be summarized in four ways: signaling, decoying, guiding, and scaffolding. LncRNAs localized in the nucleus can recruit factors for chromatin remodeling or directly bind to transcription factors and thus participate in chromatin modifications and epigenetic modifications. LncRNAs can be translocated by a mechanism similar to that of mRNAs, and those localized in the cytoplasm can regulate metabolism and organelle function by binding to organelles, such as mitochondria. LncRNAs can also bind to miRNAs or ribosomes to participate in the regulation of gene expression at the post-transcriptional level. CircRNAs are formed by reverse splicing of RNA polymerase 2 transcription products. CircRNAs can serve as scaffolds to facilitate the interaction of related proteins or participate in regulating the miRNA-mediated degradation of mRNAs.

a genetic screen of 882 non-syndromic sensorineural hearing loss patients and 836 normal-hearing controls. They identified a putative novel mutation in miR-96 that reconstructed the secondary structure of the pre-miR-96 hairpin, which significantly affected the ability of mature miR-96 to regulate selected targets. In particular, mutations in miR-96 were found to cause more severe hearing loss in mice than mutations in the other two miRs, possibly due to the creation of new target genes and the loss of normally acting target genes (Lewis et al., 2020). Kuhn et al. (2011) further demonstrated that miR-96 mutations could lead to a pre-termination of sensory hair cell maturation in mice, as well as disrupted maturation of hair cell stereocilia bundles and disorganized remodeling of auditory nerve connections within the cochlea. These indicated that miR-96 profoundly influenced the process of cochlear hair cell physiology and their morphological differentiation. Although several genes, including Clic 5, Aqp 5, Odf, Celsr 2, Ryk, Myrip, and Ptprq, have been identified as possible targets of miR-96, their regulatory roles in the inner ear remain poorly understood (Lewis et al., 2009; Gu et al., 2013; Chen et al., 2014).

By transfecting miR-182, bone marrow stem cells (BMSCs) could be induced to express mRNAs for hair cell markers such as SOX2, POU4F3, and ATOH1, as well as ATOH1 protein, thus playing a key role in the differentiation of hair cell (Mahmoudian-Sani et al., 2018). Forkhead box O3a (FOXO3a) is a transcription factor of the FOXO family, which is associated with a variety of biological processes, such as cell cycle, apoptosis, and metabolism (Habrowska-Górczyńska et al., 2021). Li et al. (2016) found that the overexpressed miR-182 could inhibit apoptosis by suppressing the translation of FOXO3a, thus providing a protective effect on cisplatin-treated hair cells *in vitro*. Also, miR-182 increased the level of phosphatidylinositol-3 kinase (PI3K) regulatory subunit p85α in outer hair cells, consequently reducing the loss of hair cells and relieving steric cilia in the drug-associated hearing loss rat model induced by kanamycin and furosemide, leading to the mitigation of perpetual threshold shifts (Chen J. et al., 2020). These demonstrated the protective potential of miR-182 in drug-induced hearing loss. Transmembrane protease serine 3 (TMPRSS3) is a gene expressed in the fetal inner ear, and its mutation is associated with non-syndromic hereditary hearing loss (Guipponi et al., 2002). TMPRSS3 could regulate the apoptosis and survival of HEI-OC1 cells through the circ-Slc41a2-miR-182-Akt cascade, thereby acting a role in preventing hearing loss (Zhang Z. et al., 2019).

MiR-183 is aberrantly expressed in hearing loss at a high frequency. After exposure to white band noise, the corti organ, outer hair cells, and ABR threshold movement of rats could be impaired (Park S. et al., 2020). The miR-411-3p, miR-183-5p, miR-377-3p, miR-20b-5p, and miR-200b-3 in the cochlear nucleus were significantly altered, andmiR-92a-1-5p, miR-136-3p, and miR-26b-5p in the inferior colliculus also experienced significant changes.

MiR-183 is also differentially expressed in idiopathic sudden sensorineural hearing loss (ISSNHL). Ha et al. (2020) found that miR-183, miR-210, miR-18b, and miR-23a were significantly differentially expressed in ISSNHL patients, and the sensitivity and specificity of their diagnostic efficacy was 80.95% (17/21) and 87.50% (21/24), respectively. This indicated that miR-183 might play an important role in the pathology of hearing loss. Further, Patel et al. (2013) found that Taok1 may be a target gene of miR-183 in NIHL using target prediction analysis. They validated the correlation *in vitro* model and concluded that the miR-183/Taok 1 target pair might participate in regulating the cochlear degenerative process after sound overstimulation (Patel et al., 2013). The impact of miR-183/miR-96 on normal synaptic transmission and the development of the auditory hindbrain was probably another mechanism that contributed to the development of hearing loss (Krohs et al., 2021). In hair cells, inhibition of Notch signaling promoted hair cell differentiation and regeneration, and miR-183 inhibition abolished this promoting effect, thus demonstrating the important involvement of miR-183 in hair cell differentiation and regeneration (Zhou et al., 2018).

3 MiR-34a in hearing loss

miR-34a, a member of the miR-34 family, is first described as a tumor suppressor miRNA that can be expressed induced by p53 (Raucci et al., 2021). With intensive investigation, the role of miR-34a has been revealed in a variety of life processes, both in neurogenesis and differentiation and in cancer progression (Chua and Tang, 2019).

Li et al. (2017) sequenced samples from SSNHL patients and normal individuals to detect differentially expressed miRNAs, and further constructed a miRNA-target-protein-protein interaction (PPI) network by combining the obtained results with database (Zhou H. et al., 2023). Hsa-miR-34a/15a/23a/210/18b/548n/143 had the most target genes in the miRNA-target-PPI network, suggesting their extensively involvement in the pathogenesis of SSNHL. MiR-34a levels in the cochlea, auditory cortex, and plasma of mice were elevated during aging, while sirtuin-1 (SIRT1), Bcl-2, and E2F3 levels were decreased with increasing age (Pang et al., 2016). In AHL, miR-34a overexpression could promote apoptosis by inhibiting Bcl-2, as well as by inhibiting SIRT1 and increasing HIF-1α and the acetylation of p53, thus contributing to the progression of hearing loss (Xiong et al., 2015; Huang et al., 2017). In HEI-OC1 cells, the overexpression of miR-34a could regulate autophagy and biogenesis of mitochondrial by suppressing SIRT1, and also lead to a reduction of ATG9A and impaired autophagic processes, while rapamycin could protect HEI-OC1 cells by restoring autophagic fluxes (Lin et al., 2017; Pang et al., 2017; Xiong et al., 2019).

In other types of hearing loss, aberrant expression of miR-34a was also involved in disease progression. The overexpression of miR-34a inhibited DRP-1 expression and led to mitochondrial dysfunction as well as exacerbation of cisplatin-induced ototoxicity (Wang et al., 2023). In the cochlea of db/db mice with diabetes mellitus, miR-34a was found to be significantly upregulated and accompanied by significant hearing threshold elevation and hair cell loss, implying that miR-34a could serve as a potential therapeutic target for diabetes-related hearing loss (Lin et al., 2017; **Figure 2**).

4 Other miRNAs in hearing loss

4.1 miRNAs in age-related hearing loss

Zhang J. et al. (2019) identified 799 differentially expressed genes in the cochlear tissue of Cmah-deficient mice, and found that the down-regulated differentially expressed genes were mainly involved in the PPAR signaling pathway. Among them, mmu-miR-130b-3p, mmu-miR-27a-3p, mmu-miR-27b-3p, and mmu-miR-721 were predicted to regulate PPARG, which may be an important mechanism in AHL. While in the miRNAs that appeared to be differentially expressed in the lateral wall of the cochlear duct in senescent mice, most of the down-regulated miRNAs were known pathways that regulate cell proliferation and differentiation, while all of the up-regulated miRNAs were associated with apoptosis (Zhang et al., 2014). MiR-29b, a miRNA significantly up-regulated in cochlear hair cells of senescent mice, was able to inhibit the expression of SIRT1 and PGC-1α in HEI-OC1 cells, leading to mitochondrial dysfunction and increased apoptosis. While MIAT could protect HEI-OC1 cells through down-regulating miR-29b (Xue et al., 2016; Hao et al., 2019). MiR-34a and lncRNA H19 were also involved in the SIRT1-mediated regulation of cytoprotective functions, indicating a possible synergistic role of specific ncRNAs in the development of hearing loss.

