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Review

Development of the inner ear and regeneration of hair cells after hearing impairment

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ABSTRACT

Hearing loss, as a sensory disorder, is the most common occurrence among humans, which has received increasing attention from society. It is mainly caused by the damage of inner ear hair cells (HCs) or the degeneration of spiral ganglion neurons. In mammals, cochlear HCs cannot regenerate naturally after injury, leading to irreversible hearing loss. Therefore, HCs are essential for hearing protection. In recent years, the protection of drug-related ototoxicity, inner ear stem cells, gene therapy, new materials, and signal regulation have become important ways to develop regeneration strategies of HCs. An in-depth study of the causes of the occurrence and development of hearing impairment and the regeneration of hearing loss for effective prevention, discovery, and treatment of deafness has great significance. This review aimed to analyze the development of the inner ear and summarize the related factors leading to HCs injury and the research progress of regeneration after injury.

1. Introduction

One of the most common sensory disorders in humans is hearing loss. In 2020, 466 million people suffered from disabling hearing impairment worldwide. According to the World Health Organization, an estimated more than 900 million people will suffer from disabling hearing loss by 2050. Hearing loss is caused by any organic or functional impairment of the auditory pathway [1], such as genetic variants, viral infection, chronic ear infection, intense noise, aging, and ototoxic drugs. Hearing loss is broadly categorized into two types: conductive hearing loss and sensorineural hearing loss (SNHL). Conductive hearing loss mainly involves the middle ear structure, while SNHL mainly involves the inner ear [2]. As hearing loss is common and hair cells (HCs) loss is permanent, understanding the damage mechanism is necessary to develop preventive and restorative therapies [3].

The inner hair cells (IHCs) are the main sensory receptors, which convert the tip cilia displacement caused by acoustic waves into depolarization signals. The outer HCs (OHCs) receive efferent stimuli from higher control centers to regulate auditory signals and help amplify system sensitivity [4]. Cochlear supporting cells (SCs) maintain the dynamic balance of cochlear ions and chemical environment. Cochlear SCs defects can also lead to HCs degeneration and hearing loss. Related research has shown that these kinds of cells cannot regenerate spontaneously, so the impairment or loss of HCs and the degeneration of spiral ganglion neuron (SGN) can lead to permanent deafness [5].

In recent years, animal studies on signaling pathway manipulation, gene therapy, stem cell transplantation, drugs, new materials, and so forth showed that HCs and SGN could be triggered to regenerate [6]. Neural stem cell transplantation plays a crucial part in cell activation, regeneration, and repair. Various new materials have been widely studied, and gene and cell therapies also have great prospects. At present, relevant research focuses on vectors, therapeutic genes, cell materials, delivery pathways, and various targets. The study of the autophagy mechanism also plays an important role in the regeneration and damage repair

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of HCs. The co-regulation of Sonic Hedgehog(SHH), Notch, and Wingless/integrated(Wnt) signaling pathways provides a resultful method for inducing the proliferation of sensory progenitor cells and HCs regeneration, as well as restoring hearing and balance functions after HCs injury in the inner ear [7].

Therefore, this study reviewed the evolution, development, and survival mechanisms of the inner ear, and the factors related to the loss and regeneration of HCs after injury.

2. Development of the inner ear

The ear consists of the inner ear, middle ear, and external ear. The external ear collects sound waves, which are mainly formed by the fusion of the first-gill groove from the head and neck ectoderm and the six ear nodules around it [8]. The middle ear conducts sound waves, which are mainly formed by the development of the first pharyngeal pouch from the endoderm. The inner ear converts sound waves into nerve impulses of different frequencies and senses position changes, which mainly evolve from otocysts formed in the head ectoderm during its embryogenesis.

The inner ear mainly comes from the surface ectoderm at the level of the rhombocerebrum. At the beginning of the fourth week of the embryo, the surface ectoderm on both sides of the rhombencephalon thickens under the induction of the rhombocerebrum to form the otic placode and then sinks into the lower mesenchyme to form the otic cup. Finally, the otic cup is closed and separated from the surface ectoderm to form otic versicle. At the beginning, the otic versicle is pear-shaped, and then extends and expands to form the dorsal vestibular organ and the ventral cochlea, and a small cystic tube grows inside the dorsal end, which is an endolymphatic duct. The epithelium of vestibular organ forms the three semicircular canals and elliptic bursa, and the epithelium of the cochlea forms the balloon and cochlear canal. In this way, the otic versicle and the surrounding mesenchyme evolve into the membranous labyrinth of inner ear. Under the induction of otic versicle, the mesenchyme around the membranous labyrinth starts to form cartilage matrix from the 8th week of the embryo. At the 3rd month of the embryo, it becomes a cartilaginous auditory sac, wrapping the membranous labyrinth. With the enlargement of the membranous labyrinth, vacuoles appear in the cartilaginous auditory sac, and the vacuoles fuse with each other to form a perilymphatic space. At about the fifth month of the embryo, the cartilaginous auditory sac ossifies into an osseous labyrinth, so the membranous labyrinth is completely sheathed in the labyrinth, and only a narrow perilymphatic space is separated between them. The receptors of executive position and auditory sense are crista ampullaris, maculae static, and spiral organ, which are all located in the membranous labyrinth. Corti organ, which occurs at the end of 2 months, is a device stimulated by sound waves formed by thickening epithelial cells on the surface of the basement membrane. Like the position sensor, the spirochete is also composed of SCs and HCs. The organ of Corti, which develops at the end of 2 months, is a device stimulated by sound waves formed by thickening epithelial cells on the surface of the basement membrane. Like the position sensor, the spirochete is also composed of SCs and HCs [9].

Supporting cells: Various kinds of SCs exist, which are mainly column cells and finger cells. The nucleus of a columnar cell is round and located at the base of the cell. Abundant tension fibrils present in the cytoplasm play a supporting role. Finger cells are columnar and also located on the basilar membrane. The inner finger cells are arranged on the inner side of the inner column cells, and the outer finger cells are on the outer side of the outer column cells. The former has only one row, while the latter has three to five rows.

Hair cells: The HCs are divided into IHCs and OHCs, located above the inner and outer finger cells, respectively. They are surrounded by the digitation of the finger cells. Corresponding to the finger cells, only one row of about 3500 IHCs and three to five rows of about 1200 OHCs exist. The HCs are columnar, the nucleus is near the base, and many static cilia are arranged in a V or W shape on the top of the cells, which are called auditory hairs. The basal part of HCs forms synapses with the peripheral processes of bipolar neurons, and the central processes penetrate the modiolus to form the cochlear nerve.

Spiral ganglion: In the fourth week of the human embryo, neural crest cells and epithelial cells of the otocyst co-differentiate into vestibular ganglion and spiral ganglion in its adjacent outer mesenchyme [10]. The ganglion is composed of bipolar cells, and its peripheral processes are the vestibular and cochlear nerves [11]. The cochlear nerve extends to the base of the spiral organ in the 7th to 9th weeks of the fetus.

The inner ear is a complex and fine structure of auditory and positional receptors. Its formation starts from the ectoderm on both sides of the rhombocerebrum. Then it develops into the dorsal vestibule and the ventral cochlea through the otic placode, otic cup, otic versicle, and other stages, finally forming a complete inner ear structure. Many molecules interact and cooperate closely, playing an important regulatory role in inner ear maturation [12]. The otic versicle produces three cell lineages: prosensory cells, neuroblasts, and non-sensory cells. The cochlear neurons and vestibular neurons are produced by common neural progenitor cells. HCs and supporting cells originate from prosensory cells. IGF-I, c-fos, c-jun, SOX2, RA, Notch pathway and BMP4 are all necessary for generating prosensory cells. Brn3.1 and Notch signaling pathways play a role in the differentiation of HCs. The expression of FGF2, IGF-I, NGF, Islet-1, and SOX2 determines the fate of neuroblasts. Cochlear neurons mainly depend on the expression of neurotrophins 3 (NT-3) and trkC, and vestibular neurons need brain-derived neurotrophic factor (BDNF) and trkB [13](Fig.1).

