

Application of gene therapy in auditory system diseases

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ABSTRACT

Prevention and treatment of auditory related diseases through genetic intervention is a hotspot in the field of hearing research in recent years. With the development of molecular technology, gene regulation has made a major breakthrough in the research of inner ear hair cell regeneration in recent years, which may provide us with a novel and efficient way to treat auditory related diseases. This review touches on the latest research on gene therapy in hereditary deafness, drug deafness, aging-related hearing loss, and noise-related hearing loss.

Keywords: Gene therapy · Hearing · Transfection vector · Auditory diseases · Inner ear

Introduction

Hearing loss is the most common sensory deficit in humans. Approximately 466 million people worldwide suffer from deafness, and the number is expected to increase to nearly 1 billion by 2050 (<http://www.who.int/mediacentre/factsheets/fs300/en/>). In recent years, with the in-depth development of research on the pathogenesis of deafness, many researchers hope to improve or rescue hearing through gene therapy. We will introduce the research progress and new findings of gene therapy in hearing in this manuscript.

Construction of animal models

In order to better assess the efficiency, safety and therapeutic effects of gene transfection, it is necessary to construct a suitable animal model with a specific disease.

Mammalian model

Mice are one of the most commonly used models in mammals. At present, we have established a variety of mouse models of hearing loss induced by genes mutations to mimic some genetic mutations in human patients. However, the evolutionary relationship between mice and humans is relatively distant, and the biological differences are large. The ears of primate animals are more anatomically similar to humans, and they are more advantageous as animal models for gene therapy (1-3).

Therefore, research on primate models has become a hotspot. Dai et al. evaluated whether the rhesus monkeys injected with sufficient amounts of fluid would suffer inner ear damage during the gene therapy process. The transfection conditions were optimized by comparing the transfection effects of the oval window stapedotomy pathway and the round window pathway (4). In addition, György et al. introduced the adeno-associated virus (AAV)-9 variant (aav9-php.b) into cynomolgus monkeys through round window membrane (RWM) and found that almost all of the inner and outer hair cells (HCs) were transfected. This is the first time that an AAV vector transfection assay has been performed on the inner ear of a primate (5).

Non-mammal model

Because non-mammals are genetically different from humans, they are rarely used in gene therapy research. However, the similarity between the zebrafish gene and the human gene is as high as 87%. Previous studies have found that *prps1a* and *prps1b* in the zebrafish genome are very similar to the *PRPS1* in the human genome, which provides support for zebrafish as a model for studying human hearing loss (6). In addition, the emergence of the CRISPR/CAS9 technology has made it possible to establish a model of hereditary hearing loss in zebrafish. In the latest report by Bing Zou et al., a zebrafish mariner mutant model was constructed using CRISPR/CAS9 (7).

Application of gene transfection vectors

Choosing the ideal gene transfection vector is a prerequisite for gene therapy. The ideal vector not only

has high transfection efficiency *in vivo*, but also can accurately deliver DNA into target cells or tissues. In addition, whether the transfection intensity and time of the vector are controllable, whether it is safe and convenient for large-scale preparation are also factors for evaluation. At present, vectors are mainly divided into two major categories: viral vectors and non-viral vectors.

Viral vectors

Viral vectors include adenovirus, adeno-associated virus, helper-dependent adenovirus, herpes simplex virus, vaccinia virus, etc. Viral vectors are the most widely used vectors due to their high transfection efficiency. However, the traditional viral vector still has certain toxicity, and the gene carrying capacity is small. In recent years, more ideal viral vectors have been discovered through the mutation of AAV virus, which has improved the effectiveness and safety in specific applications, such as AAV2/ANC80L65 (8, 9), AAV-ie (10), and AAV9-PHP.B (11), etc (**Figure 1**).

Non-viral vectors

Non-viral gene vectors mainly include cationic liposomes, nanomaterials, etc. Compared with viral vectors, non-viral vectors are simple to prepare, low toxicity and do not induce host immune responses. Non-viral vectors have no restriction on the capacity of carrying genes, and can be modified to obtain a variety of biological properties. However, the transfection

efficiency of non-viral vectors is relatively low.

Gene delivery pathway

At present, the gene delivery pathways commonly used in animal experiments are as follows: (1) perforation in the cochlea; (2) perforation in semicircular canals; (3) injection of RWM; (4) infiltration of RWM. The choice of path depends mainly on factors such as the subject, the type of viral vector used, the transfection efficiency of the target cell and the degree of damage to the inner ear. In addition, the electroporation technology has been used as an alternative method for gene delivery in the inner ear in recent years, but it is still limited to *in vitro* tissue culture and gene therapy research in the uterus (12, 13).