4.2 miRNAs in noise-induced hearing loss

Noise is a common cause of hearing loss, with an estimated 1.3 billion people worldwide suffering from hearing loss due to noise exposure (Vos et al., 2012). NIHL is a complex disease with a combination of genetic and environmental factors, so susceptibility may vary widely between individuals. Several studies have revealed that ncRNAs may be part of the pathogenesis of noise-induced hearing loss.

Miao et al. (2021) investigated the relevance of SNPs in the AKT2 gene to NIHL. They demonstrated that the SNP rs2304186 in the AKT2 3′-UTR could alter the binding affinity of hsa-miR-625-5p to the mutant region in an allele-specific manner, by which the susceptibility to NIHL might be changed. KCNQ4 is a voltage-gated potassium channel, and is critical for hearing preservation due to the capability of maintaining ionic homeostasis in hair cells. Wang et al. (2021) found that KCNQ4 levels were markedly decreased by miR-153, which was significantly increased in mice that developed sensory neural hearing loss after noise exposure. Knockdown of miR-153 could increase KCNQ4 levels and relieve impaired hearing (Wang et al., 2021). Another miRNA, miR-1229-5p, was notably elevated in the serum of patients with occupational noise-induced hearing loss (ONIHL), and its overexpression might take part in the pathogenesis of ONIHL by inhibiting MAPK1 signaling (Li et al., 2018). In addition, miR-185-5p and miR-451a had elevated levels in the plasma of NIHL patients, hoping to serve as biomarkers for NIHL (Ding et al., 2016).

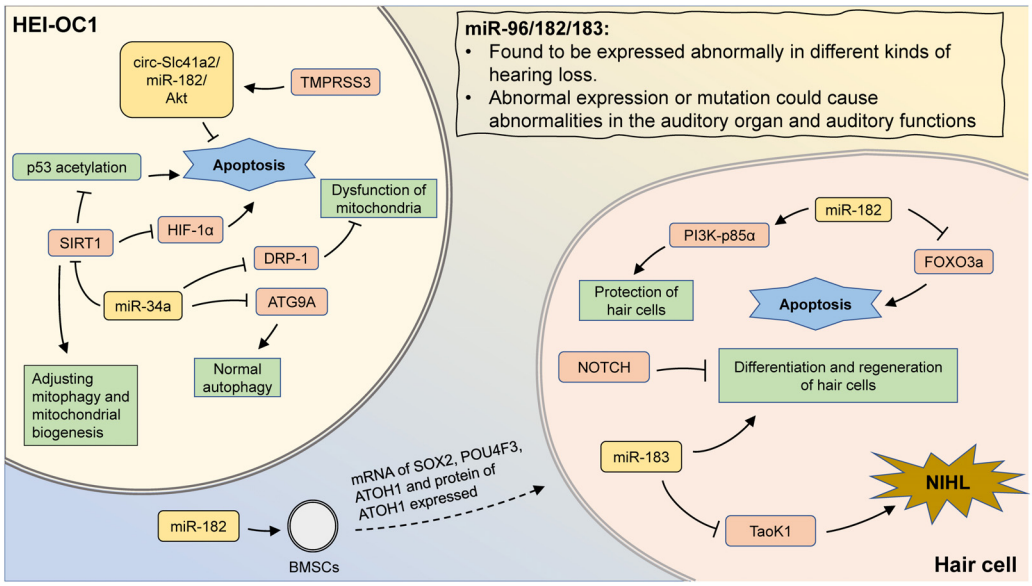

FIGURE 2
The mechanism involving miR-183 family and miR-34a in hearing loss. MiR-96/182/183 are found to be expressed abnormally in different kinds of hearing loss. Also, aberrant expression or mutation could cause abnormalities in the auditory organ and auditory functions. Transfection of miR-182 promotes the expression of hair cell-related markers in BMSCs. In hair cells, miR-182 can inhibit apoptosis by suppressing FOXO3a, as well as protect hair cells by promoting PI3K-p85α expression. Inhibition of Notch signaling promotes hair cell proliferation and differentiation, which could be abolished by miR-183 inhibition. In addition, aberrant expression of miR-183 may attenuate the inhibition of Taok1 and contribute to the development of NIHL. In HEI-OC1 cells, inhibition of DRP-1 by miR-34a can lead to mitochondrial dysfunction. MiR-34a can also regulate mitophagy and mitochondrial biogenesis by suppressing SIRT1. The inhibition of SIRT1 could promote the p53 acetylation and reduce the expression of HIF-1α, leading to cell apoptosis. MiR-34a inhibits ATG9A and lead to impaired autophagy. TMPRSS3 inhibits apoptosis through the circ-Slc41a2/miR-182/Akt pathway.

4.3 miRNAs in drug-associated hearing loss

Alterations in miRNA expression profiles in the inner ear following ototoxic drugs could contribute to understand the mechanisms by which these ototoxic drugs cause hearing loss. These altered markers also help to identify potential markers for diagnostic and therapeutic applications. In addition to the well-known miR-183 cluster, several miRs have also been identified that may play a role in drug-induced hearing loss.

Cisplatin is widely used in the chemotherapy of tumors, and is also commonly used in combination with other drugs or alone in the treatment protocols of multiple cancers (Tang et al., 2021). However, cisplatin could cause several side effects, including ototoxicity, nephrotoxicity, and neurological damage. Regulation of differentially expressed ncRNAs may be an effective way to minimize the toxic side effects of cisplatin. Liu et al. constructed a hearing loss model by intraperitoneally injecting cisplatin into mice, attempting to explore the protective effect of resveratrol against cisplatin-induced hearing loss (Liu et al., 2021). They found that miR-455-5p could be upregulated by resveratrol, thereby activating the PTEN-PI3K-Akt signaling pathway and attenuating the ototoxic effect of cisplatin. Forkhead box G1 (FOXG1) was a member of the FOX transcription factor gene family and exerted an important role in neuronal cell development and cell cycle regulation (Tigani et al., 2020). Cisplatin treatment decreased FOXG1 expression in mice and reduced cellular autophagy through its downstream target miRNAs, leading to the accumulation of reactive oxygen species (ROS) and cochlear hair cell death (Mu et al., 2023). Mu et al. (2023) rescued the reduced autophagy and relieved the ear injury caused by cisplatin by overexpressing FOXG1 and its target miRNA miR-34 and miR-183 family. In addition, Tsai et al. (2021) demonstrated that exosome injection may exert a protective effect against drug-induced hearing loss through miRNAs. After exosomal injection with umbilical cord MSCs, the mRNA levels of SMN1 and Pona were significantly up-regulated in cisplatin-treated mice, while the expression levels of mmu-miR-125b-5p, mmu-miR-125a-5p, and mmu-miR-127-3p in the inner ear tissues were also markedly improved, thus yielding a significant rescue of hearing loss in the mice.

Some antibiotics are ototoxic, principally including streptomycin and aminoglycoside antibiotics (Kros and Steyger, 2019). Lee et al. (2018) treated mice with kanamycin and furosemide to disrupt their hearing, showing that the chronological order of miR-205 elevation in the inner ear was consistent with the order of functional impairment occurring in the inner ear. This demonstrates that miR-205 was directly engaged in the process of ototoxicity and could be used as a potential diagnostic molecular marker. Ding and Wang (2022) also constructed a hearing loss model by using gentamicin in rats, demonstrating that miR-106a could promote oxidative stress-induced SNHL by targeting connexin-43.

In conclusion, several preclinical studies have confirmed the involvement of miRNAs in the process of inner ear injury in drug-induced hearing loss. Platinum drugs and some antibiotics are the most common drugs with ototoxicity, as targeted modulation of some miRNAs performed in rodent models effectively mitigated

the hearing loss induced by them, indicating that miRNAs have the potential to be therapeutic targets for Drug-Induced Hearing Loss.

4.4 miRNAs in sudden sensorineural hearing loss

Sudden sensorineural hearing loss (SSNHL) has no identifiable cause and is characterized by a hearing loss of ≥30 dB HL in at least three consecutive frequencies (Chandrasekhar et al., 2019). Treatment response in SSNHL varies widely between individuals, and seriously impacts the quality of patient survival (Kang et al., 2020; Park K. et al., 2020). Exploring the pathogenesis of SSNHL is essential for the development of novel prognoses and therapeutic options. SSNHL is associated with vasodilatation, infection, and immune abnormalities, yet its pathogenesis is poorly understood. (Yamada et al., 2022). In the absence of an accurate causative factor, it was difficult to simulate a disease model that reflects the pathological state of SSNHL, which further limits the advancement of this field.