3. Inducement of hearing loss

Worldwide, about 466 million people now suffer from disabling hearing impairment. SNHL is the most frequent hearing impairment, accounting for 85% of all hearing loss cases. Based on the development process of the inner ear and the influence of various factors, hearing loss is caused by various reasons, which can be roughly divided into drug-induced hearing loss, hereditary hearing loss, presbycusis, and noise-induced hearing loss. There are some rare types of hearing loss, such as dyslexia-related hearing loss caused mainly by abnormal spontaneous electrical activity of Spiral ganglion neurons, hearing loss caused by FAM73a or FAM73b deficiency, and hearing impairment in patients with peroxisome biogenetic disorders (PBDs) [14–16].

3.1. Drug-induced hearing loss

More than 150 drugs have been found to have ototoxic effects, and ototoxic drugs are common inducing factors of SNHL. The mechanisms of HCs injury and apoptosis induced by ototoxic drugs are complex. The accumulation of reactive oxygen species (ROS) in cells frequently activates various signaling pathways related to apoptosis, and can play a vital role in inducing HCs death [5].

3.1.1. Aminoglycosides

Aminoglycosides, such as gentamicin, sisomicin, streptomycin, kanamycin, and neomycin, are commonly used antibiotics to treat Gram-negative bacterial infections [17].

The ototoxicity mechanism of aminoglycosides is mainly through inducing HCs damage and apoptosis, which leads to permanent hearing loss and vestibular dysfunction [18]. Under physiological conditions, the ROS is eliminated through the antioxidant mechanism of HCs, which is produced by mitochondrial metabolism [19]. However, aminoglycosides increase ROS production in cochlear HCs, and ROS triggers various cell death mechanisms, including caspase-dependent or caspase-independent apoptosis and necrosis. Excessive ROS overwhelms the cell defense mechanism and finally leads to apoptosis of HCs [20]. After the

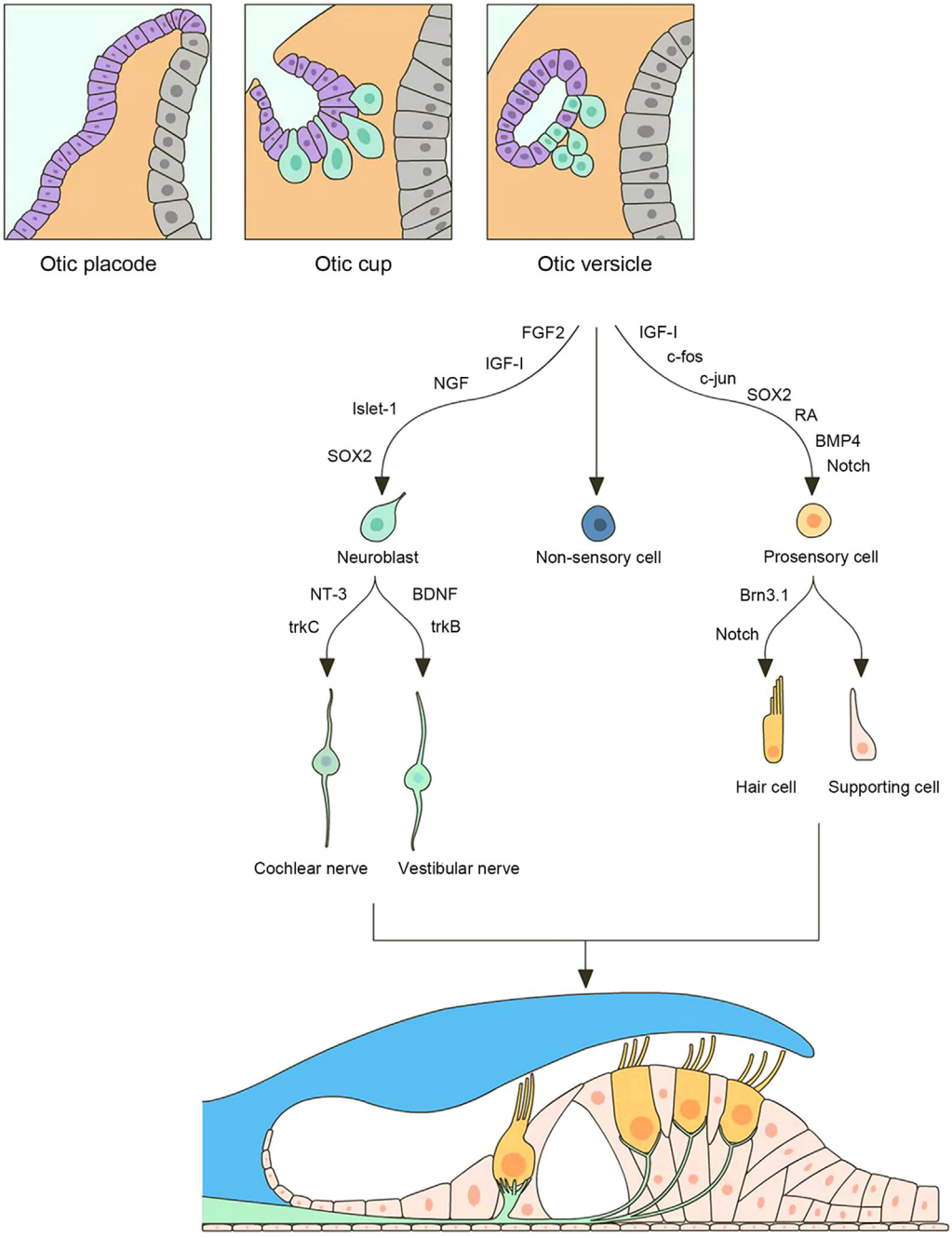

Fig. 1. Diagram of the developmental pattern of the inner ear. After the development of the inner ear goes through stages such as ear plate, ear cup, and earlobe, it differentiates into neuroblasts, non-sensory cells, and presensory cells under the regulation of various signaling pathways and transcription factors, and further differentiates into cells such as HCs, SCs and other cells, finally form a complete inner ear structure.

drug enters the cochlea, the increase in ROS and calcium ions in the inner ear sensory cells and neurons destroys the synthesis of physiological mitochondrial proteins, reduces the mitochondrial membrane potential, and then promotes cytochrome c to release into the cytoplasm [21].

3.1.2. Antineoplastic drugs

Antineoplastic drugs, such as cisplatin, carboplatin, vincristine, nitrogen mustard, cyclophosphamide, methotrexate, and bleomycin, also have ototoxicity. Research suggests that cisplatin can cause severe degeneration of inner ear stria vascularis, capillary congestion, and extensive HCs damage. In addition, cisplatin can reduce Na^+-K^+-Adenosine triphosphate(Na^+-K^+-ATPase) activity of stria vascularis cells and inhibit cochlear action potential. Cyclophosphamide can cause permanent hearing loss, methotrexate and carboplatin have cochlear and vestibular toxicity, and nitrogen mustard can cause structural damage to cochlear auditory receptor HCs, especially local perfusion. Vincristine can cause SNHL [22].

Ototoxicity caused by cisplatin mainly occurs in the inner ear and has three main targets: HCs, SGN, and stria vascularis [23]. Cisplatin induces apoptosis and subsequent hearing loss by damaging the cilia of HCs, mitochondria, and nuclei. Cisplatin can also cause ototoxicity by directly damaging and interfering with SGN function and leading to SGN loss. The increase in ROS formation and the consumption of antioxidants that Cisplatin-induced lead to HCs damage through oxidative stress [24]. Cisplatin can also change the expression of mitochondrial fusion and mitotic proteins.

