

EXPERT INSIGHT

Progressing towards a cure for deafness through gene therapy

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HEARING LOSS

Acquired and hereditary deafness affect an estimated 360 million people worldwide (World Health Organization, 2013). There are currently no pharmacological interventions on the market for hearing loss despite recent advances in our understandings of the capacity of the cochlea for repair and regeneration. However, gene therapy is emerging as a promising method to deliver factors necessary for recovery of hearing. In the cochlea, there are some challenges to overcome before gene therapy can be considered in the clinic, including efficiency and specificity of transduction and overcoming the cellular degenerative changes that accompany hearing loss.

GENE THERAPY IN THE COCHLEA

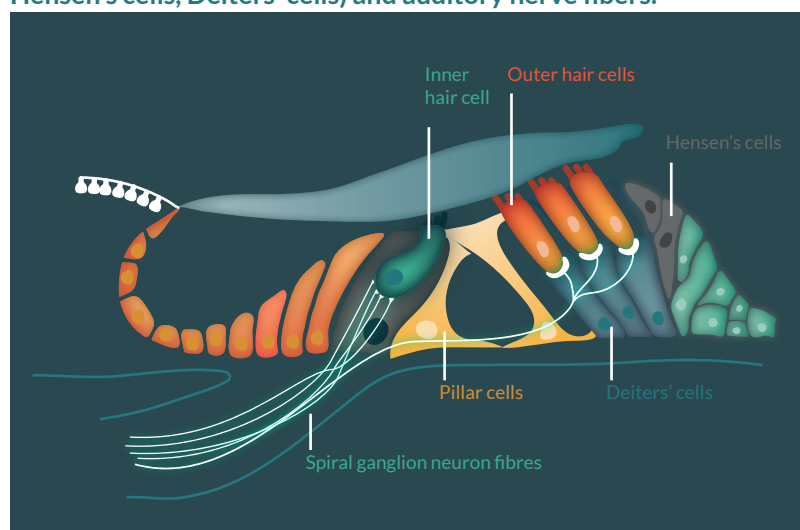
The cochlea houses the organ of Corti: the hearing sensory organ that contains one row of inner hair

cells, three rows of outer hair cells, supporting cells and innervating auditory nerve fibers from spiral ganglion neurons (Figure 1). All of these cells are susceptible to degeneration after exposure to excessive noise, ototoxic drugs or simply with aging, leading to acquired

hearing loss. Gene therapy is being studied as a means to prevent or reverse their degeneration [1]. Cells of the organ of Corti are also commonly affected in hereditary deafness, with gene therapy offering the potential to correct the genetic defect.

► FIGURE 1

The organ of Corti, showing hair cells, supporting cells (pillar cells, Hensen's cells, Deiters' cells) and auditory nerve fibers.



All of these cells are sensitive to damage and degeneration. Gene therapy can provide factors to rescue these sensory cells and recover hearing [1].

Direct injection of viral vectors (e.g., adenovirus or adeno-associated virus; [AAV]) into cochlear fluids through a small hole drilled into the cochlear wall (cochleostomy) or through the round window membrane of the cochlea ensures localised gene transduction, but with extremely variable efficiency. For example, the number of transduced hair cells can be as low as 15% or as high as 100% following injection with AAV (serotype 1 or 8) into mouse cochleae, with age being a contributing factor [2,3]. A sealed injection system is also critical, as the fluids of the cochlea readily leak out during injection and severely reduce the transduction efficiency [4]. Unfortunately, loss of residual hearing can occur with direct injection into the cochlear fluids [5,6]. To overcome this hearing loss and to maintain a sealed cochlea, the permeability of the round window membrane to some viral vectors has been exploited, with factors such as hyaluronic acid shown to enhance its permeability to adenovirus and Sendai virus [7-9]. The cochlear implant has also been used as a delivery device for gene therapy. A DNA gene construct was introduced to the cochlea via a modified cochlear implant where electrical stimulation from the implant itself was used to electroporate cells in the cochlea, with resulting gene expression in cells lining the scala tympani [10].

Cell specificity of gene expression is also important and this can be achieved in the cochlea in a number of ways: utilizing the anatomy of the cochlea (injection into different fluid chambers of the cochlea [5,11-13]); different serotypes of AAV, which naturally target different cell types of the cochlea; and

specific targeting of hair cells or neurons with cell-specific promoters [14-16].

Specific targeting of hair cells or other cells of interest and safe and efficient injection techniques will be necessary to develop effective molecular therapies for functional hearing protection and/or recovery.

GENE THERAPY FOR ACQUIRED HEARING LOSS

Two of the key cell types affected by acquired deafness are cochlear hair cells and neurons. Protecting and regenerating these cells via gene therapy are key steps towards reversing hearing loss.