Several studies suggested that miRNAs might be involved in SSNHL pathogenesis. Dicer and Drosha are two RNase III proteins that play an important role in miRNA biogenesis and have been shown to engage in a variety of diseases (Ha and Kim, 2014). Kim et al. examined the mRNA level of Dicer and Drosha in the blood of 57 SSNHL patients and 50 healthy volunteers, and found that dicer mRNA was significantly down-regulated in SSNHL patients, while no obvious change was observed in drosha mRNA (Kim et al., 2015). Similarly, AGO2, another component of the miRNA biogenesis process was found to be expressed differentially in SSNHL (Han et al., 2014). These aberrantly expressed proteins influenced miRNA processes. Although a causal relationship between these processes and SSNHL had not been confirmed, this indicated the presence of miRNA abnormalities in SSNHL. Abnormally expressed miRNAs were also present in exosomes from peripheral blood in SSNHL patients (Zhang et al., 2023b). Among them, PC-5p-38556_39, PC-5p-29163_54, and miR-93-3p showed remarkable differential expression, which might be closely related to the pathogenesis of SSNHL. In addition, the expression levels of serum miR-195-5p/-132-3p/-30a-3p/-128-3p/-140-3p/-186-5p/-375-3p/-590-5p showed a difference after the onset of sudden deafness, and this difference remained unchanged for a year thereafter (Nunez et al., 2020; Abgoon et al., 2023).

The specific role of these miRNAs in the pathogenesis of SSNHL is unknown, but has been explored with some application. Shew et al. (2019) built a machine-learning disease-specific algorithm that was able to predict the existence of sensorineural hearing loss by using the miRNA expression profiles of ectopic lymphoid lymphocytes. This potentially represents a trend in the future of disease diagnosis (Shew et al., 2019).

4.5 miRNAs in hair cell regeneration

Hair cells are a key factor in auditory conduction, as they could convert mechanical acoustic stimuli into electrochemical signals, allowing the transmission of hearing via the auditory nerve to the auditory center. Since the hair cells of mammals are non-renewable, a variety of genetic alterations and environmental factors that damage the hair cells are capable of causing irreparable hearing loss (Burns and Corwin, 2013). To date, we have a limited understanding of the mechanisms involved in the repair and regeneration of hair cells. Several combinations of growth factors and modulation of intracellular signaling pathways have been used to for testing the capacity to induce hair cell regeneration with fine results (Lambert, 1994; Yamashita and Oesterle, 1995; Zheng and Gao, 2000; Huang et al., 2009; Wu et al., 2016; Qian et al., 2021). However, these regenerated hair cells were unable to fully recover their previous quantity, while they were also lacking the normal functions of mature hair cells. Promoting the regeneration of completely functional hair cells is critical for the recovery of hearing. MiR-125 inhibited the proliferation of cochlear progenitor cell CPCs by down-regulating CDK2, compromising the regeneration of hair cells (Peng et al., 2021). LIN28B enhanced the plasticity of naive supporting cells in a mTORC1-dependent manner, thereby inducing the generation of hair cells, while this effect could be antagonized by miR-let-7g overexpression (Li and Doetzlhofer, 2020). MiR-210 was another novel factor that could promote hair cell generation (Riccardi et al., 2016). Riccardi et al. (2016) used next-generation miRNA sequencing to identify the most prominently expressed miRNAs in a mouse inner ear cell line UB/OC-1 during its differentiation to a hair cell phenotype, and validated the function of these miRNAs *in vitro*. They revealed that miR-210 could increase the production of new hair cells by promoting the transdifferentiation of supporting epithelial cells, suggesting that miR-210 was a potential therapy for hearing loss (**Table 1**).

5 LncRNA in hearing loss

LncRNA is a class of ncRNA longer than 200 bp, and is processed similarly to protein-coding genes, and has cell- and tissue-specific expression patterns (Quinn and Chang, 2016). LncRNAs are associated with a variety of diseases, including tumors, cardiovascular diseases, and inflammatory diseases. The role and mechanism of lncRNAs in hearing loss have been relatively poorly reported, compared to studies of miRNAs

With the analysis of the co-expression profiles of mRNAs and lncRNAs in the ARHL-associated RNA sequencing dataset, Kim et al. (2023) identified 112 mRNAs and 10 lncRNAs with relatively high expression in the cochlea of aged mice. Wang et al. (2017) also demonstrated that in the Chinese population, the polymorphisms in LncRNA HOTAIR also could affect the risk of NIHL. This suggested that lncRNA was involved in hearing loss. In aged mice, a total of 738 lncRNAs and 2033 mRNAs were found to be differentially expressed, with lncRNA NONMMUT010961.2 being the most significantly differentially expressed lncRNA (Zhao et al., 2020). The knockdown of lncRNA NONMMUT010961.2 reduced the expression levels of oxidative stress-related gene Ar and hearing loss-related gene Hgf in HEI-OC1 cells, proving the participation of lncRNA NONMMUT010961.2 in the pathogenesis of AHL. Oxidative stress injury associated with increasing age was a critical mechanism of age-related hearing loss, and several lncRNAs had been found to act in regulating oxidative stress in cells. LncRNA H19 was found to be notably down-regulated in

TABLE 1 NcRNAs and their roles in hearing loss.

NcRNAs	Targeted genes/proteins	Impact on hearing loss	Reference
miR-96	Possibly Clic 5, Aqp 5, Odf, Celsr 2, Ryk, Myrip, and Ptprq	Promote the development and maturation of hair cells, affect the remodeling of auditory nerve connections	Krohs et al., 2021; Lewis et al., 2020; Lewis et al., 2009
miR-182	FOXO3a, PI3Kp85α,Akt	Impact apoptosis and promote hair cell development and differentiation	Li et al., 2017; Li et al., 2022; Li et al., 2016
miR-183	Taok1, Notch	Participate in cochlear degenerative processes and promote regeneration and differentiation of hair cells	Lin et al., 2017; Mahmoodian Sani et al., 2016
miR-34a	Bcl-2, SIRT1, HIF-1α, p53, ATG9A, DRP-1	Regulate apoptosis, autophagy and mitochondrial function	Miao et al., 2021; Michels et al., 2019; Mick et al., 2014; Mittal et al., 2017; Mu et al., 2023; Nieman and Oh, 2020; Nunez et al., 2020
miR-29b	SIRT1, PGC-1α	Increase apoptosis and mitochondrial dysfunction	Pang et al., 2016; Park K. et al., 2020
miR-153	KCNQ4	Disrupt the ionic balance in hair cells	Peng et al., 2021
miR-1229-5p	MAPK1	Engage in the pathogenesis of ONIHL	Prince and Stucken, 2021
miR-455-5p	PTEN-PI3K-Akt,	Alleviate the ototoxic effects of cisplatin	Ransohoff et al., 2018
miR-106a	connexin-43	Promote oxidative stress-induced SNHL	Shew et al., 2019
MiR-125	CDK2	Inhibit the proliferation of cochlear progenitor cells	Wang et al., 2021
miR-let-7g	LIN28B	inhibit the regeneration of hair cells	Wang and Sun, 2022
miR-210	BDNF, Hoxa 1, Kctd 11, Dtx 1	Promote the regeneration and differentiation of hair cells	Weston et al., 2006

the cochlea of aged mice, and could inhibit the oxidative stress injury in cochlear hair cells through miR-653-5p/SIRT1 axis (Xie et al., 2022). Li et al. (2022) demonstrated that lncRNA Gm44593 could regulate the miR-29b/WNK axis to improve oxidative stress injury in HEI-OC1 cells. MiR-204-5p reduced the viability of spiral ganglion neurons (SGNs), which was achieved by inhibiting the expression of the TMPRSS3. In addition, the Hdac2/Sp1/miR-204-5p/Bcl-2 regulatory axis was also implicated in the apoptotic process of cochlear cells in acute hearing loss (Jiang et al., 2020; Xie et al., 2021). Jiang et al. (2020) found that lncRNA EBLN3P could competitively inhibit the effect of miR-204-5p and regulate the expression of TMPRSS3, thus effectively promoting the restoration of impaired SGN function. This revealed a key regulatory role for lncRNAs as new therapeutic targets in hearing loss.