3.1.3. Nonsteroidal anti-inflammatory drugs

OHCs are one of the main sites of ototoxicity induced by nonsteroidal anti-inflammatory drugs (NSAIDs). Large doses of salicylate can reduce the level of otoacoustic emission (OAE), causing damage to the mechanical sensory function of the OHCs of the cochlea. Salicylate-induced mild-to-moderate SNHL is due to the direct action of OHCs movements, resulting in impaired sound amplification. Perilymphatic perfusion with high concentrations of salicylate can reduce the compound action potential (CAP) threshold (an indicator of hearing level) of guinea pigs, resulting in mild-to-moderate hearing loss. NSAIDs do not affect endocochlear potential, which is an indicator of stria vascularis function [25].

Although NSAIDs may cause ototoxicity in some cases, high doses of sodium salicylate can cause moderate reversible cochlear hearing loss (20–40 dB threshold increase), tinnitus, and OAE suppression [26]. Enhancing potassium current in OHCs may prevent salicylic acid-induced transient hearing loss [27].

3.1.4. Loop diuretics

Loop diuretics, such as etaniac acid, furosemide, and budestanide, cause bilateral symmetry and transient hearing loss, accompanied by tinnitus [28]. The ototoxicity is reversible if the drug is stopped in a short time. Deafness caused by loop diuretics can occur very quickly, vestibular damage is relatively rare, and vertigo and nystagmus can occur. The acute administration of loop diuretics mainly damages the stria vascularis of the cochlea, causing cell swelling, ATPase activity reduction, changes in the concentration of endolymphatic ions, and inhibition of action potential. This can cause degeneration and produce delayed cochlear damage and permanent deafness of HCs when large doses of loop diuretics are recovered in a short time. Loop diuretics cause unique pathological changes in cochlea, form edema in the stria vascularis epithelium, interfere with stria adenylate cyclase, and inhibit the Na^+-K^+−$2Cl^-$ cotransporter in the stria vascularis, thus leading to a rapid decline in endolymphatic potential, and eventually leading to the impairment of cochlear microphonic potential, summation potential, and CAP [29].

3.1.5. Antimalarial drug

Antimalarial drugs, such as chloroquine phosphate, quinine, chloroquine, hydroxychloroquine, and pyrimethamine, mainly cause tinnitus and deafness, and the toxicity mainly affects the spiral ganglion.

The ototoxicity of antimalarial drugs has different clinical characteristics [30]. After oral administration of quinine, tinnitus and hearing loss, mainly in low-frequency areas, may appear in a short time. Most patients may recover after short-term withdrawal. Guinea pigs experiments suggest that quinine affects OHCs, IHCs, and SGN. In adult isolated mouse SGN, quinine reversibly reduced the amplitude of action potential and prolonged the duration of action potential [31]. It also blocked potassium and sodium currents in the whole cell, but not calcium currents [32].

3.2. Hereditary hearing loss

Hereditary hearing loss (HHL) is divided into syndromic hearing impairment (SHI) and nonsyndromic hearing impairment (NSHI) based on whether symptoms other than hearing defects are present. NSHI is the most common type of HHL, among which autosomal recessive inheritance is the most common [33].

SHI is a form of hearing loss associated with other symptoms, which is associated with abnormalities of the eyes, kidneys, musculoskeletal and nervous systems, and pigmented diseases [34]. More than 600 syndromes are related to SHI, including Usher syndrome, Pendred syndrome, Jervell and Lange Nielsen syndrome, Waardenburg syndrome, Alport syndrome, branchiootorenal syndrome, Stickler syndrome, Treacher Collins syndrome, and CHARGE (*C* for coloboma and cranial nerves; *H* for heart defects; *A* for atresia of the choanae; *R* for retardation of growth and development; *G* for genital and urinary abnormalities; *E* for ear abnormalities and/or hearing loss) syndrome.

In the last few decades, more than 200 hearing loss genes have been identified. Of these, five belong to the Solute Carrier (SLC) family. Most SLC genes are caused by point mutations in Gap Junction Protein Beta2 (GJB2), SLC26A4, and MT-RNR1 genes. Pendrin mutation encoded by SLC26A4 is a common cause of SHI and NSHI [35].

3.3. Noise-induced hearing loss

Noise-induced hearing loss (NIHL) is a common form of SNHL [36]. Slight or moderate noise triggers a temporary threshold shift (TTS), whereas strong sound vibration, grievous long-term exposure to harmful noise, or noise exposure can lead to necrosis and apoptosis of cochlear HCs, resulting in a permanent threshold shift (PTS). In addition to the decrease in language recognition ability and hearing sensitivity, patients with NIHL may also show headaches, tinnitus, vertigo, hypertension, and so forth [37].

NIHL is a multifactorial disease caused by genetic factors and environmental risk factors [38]. Recent studies have revealed that genetic factors, together with environmental conditions, also contribute to NIHL. A group of genes that are linked to the susceptibility of NIHL have been uncovered, involving the progression of oxidative stress, potassium ion cycling, cilia structure, heat shock protein 70 (HSP70), DNA damage repair, apoptosis, and some other genes [39]. Excessive exposure to noise leads to auditory nerve degeneration, HCs loss, and synaptic zone reduction. Depending on the severity of exposure, it may lead to temporary or permanent hearing loss. Oxidative stress, calcium overload, inflammation, energy metabolism disorders, and glutamate excitotoxicity are the main causes of NIHL [36]. A variety of candidate genes increase the susceptibility to NIHL, mainly including genes related to oxidative stress, potassium ion circulation pathway, heat shock protein, apoptosis signal, inflammation, and immune response [40].

3.4. Presbycusis

Presbycusis is also known as age-related hearing loss (ARHL). It refers to progressive bilateral symmetrical SNHL related to aging, which is most obvious at a higher frequency due to the gradual degeneration of the cochlea and auditory pathways [41].

The three main forms of ARHL are as follows: (1) SNHL, characterized by a sudden rise in the high-frequency pure tone threshold and loss of HCs at the base of the cochlea; (2) presbycusis, related to stria vascularis atrophy, which is found in patients with a slightly decreased or flat pure tone audiogram. (3) neurogenic presbycusis, characterized by loss of cochlear neurons throughout the cochlea [42]. The pathophysiology of ARHL involves complex interactions between environmental and genetic factors. The basic biological and molecular mechanisms of ARHL include membrane transport, ROS, cochlear synapses, vascular outcomes, hormones, and microRNAs. The emergence of genome-wide association research makes it possible to identify specific genomes related to ARHL.

ARHL is the cumulative impact of age on hearing. Risk factors can be divided into four categories: cochlear aging (individual age), environment (such as noise exposure and ototoxic drugs), genetic susceptibility (such as gender, race, and gene variation), and medical comorbidity (such as hypertension, diabetes, stroke, and smoking). The abnormal structure and function of ROS and mitochondria are important causes of ARHL in the elderly. They are related to the mitochondrial DNA (mtDNA) mutation caused by oxidative stress, cell apoptosis, and vascular damage [43]. The downregulation of genes related to mitochondrial respiratory chain complexes and the accumulation of mDNA mutations caused by the imbalance of free radical production may be related to energy consumption, changes in cochlear atrophy, and the procedure of inducing apoptosis, leading to the death of aging HCs and neurons. These reactive oxygen free radicals damage proteins, DNA, and

other macromolecules, and cause the degradation of tissues and organs [40]. MtDNA4977, a specific deletion, is often observed in the temporal bone of patients with ARHL, and its level is closely related to the severity of ARHL. Polymerase gamma (POLG) mutation encoding DNA polymerase, maintaining the fidelity of mtDNA replication, or optic atrophy 1 (OPA1) encoding mitochondrial dynamins like guanosine triohosphtease (GTPase) affect the stability of the mtDNA genome. These mutations also lead to early hearing loss in patients and mice. Mitochondria, as the main source of ROS, play a key role in aging [43]. Although many researchers are committed to identifying specific genes behind ARHL, strong candidate genes have not yet emerged. ARHL is caused by various combinations of inner ear and central nervous system pathology, with each disease having potentially different causes and genetic contributions. Therefore, there may be many genes and gene combinations with subtle variations, making a person more or less susceptible to ARHL [44]. Various genetic factors lead to hearing loss, and environmental factors are also equivalent factors that lead to hearing loss in the elderly. The fact that the environment plays an important role seems very encouraging, as it means that hearing loss is not an inevitable part of the aging process. Noise exposure, inflammation of middle ear, abnormal immune function, and the use of ototoxic drugs are all potential pathogenic factors for ARHL [45].