Neurotrophin gene therapy

Neurotrophins are diffusible proteins, which are critical for neuronal fiber outgrowth and patterning during cochlear development and also for continued neural survival. The necessity to promote neural survival after deafness is of critical importance as neurons are the targets of electrical stimulation from cochlear implants.

Neurotrophin gene therapy in the cochlea has been shown to prevent neuronal degeneration after ototoxic-induced hearing loss for up to 6 months, with the effect localized to the region most proximal to the injection site where the highest level of viral transduction occurs [17-20]. In addition to protecting total neuron number, neurotrophin gene therapy also promoted localized and directional peripheral fibre resprouting, highlighting the potential to enhance the nerve-electrode interface of a cochlear implant or to direct the growth of fibers back to regenerated hair cells [5,17,21].

Recent findings strongly suggest neurotrophin gene therapy is a promising approach to enhance auditory nerve survival for an extended period [17,18,21]. However, if the neurotrophin gene therapy is targeted to the organ of Corti, neuronal survival is not sustained beyond 6 months in guinea pigs due to continued degenerative changes associated with acquired hearing loss. The time between hearing loss onset and treatment, and hence the level of degeneration, has also been shown to critically reduce the efficacy of neurotrophin gene therapy [22].

Neurotrophin gene therapy has been shown to be extremely effective in protecting auditory neurons and stimulating the regrowth of auditory nerve fibres. Targeting the therapy to cells that do not degenerate after hearing loss, such as cells lining the scala tympani or glial cells will result in continual release of neurotrophins to ensure long-term outcomes. However, more research is needed to link neural survival to improved cochlear implant function and whether this improvement is clinically meaningful.

Atonal gene therapy

Atoh1 is a transcription factor necessary for both hair cell development and survival and forced expression of Atoh1 in supporting cells of the organ of Corti initiates their trans-differentiation into ectopic hair cells [11,23–27].

The rates of conversion of hair cells and hearing recovery is highly dependent on age, degree of hearing loss and method of Atoh1 transduction [11,28,29]. *In utero* gene transfer of Atoh1 in mice results in ectopic, induced, functional hair cells, but when applied after birth Atoh1

is less effective, as demonstrated by poor hearing recovery outcomes and lack of mature markers in induced hair cells [11,28,29]. However, there have been some sporadic reports of partial recovery of hearing and balance function by Atoh1 in mature animals, suggesting that recovery of function is highly dependent on cellular context and level of pathology [27,30–35]. To improve therapy efficacy, it has also been suggested that regenerative approaches could consider reprogramming the senescent supporting cells post-deafness, via a small-molecule-based approach, enabling them to better respond to differentiating factors such as Atoh1 [36].

After sudden, severe hearing loss, there is only a short window of opportunity (at least in animal hearing loss models) during which residual supporting cells can be transformed into induced hair cells with Atoh1 gene therapy due to loss of differentiated supporting cells for transdifferentiation [11,27,37]. This window of opportunity is greater in a progressive, partial hair cell loss model, which may more accurately reflect human hearing loss pathologies [12]. Interestingly, it was shown that Atoh1 gene therapy after simulated gun-shot noise exposure could improve hearing thresholds through the repair of stereocilia of residual hair cells rather than the regeneration of new hair cells, showing a function of Atoh1 beyond *de novo* regeneration that could be developed into a molecular therapy for hearing recovery [38].

Developmentally, it is known that the expression pattern and level of Atoh1 is tightly and dynamically regulated [25,39,40]. Commonly used in Atoh1 gene therapy studies are adenoviral vectors with strong cytomegalovirus promoters for

constitutive gene expression, which fail to mirror endogenous expression patterns and may impact on survival, maturation, and function of the resulting hair cells. Moreover, after transdifferentiation into induced hair cells, a continued but lower *Atoh1* expression level may be essential for maintenance of induced hair cells via its regulation of expression of other genes known to be involved in hair cell survival such as *Gfi1*, *Pou4f3*, *Neurog1* and *Barhl1* [39,41,42]. The development of doxycycline-based conditional and inducible *Atoh1* expression systems will prove useful in studying regulated *Atoh1* expression *in vivo*, but is challenging with the blood–labyrinth barrier excluding doxycycline from the cochlea, preventing its clearance, and contributing to hearing loss [43–45].

Transforming supporting cells into hair cells will negatively impact on the cytoarchitecture and function of the organ of Corti, with supporting cells being critical for cochlear function. For recovery of function, it will be necessary to first promote the proliferation of supporting cells prior to *Atoh1* gene therapy. Supporting cells from adult cochleae maintain the capacity to divide with therapeutic manipulation, with co-transfection of *Pax2* and *Atoh1* resulting in regenerated hair cells *in vitro* [46,47].