Immune abnormalities are a major cause of hearing loss. Before 1979, the inner ear was considered an immunologically exempt organ, making it rare for researchers to explore the role of immunologically aberrant mechanisms in the development of hearing loss. The reports of McCabe (1979) and Rask-Andersen and Stahle (1979) brought to light that the inner ear was not only in contact with the host immune system, but was also susceptible to immune disorders. Zhang et al. (2023a) constructed a mouse model of immune-associated hearing loss by injecting inner ear antigens, and analyzed the plasma exosomes to identify a total of differentially expressed 94 lncRNAs, 612 mRNAs, and 100 miRNAs. Through analysis and screening, a ceRNA regulatory network was constructed based on 74 lncRNAs, 28 miRNAs, and 256 mRNAs. Among these, lncRNAs Gm9866 and Dusp7, were significantly up-regulated, while miR-185-5p was decreased, with an interaction between the three. This demonstrated the potential involvement and regulation of these three ncRNAs in hearing loss.

6 Discussion and prospect

There is an underlying genetic component in most causes of hearing loss, and identifying the involvement of coding and non-coding genes is critical to the study of hearing loss. Over the last decade, advances in sequencing technologies and assays have facilitated the identification of new genes and novel mutations associated with hearing loss. The importance of ncRNAs in the field of hearing loss is well established, although overall research progress still lags behind. A variety of miRNAs, represented by the miR183 family, are extensively involved in the genesis and regulation of hearing loss at various stages. However, there are still some areas of ncRNAs in hearing loss that deserve to be deepened.

Firstly, miRNA sequencing has identified hundreds of miRNAs expressed in the mouse inner ear, but the roles of most of them remain uncharacterized. The miR-183 family is most studied in hearing loss, but the regulatory networks are still imperfectly understood. For most of the miRNAs that are significantly differentially expressed in hearing loss, we have not fully elucidated the pathways involved and the related mechanisms in hearing loss. The study of miRNA mechanism in hearing loss would help us to identify the key miRNAs involved in hearing loss, and we can also try to explore the precursors that lead to the occurrence of these abnormalities.

Second, we emphasize here the role and mechanism of ncRNAs in hearing loss, but most of the ncRNAs involved are still predominantly miRNAs, while other lncRNAs and circRNAs have not been elucidated in depth. LncRNAs and circRNAs are the upstream regulatory units of miRNAs, and can regulate the hearing loss of ncRNAs by affecting miRNAs and regulating their corresponding proteins to fulfill their biological regulatory functions. These functions are indispensable for the occurrence and

development of hearing loss. Therefore, the enrichment of potential ncRNAs in hearing loss through multi-omics and other sequencing tools is necessary for early diagnosis, effective intervention, and prognosis of hearing loss.

Thirdly, hair cells are a key component of the auditory system, and many causes of hearing loss cross over at the point of hair cell injury. There are already some treatments that target hair cells, such as promoting the conversion of support cells into hair cells, thus accomplishing the replenishment of injured hair cells. However, the induced-differentiated hair cells usually do not have fully normal function, but instead are in an intermediate phenotype between support cells and hair cells. An improved understanding of the mechanisms of hair cell damage is necessary to overcome these difficulties. Some miRNAs, such as miR-210, can serve as possible contributors to hair cell regeneration. Meanwhile, targeted delivery using ncRNAs has been performed in many clinical/pre-clinical trials, especially in the treatment of cancer. Combining ncRNAs with nanomaterials permits targeted delivery and controlled release of ncRNAs, which holds tantalizing prospects for the treatment of hearing loss (Dai et al., 2022; Wang and Sun, 2022; Zhou et al., 2022; Zhou L. et al., 2023). The development of ncRNA-targeted drugs for hearing loss treatment is also one of the main goals of related studies.

Finally, the difficulty of obtaining cochlear tissue has been hindering progress in ear disease research, as analyzed tissue samples are necessary to study disease models. Easier and quicker approaches are needed to obtain cochlear tissue samples that can be used for research. The construction of animal models is one option, but there is a lack of widely recognized disease models for hearing loss. In recent years, advances in analytical and detection techniques have allowed us to use smaller amounts of tissue for analysis, which greatly facilitated research related to hearing loss. However, this is still insufficient for the extremely valuable inner ear tissue of humans. On the other hand, using less tissue for analysis means the possibility of more unpredictable bias, thus how to prevent this from affecting the accuracy of the results needs to be considered.

In conclusion, the role of ncRNAs in hearing loss is becoming increasingly prominent. Specially, miRNAs are often found to be abnormally expressed or mutated in several types of hearing loss, including AHL, NIHL, drug-associated hearing loss, and SSNHL. The most common examples are miR-183 family and miR-34a, both of which can be involved in processes such as apoptosis, autophagy, and hair cell regeneration through downstream proteins and signaling pathways, thereby affecting the onset and progression of hearing loss. LncRNAs are also found to be widely expressed aberrantly in hearing loss, but the specific regulatory mechanisms associated with these aberrantly expressed lncRNAs remain to be further investigated. The excavation and mechanism exploration of ncRNAs will provide new insights into the pathogenesis of unheard hearing loss and novel therapeutic approaches.

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KZ: Writing – original draft. TW: Writing – original draft. SL: Writing – original draft. ZL: Writing – review and editing. YZ: Writing – review and editing. QZ: Writing – review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Enhancing regenerative medicine: the crucial role of stem cell therapy

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Stem cells offer new therapeutic avenues for the repair and replacement of damaged tissues and organs owing to their self-renewal and multipotent differentiation capabilities. In this paper, we conduct a systematic review of the characteristics of various types of stem cells and offer insights into their potential applications in both cellular and cell-free therapies. In addition, we provide a comprehensive summary of the technical routes of stem cell therapy and discuss in detail current challenges, including safety issues and differentiation control. Although some issues remain, stem cell therapy demonstrates excellent potential in the field of regenerative medicine and provides novel tactics and methodologies for managing a wider spectrum of illnesses and traumas.

KEYWORDS

stem cell therapy, secretome, regenerative medicine, mesenchymal stromal cell, regeneration

Introduction

Organ damage and degenerative diseases are caused by cell death through ageing or loss of function and can seriously affect people's lives. Examples of such conditions include degenerative diseases like Parkinson's disease, Alzheimer's disease, cirrhosis of the liver, and hearing loss, as well as injurious diseases such as myocardial infarction and skin burns. Organs such as the liver have high regenerative capacity and can regenerate sufficiently to maintain functional stability under certain circumstances (Michalopoulos and Bhushan, 2021). Mouse liver has demonstrated robust regeneration that supports liver function after partial hepatectomy (Zhang et al., 2021; Duan et al., 2022; Fan et al., 2022). Unfortunately, most tissues and organs do not have such regenerative capacity and cannot repair themselves after injury, eventually leading to loss of function. An example of this would be the hair cells in the cochlea, which do not regenerate once they are damaged, resulting in irreversible hearing loss (Warchol et al., 1993). These patients will require a cochlear implant, whereby an electronic device containing an array of electrodes and a receiver is surgically implanted into the patient's inner ear to directly stimulate the auditory nerve and recover some of the patient's hearing (Lenarz, 2017; Carlson, 2020; Weltin et al., 2022). Similarly, patients with damaged heart valves will require replacement with artificial valves made of metal or biological material in order to maintain heart function (Singh et al., 2019; Hofferberth et al., 2020; Dreyfus et al., 2022; Figure 1).

Artificial organ replacement is complex and patients can develop infections or immune rejection after transplantation (Ko et al., 2016; Bakir et al., 2022; Crespo-Leiro et al., 2022). Some inflammatory reactions, such as infectious endocarditis, can be fatal (Berisha et al., 2022). Therefore, there is a need for an immunogenically weak treatment that can effectively repair damaged tissues