4. Protection against hearing loss

Based on the different causes of hearing loss, research and development have focused on different types of hearing prevention and protection measures, and prevention of hearing loss is receiving increasing attention.

4.1. Protection of drug-induced hearing loss

Aminoglycosides and cisplatin are the most common ototoxic drugs that cause SNHL in clinics. The purpose of ear protective drugs is to protect HCs and spiral ganglion neurons from acute injury and cell death, which are usually caused by local inflammation [46].

Tetrandrine (TET) is a bioactive bisbenzylisoquinoline alkaloid extracted from TET tetrandrum. Studies have found that taking TET can significantly reduce the oxidative stress and apoptosis of HCs after neomycin exposure, and mainly fight against neomycin-induced ototoxicity by promoting the biosynthesis of steroids [47,48]. Blebbistatin is a myosin II inhibitor. Studies have found that blebbistatin can significantly reduce ROS accumulation, maintain mitochondrial function, and thus prevent neomycin-induced apoptosis of house ear institute-organ of corti 1 (HEI-OC-1) cells and cochlear HCs in vitro explant culture [20]. Fasudil (Fas) is a vasodilator and Rho-kinase inhibitor with antioxidant capacity. Fasudil may help to increase the survival rate of HCs after neomycin exposure by inhibiting the Rho signal pathway, and provide a new treatment target point for the prevention of HCs loss and hearing impairment caused by aminoglycosides [49].

Some antioxidants, including resveratrol, N-acetylcysteine, and vitamin C, can reduce the ototoxicity of aminoglycoside antibiotics [50]. After cisplatin treatment, the expression of tumor protein 53-induced (TP53-induced) glycolysis and apoptosis-regulating factor (TIGAR) in SGN increases, which is regulated by the Wnt signal. Wnt activation in SGN upregulates the expression of TIGAR and inhibits the accumulation of ROS and apoptosis induced by oxidative stress. Wnt signal can inhibit oxidative stress and apoptosis in SGN, activate TIGAR, and protect SGN from cisplatin-induced injury [51]. The selective activation of mTORC2 can protect mice from acoustic trauma and cisplatin-induced ototoxicity [52].

Ferroptosis is a cell death pathway in cisplatin-induced ototoxicity [53]. Cisplatin-induced ferroptosis of auditory cells is an autophagy-dependent cell death. Drug-inhibited ferroptosis can protect OHCs from cisplatin damage, and more importantly, it can reduce hearing metastasis, providing clues for a new treatment for cisplatin-induced hearing loss [54].

In the inner ear, heat shock proteins (HSP) induction is a key response that can protect HCs from damage. One of these protective HSPs is heme oxygenase-1 (Hmox1). Induction of Hmox1/HSP32 has been found to reduce HCs death in cisplatin-induced adult mouse cyst whole-organ culture. Drug-induced Hmox-1 may be a combined therapy to prevent hearing loss caused by cisplatin [55].

4.2. Prevention of HHL

The World Health Organization for HHL divides prevention into three levels: primary prevention to avoid adverse health conditions, secondary prevention to carry out early detection and timely treatment, and tertiary prevention to reduce the impact of established conditions and possible recovery functions [56].

The prevention of HHL mainly refers to prenatal or early pregnancy genetic counseling. Through gene screening and diagnosis of high-risk groups, the incidence of congenital deafness in children can be fundamentally reduced. The high-risk population for HHL children includes hearing loss patients and their relatives and couples with deaf children. Because of the high frequency of specific hearing loss mutations, it is also necessary to screen common hearing loss genes in normal hearing people to identify carriers of recessive gene mutations. For carriers with hearing loss gene mutations, further genetic testing is required for their spouses. If both parents have hereditary hearing loss genotypes, genetic counseling, birth guidance and prenatal diagnosis are recommended. The introduction of next-generation sequencing (NGS) has resulted in great progress in diagnostics allowing to study of all known hearing loss genes in a single assay. The advancement of molecular biology has improved the detection and early intervention of hearing loss patients [57].

A major challenge in treating HHL is its heterogeneity. As more than 100 gene mutations have been proven to cause deafness, and because each gene is different in protein function, treatment time window, and target cell population, the best method is to focus on each variant with the efforts of precision medicine. Although rare mutations only affect a few patients, they are the most common cause of deafness. At present, a breakthrough has been made on the road to gene therapy for hearing impairment, and gene tools might be used to treat and cure deafness in the future [58].

4.3. Protection of presbycusis

Presbycusis, or ARHL, is associated with degenerative changes, including stria vascularis and its vascular system, spiral ligaments, sensory HCs and high-energy cells of stria vascularis, vascular system, auditory neurons, and sensory cells, especially OHCs. Progressive functional deterioration in the cochlea is associated with ARHL. It has been proven that upregulation of endoplasmic reticulum (ER) chaperon protein HSP90AA1 mitigates ER stress-induced damages associated with aging [59]. At present, preventing noise exposure is the only effective way to reduce ARHL. Studies aimed at improving mitochondrial activity to reduce oxidative damage in the elderly might bring relief to ARHL [60]. Research shows that ears are particularly vulnerable to noise in their mature stages, and exposure to noise in childhood may increase aging speed and vulnerability. Therefore, it is also of practical significance to reduce excessive noise exposure in the first few months of life and start educating on noise and its impact on hearing, health, and learning in primary schools [61]. The drugs that have entered clinical trials mainly focus on reducing oxidative stress, manipulating cell death cascade and promoting HCs regeneration. Some drugs can correlate with different biological processes and are therefore considered suitable for treating age-related cochlear dysfunction, including drugs targeting neuronal processes, inflammation, oxidative stress, cell signaling, and other biological processes. For example, acetyldigitoxin, almitrine,bretylium,

Table 1
The causes and protection of various types of hearing loss.

Type of hearing loss	Inducement of hearing loss		Protection against hearing loss
Drug-induced hearing loss	Aminoglycosides	Increased production of ROS and calcium ions, mitochondrial dysfunction, destruction of cellular defense mechanisms, HCs apoptosis	Tetrandrine, blebbistatin, fasudi, antioxidants, drug-inhibited ferroptosis
	Antineoplastic drugs	Degeneration of inner ear stria vascularis, HCs damage,SGN functional impairment, oxidative stress, altered mitochondrial protein expression	
	Nonsteroidal anti-inflammatory drugs	OAE levels reduction, impaired mechanical sensory function of OHCs, CAP threshold reduction	
	Loop diuretics	Damaged cochlear stria vascularis, HCs denaturation	
	Antimalarial drug	Damage to OHCs, IHCs, and SGN	
Hereditary hearing loss	Genetic mutations	Point mutations, pendrin mutation	Prenatal or early pregnancy genetic counseling, birth guidance, gene screening, diagnosis of high-risk groups, precision medicine
Noise-induced hearing loss	Genetic factors Environmental factors	Oxidative stress, calcium overload, inflammation, energy, metabolism disorders, excitotoxicity of glutamate Noise exposure	Inhibition of mitochondrial calcium overload, inhibition of calcium mediated cell apoptosis, reduced formation of ROS, the calcineurin inhibitor FK506,anti-inflammatory, antioxidant, mineral, calcium antagonists, vitamins, and blood thinners
Presbycusis	Cochlear aging	Individual age	Preventing noise exposure, drugs targeting neuronal processes, inflammation, oxidative stress, cell signaling, and other biological processes
	Environment	Noise exposure, ototoxic drugs, inflammation of middle ear, abnormal immune function	
	Genetic susceptibility	MtDNA mutation, genetic variation	
	Medical comorbidity	Hypertension, diabetes, stroke, smoking	

deslanoside, and digitoxin for neuronal processes, leukotriene receptor inhibition for inflammatory processes, allopurinol inhibition for oxidative stress, abaloparatide and teriparatide for cell signal transduction, etc. [62].