Gene therapy strategy

Gene therapy mainly treats diseases through gene replacement, gene silencing, gene editing and HC regeneration. Gene replacement is the most direct way of gene therapy and is mainly used in the treatment of recessive hereditary diseases with mutation-induced phenotypic loss. For hereditary diseases associated with dominant inherited mutations, gene silencing via antisense oligonucleotide and RNA interference (RNAi) are mainly used for treatment. Currently, gene editing is mainly implemented using the CRISPR/CAS9 technology. It is the most advanced programmable nuclease for genome engineering and repair of targeted gene mutations.

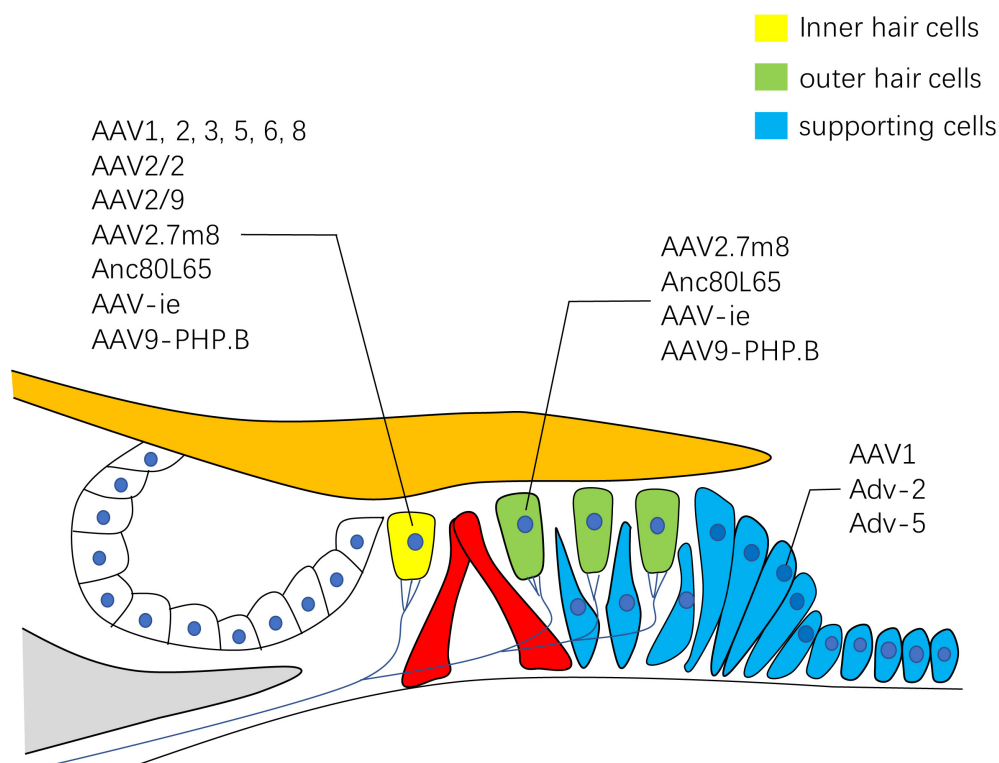


Figure 1. Viral vectors with specific transfection efficiency for different target cells. Studies have shown that AAV2.7m8, Anc80L65, AAV-ie, AAV9-PHP.B have high transfection efficiency in both inner hair cells and outer hair cells; AAV1, 2, 3, 5, 6, 8, AAV2/2, AAV2/9 also have higher transfection efficiency in inner hair cells, AAV1, Adv-2, Adv-5 have higher transfection efficiency in supporting cells; Adv: Adenovirus; AAV: adeno-associated virus.

Finally, studies have shown that HC can be regenerated by modulating specific genes. Due to the damage of HC cells in most hearing-related diseases, it is suggested that HC regeneration methods will be widely used in hearing therapy in the future.

Gene therapy in auditory diseases

The sound perception and information transmission organs in the auditory system mainly include inner ear HCs, spiral ganglia neurons, and auditory central organs. The lesions of these cells or tissues can affect the perception and transmission of sound, and ultimately lead to hearing loss. Drug-induced deafness, sudden deafness, hereditary deafness, senile deafness, and noise deafness are clinically common types of sensorineural hearing loss. At present, the treatment of sensorineural hearing loss is mainly by wearing hearing aids and cochlear implants. Gene therapy is a biology-based intervention that does not require any device to reconstruct hearing. It is more convenient and economical for patients with sensorineural hearing loss. Therefore, gene therapy is very important for the treatment of sensorineural hearing loss.