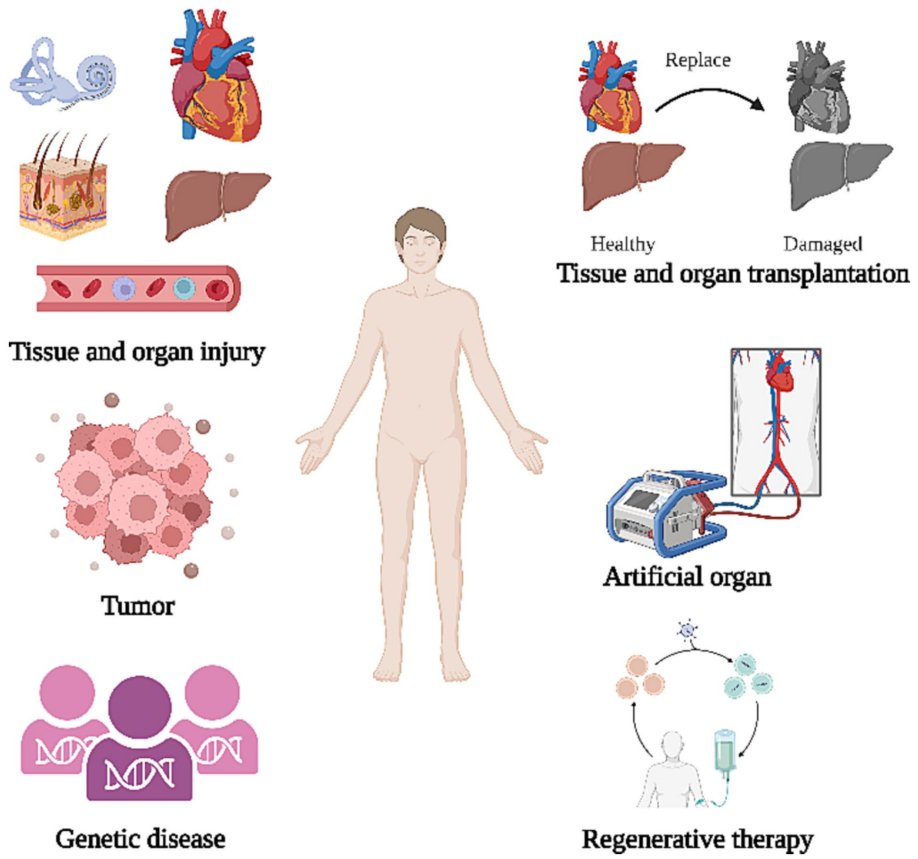

FIGURE 1
Treatment after organ dysfunction. The primary causes of organ dysfunction include tissue and organ damage, tumors, and congenital genetic diseases. To restore corresponding functions, current treatments primarily focus on organ transplantation, artificial organ substitution, and regeneration of organs.

and organs in patients, aiming to minimize the occurrence of adverse events. Stem cells (SCs), which have great potential to differentiate into a variety of cells and can proliferate indefinitely (Jin, 2017). SCs can be induced to differentiate into specific cell or tissue types *in vitro* before transplanting into the patient to replace degenerated or necrotic cells (Czajkowski et al., 2019; Selvaraj et al., 2019; Gholamigeravand et al., 2021; Melton, 2021). In addition, SCs can secrete anti-inflammatory factors, cytokines, and exosomes to suppress the inflammatory response and improve the microenvironment of the damaged area, ultimately regulating cell proliferation and differentiation (Vakhshiteh et al., 2019; Veneruso et al., 2019; Lv et al., 2021). This review provides a comprehensive overview of the mechanistic studies and clinical applications of stem cell therapy, while also pointing out pertinent issues in the field.

Classification of SCs

SCs are cells with multi-directional differentiation potential while retaining the ability to replicate and renew themselves. They can be classified according to the extent of their differentiation capability (Table 1).

Totipotent stem cells (TSCs) are a type of stem cell with the remarkable ability to differentiate into any cell type within an organism, including the placental cells necessary for embryonic development (Malik and Wang, 2022). TSCs exist at the earliest stages of embryonic development, typically at the zygote stage after fertilization when a sperm cell fertilizes an egg cell. At this point, the zygote is formed, which possesses the potential to develop into a complete organism (Baumann, 2017). TSCs often have a number of unique molecular features, including lower DNA methylation (Smith et al., 2014) and activation of endogenous retroviral components (ERVs) (Hurst and Magiorkinis, 2017). The TSC state can be induced by several methods. A mixture of the GSK inhibitor 1-azakenpaullone, the retinoic acid analogue TTNPB, and the kinase blocker WS6 can induce mouse embryonic stem cells (ESCs) to exhibit a phenotype similar to that of TSCs at the fertilized egg and two-cell stages (Hu et al., 2023). Furthermore, heterochromatin remodeling has also been demonstrated to help establish allozygous-specific H3K4me3 structural domains, thus effectively facilitating the transformation of ESCs from pluripotency to allozygosity (Yang et al., 2022). The unique ability of TSCs to differentiate into whole organisms is of great interest to developmental biology and regenerative medicine research. Still,

TABLE 1 Classification of stem cells.

Classification	Differentiation capacity	Examples
Totipotent stem cells	The most strongest differentiation potential, able to develop into complete individuals	Early embryonic stages of the fertilized egg and two-cell stage
Pluripotent stem cells	Second only to totipotent stem cells, capable of differentiating into most cells	Embryonic stem cells and induced pluripotent stem cells
Adult stem cells	Limited differentiation potential, able to differentiate into multiple cell types in a specific tissue or organ	Hematopoietic stem cells, mesenchymal stem cells, and neural stem cells
Unipotent stem cells	The weakest differentiation capacity, capable of differentiating into only one specific cell type in its tissue of origin	Skin basal cells and skeletal muscle satellite cells

there are specific ethical issues associated with their use and study (Takahashi and Yamanaka, 2006).

Pluripotent stem cells (PSCs) are known for their exceptional ability to differentiate into various specialized cell types across all three germ layers: ectoderm, endoderm, and mesoderm. Specifically, PSCs exhibit a significant capacity for differentiation into ectodermal derivatives (Yilmaz and Benvenisty, 2019). This is evidenced by their ability to generate neurons, glial cells, neural crest cells, and other cell types originating from the ectoderm (Yang et al., 2022). They are typically derived from ESCs at the blastocyst stage (Varzideh et al., 2023), or induced pluripotent stem cells (iPSCs) by reprogramming adult cells with four transcription factors: *Oct4*, *Sox2*, *Klf4*, and *c-Myc* (Takahashi and Yamanaka, 2006). The most significant advantage of iPSCs is that they are derived from differentiated somatic cells, providing the advantages of SCs while significantly avoiding the ethical issues associated with TSCs and ESCs. The indefinite self-renewal capacity of iPSCs in culture allows the generation of an almost unlimited supply of specialized cells, offering a great potential for the study of early human development, disease modeling and regenerative therapies (Chandy et al., 2022; Cho et al., 2022; Varzideh et al., 2023).

Adult stem cells (ASCs), categorized as multipotent stem cells, demonstrate a more restricted capacity for differentiation compared to pluripotent stem cells. These cells reside in various tissues and organs throughout the body, playing a role in maintaining, repairing, and regenerating tissues within their specific microenvironments (Prentice, 2019). Unlike pluripotent stem cells, which possess a broader potential to differentiate into diverse cell types from multiple germ layers, ASCs are more constrained in their differentiation scope. They typically generate cell types specific to their tissue or organ of origin and are more specialized than pluripotent stem cells. Consequently, ASCs can only generate specific cell lineages corresponding to the exact tissue of their origin, differing from the broader differentiation potential exhibited by their pluripotent stem cell counterparts (Barker et al., 2010). The most common ASCs include hematopoietic stem cells (HSCs; responsible for the production of blood cells in the bone marrow) (Cho et al., 2022), mesenchymal stem cells (MSCs; differentiate into fat, cartilage, and bone cells in various tissues) (Wang et al., 2023), and neural stem cells (NSCs; differentiate into neurons, astrocytes, and oligodendrocytes of the nervous system) (Zholudeva et al., 2021). ASCs are characterized by their relative abundance in adult tissues, their ability to regulate the microenvironment by secreting specific signaling molecules, and their ease of isolation (Ma et al., 2014; Zholudeva et al., 2021). They are, therefore, of great value for tissue and organ repair and cancer therapy (Barker et al., 2010; Liu et al., 2023; Wang et al., 2023).

Unipotent stem cells (USCs) constitute a specialized subset among stem cells, distinguished by their notably restricted differentiation potential. In contrast to pluripotent or multipotent stem cells, which are capable of generating a variety of cell types, USCs are dedicated solely to generating a single specific cell type (Lilja et al., 2018). These cells predominantly reside in specific tissues or organs, fulfilling a crucial function in sustaining, repairing, and rejuvenating the particular tissue they inhabit (Thomson et al., 1998). USCs are commonly found in adult tissues and are able to continuously replenish particular cell populations that are consumed, playing a vital role in tissue maintenance and repair. Examples of USCs include basal cells in the skin (Lin and Lu, 2021) and satellite cells in the skeletal muscle (Mierzejewski et al., 2020). The potential of USCs in the treatment of diseases is limited by their single mode of differentiation.