4.4. Protection of NIHL

Exposure to strong sound or noise may cause purely TTS or leave residual PTS, as well as changes in the growth function of auditory nerve output. The main causes of NIHL are cochlear HCs injury and associated synaptic lesions. Its mechanism has the transformation potential of drug intervention and provides multiple opportunities for preventing HCs injury or saving damaged HCs and SGN. Cochlear HCs apoptosis is a phenomenon related to noise-induced irreversible injury [63]. Therefore, regulating this process may be an effective way to prevent NIHL. Mitochondrial calcium overload is related to apoptosis. The increase in free Ca^{2+}in OHCs has been proved to lead to Bax activation, thereby altering the permeability of the outer mitochondrial membrane and promoting the release of cytochrome c, followed by caspase activation and apoptosis. Bcl-2 antagonizes Bax expression and regulates apoptosis. Exchange protein directly activated by cAMP 1 (Epac 1) induces apoptosis of cardiomyocytes and smooth muscle cells through the calmodulin-dependent protein kinaseII (CaMKII) pathway. Inhibition of Epac 1 can reduce the loss of cochlear HCs and the mitochondrial damage to HCs. ESI-09 (a novel acyclic nucleotide EPAC antagonist) reduces the expression of CaMKII and inhibits calcium-mediated apoptosis activation [64,65]. Oxidative stress plays a decisive role in the pathogenesis of NIHL. Because cell defense against oxidative stress is an energy-consuming process, glucose supplementation can reduce the formation of noise-induced ROS, thereby reducing the loss of noise-induced synaptic zones of OHCs, inner HCs and NIHL. Glucose supplementation increased ATP and nicotinamide adenine dinucleotide phosphate levels and reduced H_2O_2-induced ROS production and cytotoxicity. This shows that energy availability is critical to oxidative stress resistance, and glucose supplementation provides a simple and effective method to protect cochlear HCs from oxidative stress and NIHL. The calcineurin inhibitor FK506 (tacrolimus), which is clinically used as an immunosuppressant, can also be used to treat noise-induced HCs loss and NIHL. After moderate noise exposure in adult congregation betn abraham-jacob (CBA/J) mice, calcineurin (CaN) immunostaining in OHCs and nuclear factor activation of T-cell subtype c4 (NFATc4/NFAT3) in the nuclei of OHCs increased significantly. FK506 treatment significantly reduced the loss of OHCs and NIHL caused by moderate noise. ROS induced by moderate noise decreased significantly after treatment with FK506 [66,67].

Methods such as anti-inflammatory, antioxidant, mineral, calcium antagonist, vitamins, and blood thinners have been used to treat and prevent NIHL. Previous studies have confirmed the effectiveness of early intratympanic injections of methylprednisolone. N-acetylcysteine (NAC) is deacetylated to cysteine in the liver or local tissues, and neutralizes the effects of noise through various mechanisms. Clinical trials have also confirmed the protective effect of oral NAC on NIHL. Oral administration of magnesium Aspartic acid can provide significant protection to human with PTS and TTS. Vitamin E can reduce oxidative stress caused by noise exposure, thereby preventing NIHL. D-methionine tablets also have preventive effects [68] (Table 1).

5. HCs regeneration and protection

Previous studies on the protection of hearing loss mainly focused on the prevention of hearing impairment through drug treatment, peripheral protection or stimulation of artificial hearing aids, or the use of cochlear implants to directly send stimuli to the auditory nerve. In recent years, inner ear stem cells, gene therapy, and signal regulation have become important ways to develop HCs regeneration and protection [5].

5.1. Neural stem cells

Neural stem cells transplantation is a new therapeutic method to supply specific cells that are damaged by neurodegenerative diseases. Neural stem cells multiorgan transplantation plays a vital part in the activation, regeneration, and repair of damaged neurons. The curative mechanism of neural stem cells can be divided into the following three categories: (1) Neural stem cells gather, proliferate, and differentiate into specific cells at the injured site to realize the functional restoration of original tissues or organs. (2) Neural stem cells promote the recovery and regeneration of damaged cells by secreting related nutrients. (3) Neural stem cells restore the nerve conduction pathway by establishing or improving the synaptic connection between nerve cells [69].

Different types of stem cells (adult stem cells, induced pluripotent stem cells, and embryonic stem cells) participate in the regeneration of sensory HCs and auditory neurons. Lgr5+ stem cells can be used as progenitor cells in mammalian cochlea, and these cells have regenerative capacity in the early postnatal period [70]. Compared with Lgr5+ progenitor cells, the expansion ability of Lgr6+ HCs progenitor cells is weak, and the ability of Lgr6+ progenitor cells to produce HCs is significantly higher than that of Lgr5+ progenitor cells. Lgr5+ progenitor cells from sensory epithelium produce HC-like cells and SCs, but do not produce neurons or glial cells [71]. In the cochlea of newborn mice, the overexpression of Atoh1 in Sox2-positive cells was found to induce many SCs to transdifferentiate into HCs [1]. A few HCs are generated by mitosis in the larger epithelial ridge, and the combination of Atoh1 overexpression and Wnt overexpression as well as Notch inhibition leads to an extensive production of mitotic HCs [72]. The implantation of neural stem cells into the inner ear to regenerate spiral neurons and synaptic connections is expected to become a clinical treatment for SNHL. Combined with important regulatory genes in HCs regeneration-related pathways during inner ear development, such as Atoh1, Noth, Foxg1, Hedgehog, and Pou4f3, it can affect the proliferation of neural progenitor cells in the inner ear. In addition, simulating human diseases in vitro through genetic manipulation of stem cells is expected to help elucidate the pathogenesis of diseases.

5.2. New materials

Neural stem cells and inner ear progenitor cells are indispensable for the regeneration of HCs and nerve repair. Many studies have focused on the use of new materials for the proliferation of neural stem cells in vitro, the differentiation of inner ear cells and the regeneration of HCs. For example, regenerated silk fibroin (RSF) mats have excellent biocompatibility with inner ear progenitors and help the inner ear progenitors maintain their stemness. Therefore, it plays an important role in the differentiation of inner ear progenitors into HCs. These materials can simulate the biophysical characteristics of the relevant environment in the human body. Because the cell microenvironment directly defines the cell structure and function and regulates multiple signal cascades involved in stem cell differentiation with surface chemistry to control stem cell differentiation and HCs regeneration. The clinical practice of neural stem cells has been promoted by new technologies and materials for treating hearing diseases with neural stem cells [73]. By using graphene as a scaffold material or nanocomposite carrier for neural stem cells, the proliferation and differentiation of neural stem cells and the directional growth of neuronal axons can be achieved, and ultimately leading to the formation of biologically functional tissues. Likewise, the growth of neural stem cells can be facilitated by artificial photonic crystal materials with special topological properties and electrical signals. Apart from these new materials mentioned above, anisotropic anti-opal is a material that modifies the behavior of neural stem cells by changing their surface morphology. The special 3D porous structure of anisotropic anti-opal can make neural stem cells spheres have better directional differentiation, a significantly higher dendritic complexity index, stronger proliferation ability and more orderly cell arrangement, compared with isotropic anti-opal. Through the 3D culture system, the microenvironment of the inner ear can be simulated to promote the stem cells to fully form the functional structure and morphology of the inner ear [69].