The prospect of regenerating hair cells after hearing loss is exciting and understanding the molecular dynamics involved in creating a mature functional hair cell from existing cells in the cochlea will help to develop a therapy that might require more sophisticated regulation of *Atoh1* expression or more than one gene.

GENE THERAPY FOR HEREDITARY DEAFNESS

At least half of all childhood deafness is inherited, with at least 80 deafness genes identified to date. Using mouse models of human hereditary deafness, a number of recent reports have shown significant progress towards hearing recovery following gene therapy.

In *VGLUT3* knockout mice, there is a defect in the release of glutamate from inner hair cell afferent synapses. When AAV1–*VGLUT3* was delivered to the round window membrane of the cochlea of mice at postnatal day 1–10, there were measurable improvements in hearing function within 7 days and complete restoration of function by 14 days, extending up to 9 months after gene delivery [2]. Connexin26 mutations account for a significant number of hereditary deafness cases and deletion of connexin26 in mice results in significant degeneration of the organ of Corti and auditory neurons. Injection of AAV–*Cx26* into the scala media of *Cx26* knockout mice at P0–1 improved morphology and cell communication in the organ of Corti but did not recover hearing, while degeneration of auditory neurons was reversed by neurotrophin gene therapy in 1 month old mice [48,49].

Many deafness syndromes are the result of mutations in hair cell stereocilia such as DFNB31 or type II Usher syndrome and are modelled by Whirler mice with affected stereocilia. Injection of AAV8–Whirlin through the round window membrane resulted in Whirlin expression in the tips of the stereocilia and restoration of stereocilia structure. Likewise, the *Tmc1* gene (affected in human DFNB7/11 and DFNA36) was introduced into mice with a

targeted deletion of *Tmc1* (Beethoven mice) via AAV2/1 (via the round window membrane of P0–2 mice), with resulting expression in the tips of stereocilia and recovery of hearing thresholds, although not to wild-type levels [50].

These studies emphasize the potential for gene therapy to counteract the functional defects of various underlying genetic faults that lead to loss of hearing. With further developments gene therapy could result in restoration of normal hearing function for many types of hereditary deafness with long-term or even life-long benefit.

CONCLUSIONS

The prevalence of hearing loss necessitates the development of a therapy that can prevent the degenerative changes associated with hearing loss and even restore hearing. Gene therapy is of particular interest for the correction of genetic defects that affect hearing and for the restoration of hair cells and neurons after acquired hearing loss.

For translation of potential gene therapies for hearing loss there is the need to optimise methods of gene transduction. This includes improving gene targeting to specific sub-populations of cells in the cochlea, controlling induced gene expression and optimising transfection efficiency through improved viral vectors and method of injection [1,51]. The round window membrane of the cochlea will be the most likely method of delivery of gene therapy in humans due to its accessibility and potential for hearing preservation, but overall transduction rates need to be increased for hearing recovery to be clinically meaningful [52–54]. This

will necessitate the development of a gene therapy delivery system for the cochlea that prevents losses during injection and/or indirect methods that do not compromise the cochlea such as diffusion across the round window membrane.

A major challenge to overcome is the rapid decline in the efficiency and effectiveness of gene therapy with age. Most gene therapy studies with reported hearing recovery are performed in neonatal and early post-natal mice, highlighting the need for early diagnosis and intervention for congenital deafness and more research on gene therapy in mature subjects; the main group in the population with acquired hearing loss.

In parallel to improving general gene therapy techniques, *Atoh1* gene therapy in particular will benefit from a greater understanding of the timing and dosage of *Atoh1* and the co-factors required for mature hair cell formation, both developmentally and after injury to the cochlea [35]. A one-gene approach may have some role, perhaps in hair cell repair, but the best chance of recovering hearing may come from co-expression of *Atoh1* with other factors that will initially help supporting cells to divide but also other factors such as *Gfi1*, *Pou4f3*, *Neurog1* and *Barhl1* that will help induced hair cells to fully differentiate, attract nerve fibers and survive long-term.

Collectively, these studies demonstrate that gene therapy holds promise for curing deafness, however, the efficacy of cochlear gene therapy in a human patient population remains to be determined. Medical researchers are currently conducting a clinical trial to assess the safety and efficacy of *Atoh1* therapy in those suffering severe acquired hearing loss. The findings of this study will provide useful

insight into the overall safety and efficacy of viral-mediated gene therapy. However, critical factors such as expression levels and duration, degree of pathology and age outlined above go beyond the scope of this initial trial and will also need to be addressed to more fully elucidate the efficacy of Atoh1 gene therapy in the desired patient population. There is much to be hopeful for and a deeper understanding of the molecular nature of hair cell formation and function, along with engineering advances in viral delivery techniques, will lead to great advances in the near future.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

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