The technical route to SC therapy

To employ SCs for therapy, it is essential to first consider the source of the SCs. Considering their differentiation capacity and ethical issues, the most widely used SCs for treatment are currently PSCs and ASCs. ESCs are typically derived from the inner cell mass of a blastocyst (Bacakova et al., 2018). iPSCs are generated as described above and ASCs are derived from a variety of adult tissues, including adipose tissue, bone marrow, neural tissue, blood, skeletal muscle, etc., which provide convenient cell sources (Bacakova et al., 2018).

In order to maintain the multigenerational self-renewal capacity and differentiation ability of SCs, specialized culture systems are required to support this. Different biomaterials in the culture medium can impact the differentiation potential and the amplification capacity of SCs. Media containing oligopeptide-grafted hydrogels have been shown to enhance the proliferation and pluripotency of human ESCs and iPSCs (Chen et al., 2017). The use of culture systems containing human plasma and human embryo extracts maximizes the number of passages while maintaining the self-renewal and differentiation potential of iPSCs (Wang et al., 2012). In addition, compared to 2D cultures, 3D culture systems can better mimic the microenvironment of SCs *in vivo* and enhance the stemness of different SC species (Al Madhoun et al., 2016; Thakur et al., 2022). For example, a combination of 3D cell culture and natural brain tissue extracts can accelerate the differentiation of SCs into neuronal phenotypes (Azizi et al., 2018).

For SC therapy, the most crucial step is to direct the differentiation of SCs toward the target cell type by regulating culture conditions and signaling molecules. This can be achieved by mimicking the signaling pathways and microenvironment during embryonic development. Studies have shown that inner ear development is closely linked to fibroblast growth factor (FGF) signaling (Alvarez et al., 2003). Stimulating this pathway in human ESCs can induce two types of ear

progenitor cells that differentiate into inner ear hair-like cells and auditory neurons, respectively (Chen et al., 2012). Mild activation of Wnt signaling promotes the differentiation of MSCs into chondrogenic cells (Schizas et al., 2021). The adhesion and growth characteristics of cells can also be influenced by culturing them on the surface of nanomaterial composites, which triggers mechanotransduction-induced changes in gene expression through changes in cytoskeletal structure. Mouse kidney-derived SCs have been induced to differentiate into podocytes or proximal tubular cells in this way (MacGregor-Ramiasa et al., 2017). In contrast, in neural differentiation of SCs, chemical inducers or growth factors, including retinoic acid (RA), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF), are required (Gupta and Singh, 2022). Finally, the most direct way to induce directed differentiation of SCs is through transcription factor regulation. In addition to the above-mentioned transcription factors that can reprogram fibroblasts to iPSC, different transcription factors are required to induce differentiation of the required cell population (Ng et al., 2021). Overexpression of *NR5A1* and *RUNNX1* or *RUNX2* induces the differentiation of iPSC into human ovarian granulosa cells (Pierson Smela et al., 2023). The combined action of transcription factors, *GATA4*, *Tbx5*, *MEF2C*, and *Hand2*, reprograms mouse tail-tip and cardiac fibroblasts to cardiomyocyte-like cells with cardiac function *in vitro* (Song et al., 2012). Overexpression of *GFI1*, *Pou4f3*, and *ATOH1* directly induces the transformation of human fibroblasts into inner ear hair cell lineages (Duran Alonso et al., 2018).

Following combination and optimization of the above methods, directed differentiation of SCs can be achieved. After ensuring the validity and stability of the differentiation process, cell identification and functional validation, including cell phenotype analysis, gene expression analysis and functional assessment, are required to confirm that the differentiated cell types are as expected, thus ensuring that the resulting cells have the desired properties and functions.

Finally, differentiated and validated SCs are transplanted into patients via different vectors and scaffolds. At this stage, enhancing the retention of SCs in tissues is critical to the efficacy of the therapy. The most commonly used modality is injecting a saline suspension of the SCs directly into the target organ or tissue (Mousaei Ghasroldasht et al., 2022). However, due to the low adhesion of saline, only a small number of cells may remain in the tissue following injection. Therefore, a medium with higher adhesion properties is needed as a vehicle for SCs transplantation, such as a hydrogel (Nayagam et al., 2012; Niu et al., 2019). Nanohybrid hydrogels containing sufated glycosaminoglycan-based polyelectrolyte complex nanoparticles (PCN) are able to mimic extracellular matrices and contain a variety of bioactive factors to improve the implantation rate of neural SCs, while enabling cellular responses after central nervous system injury (Jian et al., 2018). Gelatin methacrylate (GelMA)/sodium alginate (Alg) (GelMA/Alg) hydrogels also contribute to the reduction of cellular damage after the implantation of neural SCs (Chen et al., 2023). Hydrogels of different compositions have played essential roles in cardiac infarction, skin regeneration, liver regeneration, etc., (Mardpour et al., 2019; Ke et al., 2020; Gong et al., 2022).

In summary, a complete SC therapeutic process comprises three significant aspects: SC generation and amplification, targeted differentiation and application, and selection of the optimal technical route to achieve regeneration and functional recovery of damaged tissues and organs is required for different clinical areas (Figure 2).

Applications of SC therapy

Cell therapy for organ and tissue regeneration encompasses a range of methods aimed at repairing or regenerating damaged tissues or organs by introducing exogenous cells into the body. Stem cell therapies, among other approaches, constitute a significant aspect of this field, harnessing the regenerative potential of specific cell populations to restore tissue function in conditions ranging from degenerative diseases to injuries.

Cell therapy for organ and tissue regeneration

Cell-based therapies operate through various mechanisms, encompassing cellular differentiation, secretion of bioactive molecules like growth factors and cytokines, modulation of immune responses, and facilitation of tissue repair and remodeling. Degenerative and injurious diseases, including circulatory, endocrine and neurological disorders, have the potential to be restored through SC therapy (Rossi and Cattaneo, 2002; Boyle et al., 2006). The first clinical applications were in the hematological sector, involving transplantation of hematopoietic stem cells (HSCs) from the blood system (Eaves, 2015). HSC transplants have now become the standard of care for hematological malignancies and hereditary blood cell disorders (Bordignon, 2006). Graft-versus-host disease (GVHD) can be minimized by analyzing genes within the human leukocyte antigen (HLA) region to find the best HLA-matched donor and recipient. To avoid the limitations of donor matching and potential immune complications, genetic correction or gene editing of patient's own HSCs has dramatically improved the efficiency of transplantation therapy for hematological disorders (Morgan et al., 2017). Wiskott-Aldrich syndrome (WAS), characterized by macrothrombocytopenia, eczema, autoimmunity, and lymphoid malignancies, is caused by the expression of mutated forms of the *WAS* gene. This mutation has been corrected in the patient's own HSCs by lentiviral transfection of the correct gene, followed by infusion of the modified HSCs into the patient, who showed improvement in immune function and clinical symptoms (Aiuti et al., 2013). In sickle cell disease, the hemoglobin abnormality is reversed by the introduction of the globin genes (γ-globin, γ/β-globin hybrids, and anti-sickle β-globin) into HSCs via γ-retroviral and lentiviral vectors or by directly targeting the fetal γ-globin suppressor gene *BCL11A* (White et al., 2023).

SC therapy has also shown strong potential in the treatment of deafness. Combined treatment of ESCs with insulin-like growth factor-1 (IGF), epidermal growth factor (EGF), and bFGF can induce ESCs to express markers of inner ear progenitor cells, including *ATOH1* (Li et al., 2003). After co-culture of ESCs/iPSCs and stromal cells from embryonic chicken egg sacs, Oshima et al. identified a class of hair bundle cells with short microvilli that have electrophysiological properties resembling immature hair cells (Oshima et al., 2010). This method further completes the progressive differentiation from SCs to hair cells. Treatment of hereditary hearing loss with SCs also requires the aid of gene editing. In deaf patients with *MYO7A mutation*, CRISPR/Cas9 gene correction in iPSCs is required to restore normal morphology and function of the differentiated hair cell-like cells (Tang et al., 2016).