5.2.1. Graphene-based materials

Extensive tests on the toxicity and biosafety of graphene-based materials have shown their potential in medical applications, including drug delivery, photothermal therapy, and nerve repair and regeneration. Considering the neural stem cells cultured on graphene as the research object, this study explored the influence of cochlear implant–based electric-acoustic stimulation (EAS) on the neuronal differentiation, survival and proliferation of neural stem cells in vitro. It is a promising and vital approach to treating hearing impairment through the cochlear implant and stem cell transplantation combined to treat SNHL so as to achieve the restoration of hearing function [69]. Three-dimensional graphene foam (3DG) not only supports the growth of neural stem cells but also promotes neural stem cells to differentiate into astrocytes, especially neurons, which has been proven to be a powerful scaffold for culturing neural stem cells in vitro. 3DG may have strong therapeutic promise in treating neuronal diseases and neurodegenerative diseases. A new form of bioactive hydrogel incorporating topological graphene oxide (GO) and TEMPO-oxidized bacterial cellulose (GO/TOBC hydrogel) was developed to provide a favorable microenvironment for SGN neurite outgrowth and had the potential for constructing biomimetic nerve grafts for repairing or replacing nerve defects [74]. The Ti3 C2 Tx MXene Matrigel composites (MXene-Matrigel) regulate the development of Cochlear Organoids (Cochlea-Orgs), particularly in promoting the formation and maturation of organoid HCs. Research has demonstrated that the feasibility of electrically conductive NGCs based on the rGO/BDNF/GelMA-integrated *Morpho* butterfly wing as functional nerve regeneration conduits, which may have potential value for application in repairing peripheral nerve injuries [75–79]. Ti3C2TxMXene is an efficient interface for the proliferation and neural differentiation of NSC and the maturation of NSC-derived neurons, which expands the potential uses of the MXene family of materials and provides new strategies for stem cell studies. The incorporation of Ti3 C2 Tx MXene nanomaterial into Matrigel regulates the properties of Matrigel and exhibits satisfactory biocompatibility. The Ti3 C2 Tx MXene Matrigel composites (MXene-Matrigel) regulate the development of Cochlear Organoids (Cochlea-Orgs), particularly in promoting the formation and maturation of organoid HCs. The reduced graphene oxide (RGO) substrate maintains embryonic stem cells (ESC) pluripotency by promoting E-cadherin-mediated cell-cell interaction and Wnt signaling [80,81,75,82].

5.2.2. Magnetic nanochain

Magnetic nanoparticles have a large specific surface area, good surface modification, and excellent supermagnetism. Magnetic nanochains can be used to guide the directional growth of neural stem cell-derived neurons. Under the guidance of magnetic nanochains, it was found that neural stem cells showed a good arrangement and neurons derived from neural stem cells showed good directional growth along the direction of nanochains.

Fe_3O_4 nanoparticles are coated with a thin layer of silica. Then, the dispersed Fe_3O_4 and SiO_2 nanoparticles are arranged by the magnetic field to form magnetic nanochains, which are fixed by a laminin coating. The monodisperse solution of neural stem cells was dropped onto the immobilized magnetic nanochains. After adhesion, proliferation, and differentiation culture were carried out in turn. The adhered neural stem cells showed a good directional arrangement, and the newly derived neurons grew along the direction of magnetic nanochains. Magnetic nanochains also have a good guiding effect on the neurite extension of newly derived neurons [83].

5.2.3. Superparamagnetic iron oxide

Nerve regeneration is a major challenge for neuroscience to treat degenerative diseases and repair damaged nerves. Physical stimulation is important for the growth and development of neurons. Superparamagnetic iron oxide nanoparticles (SPION) and magnetic fields (MFs) are used to orient neurons and grow neurites. SPION can orient neurites. SPION and MFs have the potential to regulate SGN, especially in neuronal orientation and neurite growth [84].

5.3. Gene therapy for hearing loss

Gene and cell therapy have become promising methods to treat hereditary diseases such as hearing loss [72]. At present, gene transfer technology involves two main ways: viral vectors and the use of non-viral vector transfection. So far, many different viral vectors have been

used to transduce the inner ear, including adenovirus, adeno-associated virus (AAV), lentivirus, herpes simplex virus, and newly developed synthetic AAV vectors. The main limitation when using viral vectors is their immunogenicity and cytotoxicity [42].

In treating hearing loss, gene therapy must overcome several obstacles related to the structural characteristics and anatomy of the inner ear. The cochlea is a closed, bone-covered, fluid-filled cavity that is very vulnerable to changes in the amount and composition of the inner ear fluid. Therefore, it is feasible to deliver therapeutic materials into the cochlea without destroying homeostasis. Another method is to implant an artificial cochlea in the cochlear ostomy [85].

Gene therapy depends on the identification of gene delivery vectors. The United States has approved gene therapy mediated by AAV vectors to treat a rare hereditary eye disease. The researchers screened and identified an AAV, that is, mutant AAV-ie-K558R, which could efficiently transduce HCs and stem cells in the cochlea of newborn mice. AAV-ie-K558R partially recovered hearing loss in prestin knockout mice. Importantly, it could transfer Atoh1 to the stem cells of the cochlea to generate HC-like cells. The results showed that AAV-ie-K558R had clinical potential for treating hearing loss caused by HCs death [83].

The use of genetically modified human-induced pluripotent stem cells to differentiate into auditory neuron-like cells can provide a promising complementary tool for the study of HHL. This will expand the possibility of studying deafness-related genetic mechanisms in hearing loss and seeking new cell therapies for HHL [83]. There are currently some Food and Drug Administration (FDA) approved gene therapies, such as Luxturna and Zolgensma, which use AAV as a carrier. They can effectively transduce many different types of cochlear cells, including neurons and HCs. At present, standard AAV vectors are unable to treat diseases caused by gene mutations larger than 3.5 kb. The relatively small packaging capacity is the biggest limitation of AAV as a gene therapy carrier. By using double or even triple AAVs to deliver large therapeutic genes to the inner ear or other areas, these size limitations of AAV vectors can be avoided. The application route of AAV vector will also significantly affect the distribution and degree of transgenic expression, thus determining the treatment result [86]. When injected into the inner lymph node locally, the rAAV vector based on the ape AAV8 capsid can effectively transfer the IHC and OHC of the mouse inner ear, but when applied in the whole body, it mainly transfers the mouse liver [87]. Net1 may be a potential target for HCs regeneration. AAV-mediated gene regulation can promote mouse cochlear sertoli cells to differentiate into HCs [88]. The research on the development, repair, and regeneration of the inner ear provides molecular pathways for the treatment of inner ear diseases. Implementing local gene delivery therapy requires other methods that affect the expression of inner ear genes. Therefore, research on gene carriers that can be used for inner ear gene therapy is particularly important. Gene modification therapy provides a complementary tool for the treatment and genetic mechanism research of HHL. The neurotrophic gene therapy technology can increase the survival number of SGN, enhance biological function, and induce the growth of auditory nerve fibers, so it can improve the benefits of cochlear implant treatment.

5.4. Progress of autophagy in preventing SNHL

Autophagy is a catabolic mechanism through which cytoplasmic components in cells, including proteins, organelles, aggregates, and many other intracellular substances, are transported to lysosomes for degradation. The autophagic proteins autophagy-relatedgene (Atg)5, Atg4b, Atg9a, Beclin-1, and LC3B (microtubule-associated protein 1 light chain 3 beta) are expressed in the cochlea and vestibule of mice and chickens from the developmental stage to adulthood. The decrease of autophagic flux in the inner ear by sequestosome-1 (SQSTM1)/p62 and the increase in LC3-II relative level indicate that autophagic flux is regulated during development and reaches a stable level at 2 months. In the cochlea of adult mice, the expression of LC3II was found to mainly relate to SGN, and its upregulation was accompanied by the accumulation of lipofuscin, especially in SGN. However, during aging, it did not occur in the Corti organ or stria vascularis of senescence accelerated-prone mouse 8 (SAMP8) mice. In conclusion, the results above suggest that autophagy may be a necessary housekeeping mechanism for SGN activity [42].