Gene therapy for hereditary deafness

Hereditary deafness refers to hearing organ developmental disorders and hearing impairment due to abnormalities in genetic factors such as genes or chromosomes. Currently, more than one hundred non-syndromic hearing loss genes have been identified, including 71 autosomal recessive genes, 45 autosomal dominant genes, and 5 X-linked genes (<https://www.hereditaryhearingloss.org>). About 30% of hereditary hearing loss is associated with syndrome (14). There are currently 11 syndromes related to hearing loss, and there are 47 related genes, of which 27 are autosomal recessive, 13 are autosomal dominant, and 4 are autosomal dominant or recessive, 2 are X-linked recessive inheritance (15). Human disease is modeled by establishing animal models of multiple hereditary deafness, followed by gene therapy and intervention. Gene therapy for hereditary deafness has made some progress. Current research results have shown that gene therapy was effective in animal models of multiple hereditary deafness and is expected to be clinically transformed in the future. The popular genes currently studied include *Gjb2* (16), *Vglut3* (17), *Pou4f3* (18), *Tmc1* (19), *Kcnq4* (20), *Ush1c* (21) and *MsrB3* (22). In addition, some potential genes have recently been found to be associated with hearing loss, which may be the new direction of our research in the future, such as *Clarin-2* (22, 23), *Pls1* (24), *Cldn9* (25), etc. However, there are some limitations in the current research involving neonatal mouse models. Mice mature after a few weeks of birth, whereas humans are well-developed at birth, so intervention on newborn mice is similar to intervention at the human fetal stage. To better mimic postnatal genetic deafness, we need to expand the time span of intervention. Recently, Yoshimura et al. reported on the study of the adult mice deafness model of *Tmc1* mutation. They injected microRNA into the cochlea via the AAV vector and showed that RNAi-mediated gene

silencing inhibited hearing loss and increased survival of inner hair cells (IHC) (26). This result illustrates the feasibility of gene therapy in adult mouse models.

Gene therapy for drug-induced deafness

Drug-induced deafness is a sensorineural hearing loss caused by the application of antibiotics, antineoplastic drugs, etc. Common ototoxic drugs include aminoglycoside antibiotics and chemotherapeutic drugs such as cisplatin. For drug-induced deafness, there is still no optimized treatment yet. The best way to deal with drug-induced deafness is to reduce chances of its occurrence through early prevention. In recent years, drug deafness has been found to be associated with genetic variation, which may provide a reference for new treatments. Aminoglycoside antibiotics are commonly used antibiotics that often cause ototoxicity, leading to permanent HC loss and hearing damage. The ototoxic effects of aminoglycosides are related to oxidative stress. Studies have confirmed that antioxidant gene therapy has an inhibitory effect on aminoglycoside-induced oxidative stress in the inner ear, thereby promoting HC survival (27). Ototoxicity caused by cisplatin is a serious side effect in patients undergoing cisplatin therapy. However, ototoxicity caused by cisplatin shows significant differences among individuals, which may be due to different genetic backgrounds. Genome-wide association studies (GWAS) have found that *ACYP2* (28) and *WFS1* (29) variants are closely related to cisplatin-induced ototoxicity, and some studies have reported *SOD2* mutations associated with ototoxicity in cisplatin-treated children with medulloblastoma (30). Britt I. Drögemöller et al. have demonstrated that a variant of *Slc16a5* is a novel genetic risk factor for testicular cancer patients. Silencing of *Slc16a5* *in vitro* can alter cisplatin-induced cellular responses, suggesting that *Slc16a5* plays a role in cisplatin-induced ototoxicity (31). Other studies have shown that overexpression of X-linked inhibitor of apoptosis protein in a mouse model also has an inhibitory effect on cisplatin-induced hearing loss (32-34).