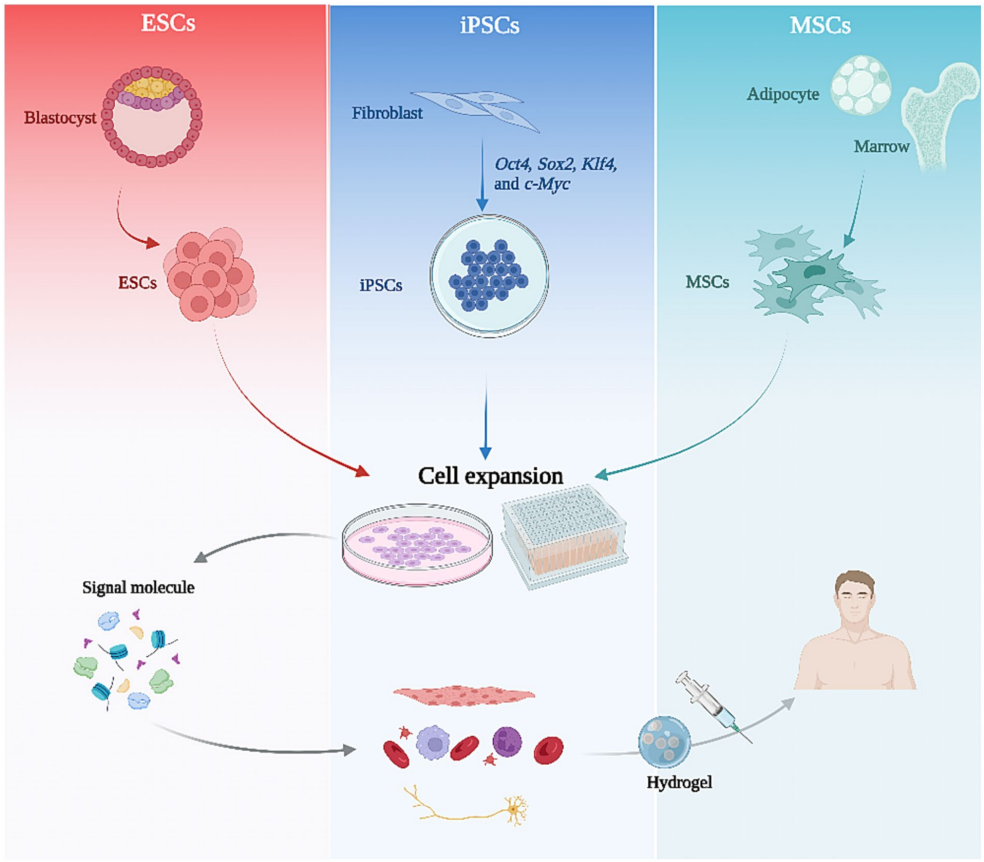

FIGURE 2
The flowchart of stem cell therapy. Various stem cell types are initially isolated from tissues and subsequently expanded through 2D or 3D culture. Following the regulation of culture conditions and signaling molecules, these stem cells can be directed towards specific differentiation pathways, ultimately resulting in tissue-specific cells that can be utilized for therapy via hydrogel encapsulation injection.

Cell-based SCs therapies have also been gradually refined for the treatment of heart and skin diseases. Cardiomyocytes are fully differentiated cells and have a limited regenerative capacity that determines the irreversible loss of cardiac function after injury (Eschenhagen et al., 2017). The re-differentiation of cardiomyocytes from ESCs and iPSCs is expected to further improve the function of damaged cardiac tissues (Mummery et al., 2012). With the addition of gene editing, SCs have been used to improve the treatment of cardiac diseases, including the introduction of *Akt1* to enhance the proliferation of cardiac progenitor cells (Noiseux et al., 2006), and modification of the *SDF-1/CXCR4* genes to facilitate the recruitment of cardiac SCs (Zhang et al., 2008; Tang et al., 2009). In terms of skin wound healing, SCs treatment mainly reduces healing time, risk of wound contracture and scar formation (Nourian Dehkordi et al., 2019).

In summary, cell-based SCs therapies work by direct replacement of the damaged tissues with cells derived from differentiation of normal SCs to restore tissue function or by correcting the abnormal SCs with gene editing so that normal tissue and organ function can be restored. While demonstrating potential in facilitating tissue regeneration and potentially reversing specific pathological conditions, several challenges persist, encompassing immune rejection concerns, ethical considerations, and the risks associated with unregulated cellular behavior subsequent to transplantation.

Cell-free paracrine therapy

There is growing evidence that in addition to direct cell replacement therapy, SCs, particularly MSCs, secrete proteins, growth factors, cytokines, and chemokines that exert influence on the surrounding cells or microenvironment to support tissue regeneration (Han et al., 2022). These secretory products, collectively known as the SCs secretome, are able to modulate the microenvironment of the damaged tissue by affecting the signaling pathways in different cell types, including tissue-specific cells, immune cells, vascular endothelial cells, and fibroblasts in the extracellular matrix (ECM) (Konala et al., 2016; Praveen Kumar et al., 2019; Daneshmandi et al., 2020). Due to their immunomodulatory properties, MSCs produce different regulatory factors to modulate the immune response in the microenvironment after homing and migrating to sites of injury (Volarevic et al., 2017). Immune checkpoint inhibitors, such as anti-PD-1/PD-L1, used for cancer therapy can also induce autoimmune type 1 diabetes, while MSCs-derived exosomes significantly prevent anti-PD-1/PD-L1-induced diabetes in mice (Kawada-Horitani et al., 2022). Bone marrow-derived MSCs were found to promote tendon-bone healing in the rotator cuff of rats by secreting TGF-β to regulate macrophage polarization via the Smad2/3 pathway. Inhibition of the M1 macrophage phenotype and promotion of the M2 phenotype was thought to contribute to tissue regeneration (Chen et al., 2021). In

addition, human MuSCs, a type of muscle SCs, can secrete mediators such as heme oxygenase-1 and prostaglandin E2 to inhibit T lymphocyte proliferation, induce Treg-like cell production and suppress the cytotoxic response of CD8+ T lymphocytes (Charrier et al., 2022). Thus, SCs can produce various mediators to act on multiple immune cells, including macrophages and T cells, and play a pivotal role in regulating the immune microenvironment.

SCs can also secrete factors that promote angiogenesis in the microenvironment while they themselves differentiate into suitable cell types to replace the damaged cells (Xia et al., 2019). This is the case in fetal skin where the SC secretome promotes HUVEC cell proliferation and angiogenesis by enhancing the transcriptional activity of targeted genes associated with fetal skin regeneration and angiogenesis, including *VEGF*, *Ang-1*, *Ang-2*, and *PLGF* (Boyle et al., 2006). ESCs-derived MSCs can promote angiogenesis and nerve regeneration through paracrine secretion, thus, improving neurological deficits and reducing infarct volumes in ischemic rats (Asgari Taei et al., 2021). Further proteomic analysis revealed that the cysteine-rich protein Cyr61 (also known as CCN1) is a pro-angiogenic factor that mediates vascular endothelial cell migration and angiogenesis through integrins $\alpha_v\beta_3$ and AMPK (Estrada et al., 2009; Park et al., 2015; Li Z et al., 2019). Similar to Cyr61, MSC-derived heparinases also promote angiogenesis via integrin pathways (Hu et al., 2015). The microenvironment also contains transport systems such as lymphatic vessels which can be regulated by SCs. When quiescent SCs shift to the activated phase, they can change the expression of angiopoietin-like protein 7 (*Angptl7*) to *Angptl4*. This results in a switch from promoting lymphatic drainage to promoting lymphatic dissociation and reducing drainage, thus mediating lymphatic remodeling (Gur-Cohen et al., 2019). In summary, the paracrine secretions from SCs can regulate lymphatic drainage and promote angiogenesis to ensure nutrient supply while replenishing damaged cells.

In addition to the normal microenvironment, SCs can also regulate the tumor microenvironment (TME). MicroRNA-100-rich exosomes derived from MSCs can inhibit the expression of VEGF in breast cancer cells through mTOR/HIF-1α signaling, ultimately inhibiting angiogenesis in the TME (Pakravan et al., 2017). However, in bladder cancer, the secretome of adipose-derived MSCs promotes the proliferation and invasion of cancer cells *in vitro* (Maj et al., 2018). Thus, regulation of the TME by the secretome of SCs can be bidirectional depending on the tissue involved. However, based on the secretory property of MSCs, therapeutic modalities that use MSCs as carriers for targeted delivery of treatment agents are now emerging (Hu et al., 2010). From delivery of cytokines such as IFN-β (Studeny et al., 2004) and IL-2/IL-12 (Gao et al., 2010; Bae et al., 2022) to regulate the immune microenvironment ($CD8^+$ T cells, NK cells), to delivery of drugs such as paclitaxel (Pessina et al., 2013), doxorubicin (Zhao et al., 2017), and photoresponsive agents for photodynamic therapy or photothermal therapy (Ouyang et al., 2020), to today's delivery of suicide genes such as TRAIL (Li M et al., 2019) and herpes simplex virus-thymidine kinase (*HSV-TK*) (Oraee-Yazdani et al., 2023), the use of MSCs as a therapeutic vector has been progressively refined.