Macrophages can engulf cell fragments and dead cells in the spiral organ, and promote the regeneration of HCs in damaged sensory epithelium. When the cochlea is damaged, the monocytes migrate from the circulatory system to the cochlea and transform into mature macrophages. Macrophages not only activate HCs but also play a part in their regeneration. It can also protect SGN and ribbon synapse after being damaged [89]. Autophagy plays an antioxidant role in preventing SNHL. It also protects against ototoxic drug–induced injury in SGN. The application of kanamycin and furosemide can restore autophagy by damaging autophagy flux and lysosomal biogenesis, as well as by promoting the translocation of transcription factor EB (TFEB) to the nucleus, resulting in HCs loss and subsequent SGN denaturation, thus weakening SGN denaturation. In cisplatin-induced SGN injuries, rapamycin-activated autophagy can reduce SGN apoptosis and hearing loss. Therefore, autophagy can mitigate the HCs loss, SGN degeneration, and subsequent hearing loss. Studies have shown that neomycin disrupted mitophagy through transcriptional inhibition of Pink1 expression, the key initiator of mitophagy. In addition, neomycin impaired mitophagy by inducing ATF3 expression. Importantly, treatment with a mitophagy activator could rescue neomycin-treated hair cells by increasing mitophagy, indicating that genetic modulation or drug intervention in mitophagy may have therapeutic potential for aminoglycoside-induced hearing loss [84,90].

5.5. Signaling pathways related to hearing regeneration

It has been found that there are many transcription regulators and signal pathways that are critical to the development and regeneration of HCs in the cochlea. The progress made in understanding the signal network involved in the development and regeneration of HCs will contribute to the development of new strategies for regeneration of HCs. For example, the Notch, Wnt, Hedgehog signaling pathways that regulate the development of the inner ear, Hippo/YAP, and the Lin28 signaling pathways play an important role in reducing HC damage and maintaining auditory function. Similarly, transcription factors such as FoxG1, Atoh1, Pou4f3, Ikzf2, and Insm1 also play vital roles.

5.5.1. Co-regulation of WNT and Notch signals

Notch signal plays an important part in the upgrowth of inner ear sensory organs. In the early stage, Notch is required to designate the sensory progenitor cell bank that produces HCs, which are responsible for mechanical nerve conduction. Later, Notch sets up the mechanism of cell fate determination and establishes the well-known spatial model of HCs and stem cells [91].

In the inner ear, $Lgr5^+$stem cells are HCs progenitor cells. In the neonatal stage, they can regenerate new HCs. The $Lgr5^+$progenitor cells have limited regeneration efficiency and ability, indicating that there are other signaling pathways or factors involved in HCs regeneration. As the accented phase of gene expression regulation is the transcription extension stage, the transcription regulation of genes regulating development is the central part between embryonic stem cell differentiation and organ formation [92]. Compared with the untreated $Lgr5^+$progenitor (ULP) cells, neomycin-treated $Lgr5^+$progenitor (NLP) cells are more capable of regenerating HCs, and the proliferation ability of NLP cells was slightly higher than that of ULP cells. The research has identified the genes that may regulate the proliferation of $Lgr5^+$progenitor cells and HCs regeneration after neomycin injury. The role and mechanism of these genes in the cochlea should be studied in the future to determine the potential therapeutic target of HCs regeneration [93]. Hedgehog signal plays a key role in the upgrowth of the inner ear embryo and

participates in the proliferation and differentiation of progenitor cells. Hedgehog signal cannot initiate new HCs formation or cell multiplication in the intact cochlear sensory epithelium, but it can indeed promote the HCs regeneration and proliferation of SCs after neomycin-induced HCs injury. The occurrence of this phenomenon may have relevance to the change in the gene expression profile of progenitor cells after neomycin-induced HCs deletion [93]. Ribosomal protein S14 (Rps14) overexpression in the mice cochlea could promote SCs proliferation by activating the Wnt signaling pathway [94]. In the normal and neomycin-damaged cochlea, Notch inhibition after Wnt activation strongly promotes mitotic regeneration of new HCs, while partially retaining the number of stem cells. Most mitotic-regenerated HCs are derived specifically from $Lgr5^+$progenitor cells with or without HCs damage. The co-regulation of Wnt and Notch signals may provide a better way to regenerate HCs from the mitosis of $Lgr5^+$progenitor cells [95]. Atoh1 is a basic helix-loop-helix (bHLH) transcription factor that is transcriptionally regulated by several signaling pathways, including Notch and Wnt signalings. Transcriptional regulation of Atoh1 by Wnt or Notch signaling or post-translational regulation by the Huwe1-ubiquitin-proteasome pathway has been shown to successfully convert inner ear progenitor cells into HCs. The enhanced expression of Espin can optimize the developmental process of stereocilia in Atoh1-induced HCs and can attenuate the damage to native HCs induced by Atoh1 overexpression [96]. Pou4f3 is a transcription factor belonging to the Pou family, an essential gene for the formation of HCs, and a downstream target for Atoh1 activation in HC. Research evidence suggests that the function of Atoh1 in HCs differentiation is regulated through interactions with Gfi1 and Pou4f3. The coexpression of Gfi1, Pou4f3, and Atoh1 can effectively induce direct programming of mouse embryonic stem cells into HCs fate in vitro [97].

The Wnt, Shh, and Notch signaling pathways play crosstalk roles in promoting the mammalian cochlear sensory cells to regenerate, and Notch inhibition in combination with Shh signaling and/or Wnt activation affects the multiplication and regeneration of cochlear sensory cells in injured mice. Notch pathway inhibition promotes the HCs to be derived from inner ear progenitor cells, while Wnt pathway activation induces the multiplication of newborn mouse cochlear progenitor cells. The inhibition of the Notch signaling pathway can promote the induction of the proliferation of stem cells and the regeneration of HCs mitotic, while the inhibition of the Wnt signaling pathway can reduce stem cells proliferation and HCs regeneration. This means that Notch inhibits the above multiplication induced by the activation of the Wnt signaling pathway. After HCs are damaged, stem cell multiplication and new HCs regeneration are induced not only by inhibiting the Notch signaling pathway and activating the Wnt signaling pathway but also by upregulating SHH signaling pathways. The co-regulation of the above three signaling pathways may provide a novel and efficient approach to induce the proliferation of sensory progenitor cells and the regeneration of HCs after HCs injury in mammalian inner ear, as well as restoring and improving hearing and vestibular functions [98].

5.5.2. Role of FoxG1 in maintaining the formation of HCs in the inner ear

During the development of the inner ear, forkhead box G1 (FoxG1) is extremely important for maintaining the formation of HCs in the inner ear and ensuring normal cochlear morphology. The knockout of FoxG1 in HCs has a negative impact on cell survival after birth. The research results showed that FoxG1 affected the reactivity of simulated senescent HCs to inflammation by regulating the autophagy pathway. The nuclear transcription factor FoxG1 is chiefly involved in regulation of HCs, cochlear spiral neurons, and SCs formation, so as to maintain the function and morphology of the cochlea, in the inner ear. FoxG1 is involved in mitochondrial function, autophagy, and related signaling pathways. Therefore, further exploration and intensive study of the role of FoxG1 in the inner ear improves its application as a therapeutic point for HCs regeneration and SNHL [99]. FoxG1 is expressed in almost all cell types of the cochlea, utricle, sacculus, and cristae in the inner ear. Cochlear stem cells, including cochlear progenitor cells, have been proven to be a promising resource for HCs regeneration [100].

The activation of cochlear precursor cells is a promising method for HCs regeneration and hearing recovery. Studies found that most proliferative stem cells and HCs generated by mitosis induced by Notch inhibition came from Wnt reactive leucine-rich repeat containing G-protein-coupled receptor 5 (Lgr5+) progenitor cells. Notch inhibits and promotes the mitosis of Lgr5+ progenitor cells to generate new HCs. It provides a new way to regenerate HCs from progenitor cells [96]. A study found that FOXG1 plays an important role in the auditory degeneration process through regulation of macroautophagy/autophagy (Fig. 2).