Gene therapy for senile deafness

Senile deafness, also known as age-related hearing loss (ARHL), is a cumulative pathological and physiological change in the age-related auditory system. Senile deafness is a progressive and irreversible hearing loss, especially at high frequencies. According to the World Health Organization, senile deafness is the second most common disease among the elderly and the third most prevalent disease in the world. It is estimated that by 2025, more than 500 million people will suffer from senile deafness. At present, the etiology of senile deafness is not very clear, and it is generally considered to be caused by interaction of various factors. A large number of studies have shown that mutations in some genes may increase the susceptibility to senile deafness. GWAS have been conducted to identify genes that may be associated with ARHL and found significant mutations in *Prkce* (35), *Tgfb1* (35), *Nrf2* (35), *Grm7* (36), and *Cdh23* (37) in ARHL. In addition, mtDNA4977 (38), *Nkcc1* (39),

mucolin 1 and 3 (40), *Wfs1* (41), *Slc7a8* (42), *Cox3*, *Gjb2* (43), *Idh2* (44), neuropilin-1 (45), *Igf1* (46, 47), *Essrg* (48), *P2rx2* (49), *Kcnq5* (49), *ErbB3* (49), *Socs3* (49), *Slc26a4* (50), *Spatc11* (51), *Bak1* (52), *Ccr3* (53) and *Gilz* (53) are also associated with ARHL, which may be potential target genes for the treatment of senile deafness by gene therapy in the future. In addition, in the research of animal models, it is found that activation of *Sirt1* expression activates autophagy pathway, reduces HC death and hearing loss, and prevents the development of senile deafness (54). Senile deafness is closely related to the irreversible death of HCs. The treatment of senile deafness by the protection and regeneration of HCs has attracted the interest of many researchers. With the extensive development of HC regeneration research, a number of potential gene therapy targets have been discovered, the most representative of which is the *Atoh1* gene. The *Atoh1* gene is a transcriptional regulator required for HC differentiation. A number of animal studies have demonstrated that the overexpression of the *Atoh1* gene in the inner ear can induce the trans-differentiation of the supporting cells (SCs) in the Corti to the HCs to achieve the purpose of HC regeneration in the cochlear and vestibular (55). At present, the intervention methods for senile deafness are still mainly to improve hearing, such as wearing hearing aids, but in the future, gene therapy is expected to be an effective method for treating ARHL.

Gene therapy for noise deafness

Noise-induced hearing loss (NIHL) is a slow progressive sensorineural deafness caused by prolonged exposure to noise stimuli. It is generally believed that the impairment of noise on hearing is related to the acoustic properties of the noise, the noise environment and individual conditions. Studies have shown that individuals' sensitivity to noise-induced hearing loss is related to genetic factors. CAT, GSTM1, PON2, and SOD2 are significantly associated with activation of the oxidative stress pathway, while HSP70-1 and HSP70-2 mediated heat shock responses provide hearing protection in noise damage (56). It has also been highlighted that P2RX2 is an important protein that attenuates sound transmission in the context of high-intensity noise (57). Noise can cause HC morphology change or even death, accompanied by degeneration of pillar cells, SCs, vascular streaks, peripheral nerve, and ganglion cells. Therefore, the protection and regeneration of hearing-related cells (such as HCs, SCs, etc.) is a key factor in the treatment of NIHL. In addition to the above-mentioned treatments using ATOH1 to induce HC regeneration, brain-derived neurotrophic factor (18) and neurotrophin-3 (NT3) (58) have been shown to promote the growth and maintenance of ribbon synapses and vestibular epithelium. Studies have found that up-regulation of NT3 expression in postnatal mice can regenerate the ribbon synapses, thereby promoting the recovery of cochlear function after hearing injury (59). Although the current application of gene therapy to NIHL is still in the exploratory stage, it has detected significant hearing protection effects after inhibiting hearing loss

susceptibility-related genes, up-regulating HC protection-related genes and HC regeneration-related genes.

Conclusion

Gene therapy in the field of hearing is a research hotspot in recent years. Although some progress has been made, most studies are still limited to cellular level or animal models. There are still many mechanisms for clinical transformation to explore. The current limitation of gene therapy is that animal models, gene vectors and delivery pathways need to be further optimized. In addition, current research on gene therapy mainly focuses on sensorineural deafness, but less on other diseases of the auditory system. Gene therapy has potential clinical application prospects, in addition to correcting gene mutations, protecting HCs, SCs and spiral ganglion neurons from various damages, gene therapy may also induce HC regeneration. Currently, there are more than 2,500 clinical trials on gene therapy, including approved, ongoing, and completed, of which 2 are related to hearing treatment (<https://clinicaltrials.gov/>). With more and more hearing-related genes being identified and the development of gene therapy technology, more hearing-related clinical trials are expected to be conducted. Although gene therapy still faces various problems in the treatment of hearing disorders, in the future, this treatment will become a highly effective and safe treatment for hearing-impaired patients.

Conflict of interest

The authors declare no competing financial interests.

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