In summary, cell-free therapies based on SCs have shown great promise with their ability to modulate the tissue microenvironment for the treatment of more diverse diseases than cell therapies in which SCs are re-differentiated to replenish damaged cells. Cell-free paracrine therapy offers several advantages over cell-based therapies, including reduced risk of immune rejection, simplified storage and administration processes, and potentially fewer safety concerns (Table 2).

Current challenges

In the current phase of rapid development in SC-based therapies, it is still important not to overlook some of the problems they pose. The first issue to be considered is the source of SCs as there are ethical and legal considerations (King and Perrin, 2014). The use of ESCs is subjected to ethical debates and legal limits, while the acquisition and amplification of adult SCs are technically tricky and have quality control issues (Chen et al., 2020). Another issue arising from prolonged continuous culture is the loss of cell viability, leading to reduced proliferative and differentiation capabilities. Addressing this necessitates the use of new materials, such as silica nanoparticles, for the long-term preservation of stem cells in a desiccated state (Gallina et al., 2015). Secondly, the efficiency and direction of differentiation of SCs is a major issue as this determines the effectiveness of the treatment. Directed differentiation is a complex process that we do not yet fully understand and many factors, such as cell culture conditions, cytokines, and signaling pathways can influence the process (Kim et al., 2016). Therefore, more research is still required to better control the direction and quality of differentiation of SCs to prevent adverse events such as tumorigenesis (Andrews et al., 2022). Another crucial determinant of stem cell therapy is the capacity to target cellular migration. Prior to assuming their role in differentiation, stem cells must be effectively delivered to the intended site. Currently, most stem cell therapeutic approaches employ intravenous drug delivery, which

TABLE 2 Applications of stem cell therapy.

	Cell therapy	Cell-free paracrine therapy
Treatment principle	The inherent ability of stem cells to undergo self-renewal and differentiation	The capacity of stem cells to secrete and generate various bioactive substances
The main acting substance	Stem cells	Stem cells secretome (proteins, exosomes, and active factors)
Therapy method	Replace damaged or abnormal cells	Regulate the microenvironment
Clinical application	Hematopoietic stem cells transplantation; regeneration of inner hair cells, cardiomyocyte, and hypodermal cell	Suppressing autoimmunity in type 1 diabetes; promoting angiogenesis of skin and brain tissue; regulating the tumor microenvironment
References	Oshima et al. (2010), Eaves (2015), and Nourian Dehkordi et al. (2019)	Boyle et al. (2006), Pakravan et al. (2017), Maj et al. (2018), Asgari Taei et al. (2021), and Kawada-Horitani et al. (2022)

exhibits limited efficacy in facilitating targeted migration from blood circulation to tissues (Liu et al., 2020). Survival of the transplanted SCs is another major issue facing SC therapy. SC therapy, characterized by its low expression of MHC and HLA, holds the potential to achieve reduced immunogenicity and significantly enhance the suppression of the graft-versus-host response. However, owing to the limitations in pre-expansion technology associated with SC therapy, its immune privilege is progressively compromised. Upon infusion into the human body, the presence of inflammatory factors within the body further escalates the immunogenicity of SCs, thereby elevating the risk of rejection (Barrachina et al., 2017). Cell survival and growth after transplantation are influenced by the host immune system, since the host immune responses to the allogeneic cells directly contributes to graft rejection (Sanz-Ruiz and Fernández-Avilés, 2018). A possible solution for allo-rejection is to knockout immune-related genes by gene editing to generate immune-compatible SCs (Ye et al., 2020). Further research will be required to resolve these and other challenges to successfully translate SCs therapies to the clinics.

Summary and perspectives

Since Ernst Haeckel first identified SCs in 1868, the development of these cells had gone through several critical stages. Initially, SCs were isolated and identified from various tissues, followed by the development of iPSCs and the combination of gene editing with SCs, leading to the progressive refinement of SC therapy. The most direct application for SCs is cell-based therapy, owing to their multi-directional differentiation capabilities. This approach involves the injection of SCs, both allogeneic and genetically modified autologous SCs, into the sites of disease or injury to promote tissue regeneration and functional recovery. The administration of cardiopoietic stem cell injection, induced by a cardiogenic growth factor, effectively enhanced cardiac function in patients with chronic heart failure during a clinical trial. Notably, no adverse effects on the heart or systemic toxicity were observed among the subjects (Bartunek et al., 2013). The deficiency of arylsulfatase A (ARSA), an inherited disorder known as metachromatic leukodystrophy (MLD), can be addressed through *in vitro* lentiviral transduction of autologous hematopoietic stem cells with cDNA encoding ARSA. This approach leads to enhanced ARSA activity and reduced brain damage (Fumagalli et al., 2022). An alternative application is cell-free therapy, utilizing the secretory ability of SCs, is also a critical approach. SC secreted factors can modulate the target tissue cells and the microenvironment, including the immune microenvironment and angiogenesis. Allogeneic expanded adipose-derived mesenchymal stem cells (Cx601) have been proven to secrete immunomodulators and anti-inflammatory factors, and have certain potential in the treatment of inflammatory bowel disease, especially in the treatment of anal fistula in patients with Crohn's disease (Panés et al., 2018). The latest therapeutic approach is to use SCs as vehicles for the targeted delivery of effectors, drugs, and genes into damaged tissues or tumors to exert the appropriate regulatory effects. The potential of oncolytic adenovirus as an antitumor therapy is limited in central nervous system tumors due to the presence of the blood–brain barrier. However, a clinical trial demonstrated that delivery via neural stem cells (NSC) facilitated safe and efficient transportation of oncolytic adenovirus to the tumor site (Fares et al., 2021).

However, SC therapy also faces a number of safety issues. Allogeneic SCs can trigger the patient's immune system, leading to graft rejection, while excessive proliferation and differentiation of transplanted SCs may lead to tumor formation. Ensuring the safety of SC therapy is, therefore, a significant challenge. It is also crucial in SC therapy to ensure that SCs can differentiate directionally into target cell types and maintain their function and stability. Further research and improved differentiation techniques are needed to ensure that differentiated cells have the desired characteristics.

In conclusion, as an essential therapeutic tool in regenerative medicine, SC therapy plays a vital role in a number of ways, both in the cells themselves and in their secreted components. With a better understanding of the properties and functions of SCs, it is expected that more diseases and injuries will be able to benefit from SC therapy.

Author contributions

JW: Writing – original draft. GD: Writing – original draft. SW: Project administration, Supervision, Writing – review & editing. SL: Data curation, Investigation, Writing – review & editing. PS: Formal analysis, Investigation, Writing – review & editing. KL: Data curation, Formal analysis, Writing – review & editing. XX: Funding acquisition, Project administration, Writing – review & editing. ZH: Conceptualization, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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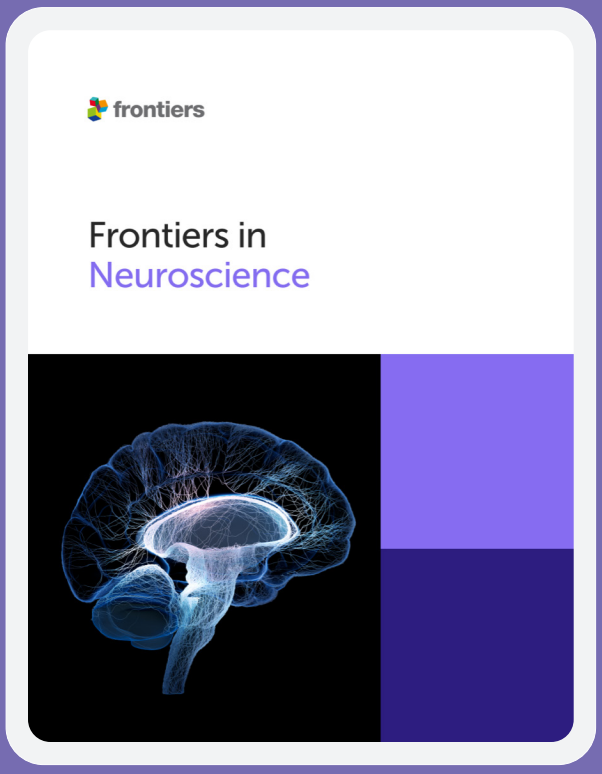

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