6. Discussion and conclusion

Hearing problems are receiving increasing attention with the continuous progress of society and improvements in personal living standards. Therefore, based on the structure of the inner ear and its genesis and development mechanisms, studying the genesis and development of hearing impairment is of great significance for effective prevention, discovery, and treatment of hearing loss [101].

Deafness is the most frequent sensory disorder in the world. Most hearing loss is due to the loss of OHCs caused by the degeneration of inner ear mechanoreceptor sensory HCs, which is influential to the amplifier function of the cochlea. The loss of IHCs or their synapses causes an inhibiting effect on the sound signal coding. The absence of a spiral ganglion interferes with the coding or transmission of sound signals [69]. In mammals, the main cause of SNHL is the irreversible damage of cochlea HCs [95].

The known types of acquired hearing loss include drug-induced hearing loss, ARHL, and noise-induced deafness. The pathological characteristics of different types of hearing loss are different. The main mechanism of drug-induced hearing loss is HC loss. The mechanism of HC injury and apoptosis caused by ototoxic drugs is complex. The accumulation of ROS in cells activates a variety of signaling pathways related to apoptosis, such as the ROS/c-Jun N-terminal kinase (JNK) and caspase-independent apoptosis pathways, and can play an important role in inducing HCs death. When the production of ROS exceeds the cell repair limit, cell damage occurs, and the accumulation of ROS species leads to cell death [5].

The deletion of the vascular striatum cells and spiral ganglion is related to ARHL. Noise-induced deafness involves two main mechanisms: mechanical injury and loss of HCs and spiral ganglions, which are caused by excessive exposure to noise. Programmed cell death (PCD) seems to play a key part in the development and disease of the inner ear. When the nucleus is seriously damaged, the initiation of PCD leads to irreversible changes, such as metabolic stagnation and structural damage, thus balancing cell death and survival. The excessive accumulation of ROS and associated inflammation in the cochlea are the basis of various forms of SNHL. Therefore, active oxygen quenchers, antioxidants and anti-inflammatory drugs, inflammatory cytokines, or apoptosis pathway molecules that aim to reduce the production of ROS constitute the main potential drug categories to prevent various types of SNHL.

Many microRNAs and proteins were discovered to be related to inner ear development, anatomical structure development, and auditory nervous system development [102]. Based on an understanding of the development process of the inner ear, modern methods of hearing recovery focus on the generation of HCs from existing cochlear stem cells or endogenous stem cells through stem cell therapy, gene therapy, and molecular therapy, and by manipulating endogenous signaling pathways, HCs, and auditory neurons in the stem cells. Another method is to induce cell replication to regenerate HCs and stem cells [103]. In recent years, inner ear stem cells, gene therapy, signal regulation, and new materials have become the main ways to study HCs regeneration and hearing protection [5]. In particular, the 3D cell culture system of stem cells and new materials has become increasingly important in tissue engineering

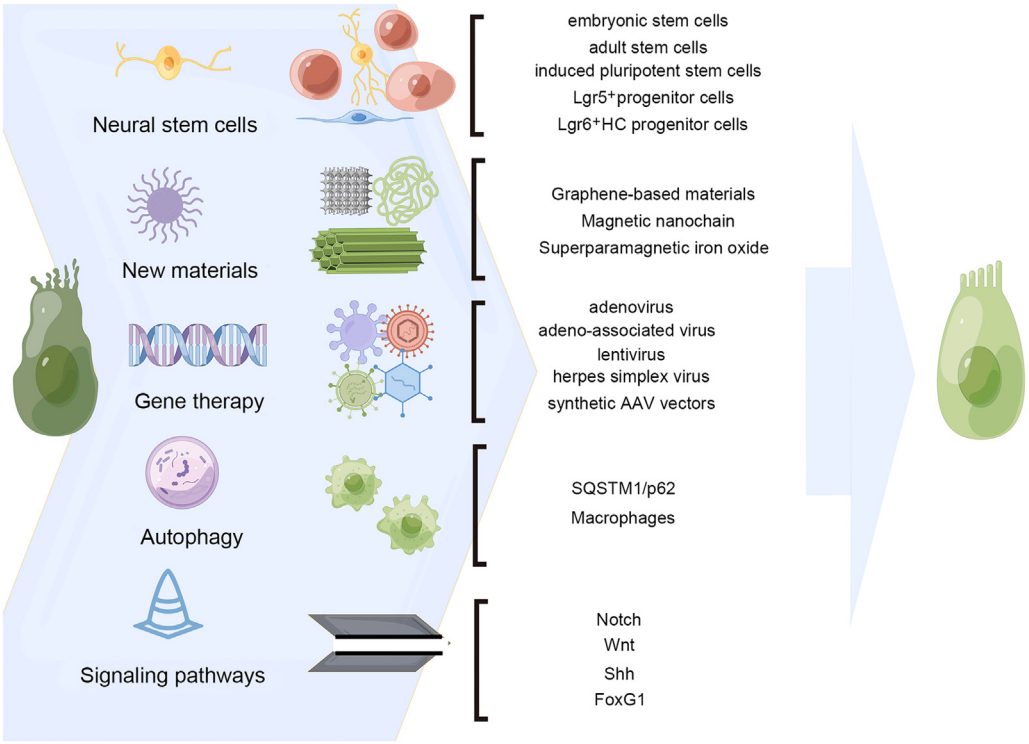

Fig. 2. Regeneration of damaged inner ear HCs. After injury, HCs in the inner ear can be regenerated through neural stem cells, new materials, gene therapy, autophagy, and signal transduction regulation.

and drug testing because of its advantages in providing physiological-related information. Using these 3D cell cultures, primary cultures that are more stable and have a longer life span than conventional 2D cultures are maintained. Further research should be carried out to prolong the survival time of HCs and eliminate antibiotics and other factors that can cause hearing loss [104]. In the process of the treatment of auditory diseases by using neural stem cells, more new technologies and materials have been developed, which have promoted neural stem cells to realize clinical application [105]. Researchers have invested a lot of energy and made many efforts to combat hearing impairment, including new materials, gene therapy, antioxidant drugs, HCs regeneration, and cochlear implantation, which is the ultimate research goal in this field. The treatment of inner ear diseases has always been a challenge for researchers. Nowadays, the treatment of hearing loss in clinical practice is still limited to the use of drugs. We have made exciting progress in the research field of attempting to restore hearing or prevent deafness and HCs regeneration. However, the existence of various physiological barriers, mainly the blood labyrinthine barrier, limits the accessibility of the inner ear and hinders the efficacy of various drug treatments. The current research focuses on local drug delivery, gene and cell therapy, but there is still a long way to go for the above methods to be applied in clinical practice. However, the current research is more limited to cell lines and animals. In clinical practice, we still need to further understand the potential mechanisms of auditory genetics, the continuous research on human genome, the improvement of transmission technology and the standardization of treatment strategies.

This study started with the genesis and upgrowth of the inner ear and reviewed the anatomical structure of the normal development of the mammalian inner ear, including the analysis of the inducement mechanism of hearing loss from the aspects of medicine, genetics, age, gene, and so forth. The technology and application of regeneration of inner ear HCs after hearing damage were described in terms of stem cells, gene therapy, signal transduction, new materials, and so forth, to provide new references and research targets for the research of HCs regeneration and hearing protection.

CRediT authorship contribution statement

Xin Bing: Writing – original draft, Conceptualization, Project administration, Supervision, Resources. **Chengcheng Liu:** Conceptualization, Project administration, Writing – original draft, Resources. **Xue Cao:** Conceptualization, Validation. **Chengzhilin Li:** Resources, Conceptualization. **Xiaochen Gao:** Resources. **Fangyuan Zhu:** Data curation. **Xinhao Wu:** Validation. **Na Guo:** Investigation. **Houyang Hu:** Visualization. **Ming Xia:** Writing – review & editing, Funding acquisition. **Miaoqing Zhao:** Conceptualization, Writing – review & editing, Resources, Funding acquisition, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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