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Vestibular regeneration – experimental models and clinical implications

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Abstract

Therapies aimed at the protection and/or regeneration of inner ear hair cells are of great interest, given the significant monetary and quality of life impact of balance disorders. Different viral vectors have been shown to transfect various cell types in the inner ear. The past decade has provided tremendous advances in the use of adenoviral vectors to achieve targeted treatment delivery. Several routes of delivery have been identified to introduce vectors into the inner ear while minimizing injury to surrounding structures. Recently, the transcription factor Atoh1 was determined to play a critical role in hair cell differentiation. Adenoviral-mediated overexpression of Atoh1 in culture and *in vivo* has demonstrated the ability to regenerate vestibular hair cells by causing transdifferentiation of neighbouring epithelial-supporting cells. Functional recovery of the vestibular system has also been documented following adenoviral-induced Atoh1 overexpression. Experiments demonstrating gene transfer in human vestibular epithelial cells reveal that the human inner ear is a suitable target for gene therapy.

Keywords: vestibular hair cells • gene therapy • adenoviral vectors

Introduction

Dizziness and vertigo are extremely common symptoms affecting more than 90 million individuals each year in the United States [1]. In 1991, nearly 5.5 million outpatient visits were determined to occur for the evaluation of vertigo [2]. Moreover, it has been stated that dizziness is the most common presenting complaint in patients 75 years and older [1, 2]. Vestibular disturbances also contribute to the occurrence of falls, which are the sixth-leading cause of death in patients over 75 years of age [1–3]. Falls result in greater than 200,000 hip, vertebral, skull and extremity fractures in the elderly, and falls are attributed to an estimated healthcare expenditure of $19,000/year/patient [3].

Although different factors are involved in the pathogenesis of balance disorders, the loss of vestibular hair cells represents a common pathway leading towards balance dysfunction [4]. Viral disorders of the labyrinth such as herpes zoster oticus [5], aminoglycoside toxicity [6], autoimmune disease [7], degenerative disorders and progressive disorders such as ageing all result in loss of vestibular hair cells [8]. Signs and symptoms of hair cell loss depend on the timing and severity of the injury to vestibular receptors. Vestibular injuries may be sudden, progressive, fluctuating, unilateral, or bilateral in nature. Central compensation is a normal component of the recovery process and occurs soon after injury to the peripheral vestibular system [9]. However, in bilateral vestibular lesions, central compensation is unable to accommodate total or near-total loss of vestibular function, and patients experience severe visual disturbances upon head

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movement (oscillopsia) [9, 10]. Currently, no clinical treatments are available for patients with a complete loss in vestibular function [10]. Currently, complete hearing loss is treated with amplification or cochlear implantation depending on the severity of the lesion. Unfortunately, there is currently no vestibular equivalent to a cochlear implant, and the engineering challenges in creating such a device are considerable [11, 12]. Baker *et al.* recently emphasized that complete recovery from vestibular loss might be possible by replacing the missing vestibular sensory cells [13, 14]. Nevertheless, the vestibular system as a whole may have a latent potential for repair, which would explain the periodic reports of balance recovery following aminoglycoside ototoxicity in humans [13]. This latent potential may be further enhanced by new treatment strategies that have emerged over the past decade.

Cellular morphology and physiology of the vestibular sensory cells

The original vertebrate hair cell (HC) probably evolved in aquatic animals before the evolution of terrestrial vertebrates. Its function was, as it is now in many species, to monitor water currents relative to the body surface. The apical portions of the HC, equipped with cilia, were exposed to the water, whereas the basolateral cell membrane was contacted by extracellular fluid [8]. Because of the differences in ionic concentration between the exterior water, the intracellular, and the extracellular space, continuous ionic currents existed. Deflection of the cilia could alter those currents [15].

When the human embryo reaches the seven-somite stage (about 22 days), surface ectoderm overlying the future site of the inner ear thickens to form the otic placode. The otic placode undergoes transformation into the otic pit. At about 30 days, the otic pit becomes pinched off, forming the otic vesicle or otocyst [4]. Concurrently, a portion of the neural crest migrates to the vicinity of the otocyst and becomes the vestibulocochlear ganglion. While morphogenesis proceeds within the otocyst, histogenesis of the sensory epithelia is occurring [8]. The arrival of afferent endings in the epithelium precedes HC differentiation. At 9 weeks, the HCs in the vestibule end organ are well differentiated and may exhibit typical synapses with nerve endings [15].

There are two morphologically and physiologically distinct types of HCs: type I or chalice HCs are more recent and are concentrated in the central portions of the sensory epithelia. Type II or cylindrical HCs are phylogenetically older [8]. The body of a type I HC is entirely engulfed by one afferent terminal. Efferent innervation is indirect, as the efferent nerve has its synapse on the afferent nerve ending. Type II HCs can have one or more afferent nerve endings on the body of the cell. Type II hair cells can also be directly or indirectly innervated by vestibular efferent terminals [16].

The 'hairs' of these cells, the cilia, extend from the apical surface and greatly increase the membrane surface area. Vestibular hair cells typically have 40–200 stereocilia and one kinocilium. The kinocilium, capable of active motion, is located at the end of the stereocilia bundle. As with other mobile cilia, it is equipped with the typical nine-plus-two axoneme of microtubules that distinguishes mobile cilia in the respiratory tract, sperm cells and elsewhere [8]. Mammalian vestibular HC kinocilia are longer than the longest stereocilia and extend into the gelatinous substance of the cupula, thus mediating displacement of these structures relative to the epithelial surface [16].

The neuroepithelium contains other cell types as well [16]. Supporting cells (SC) have the nuclei located at the basal end of the sensory epithelial, above the basement membrane.

The HC is morphologically polarized. Deflection of the stereocilia towards the kinocilium opens potassium channels and thus decreases the potential difference that exists between endolymph and the sensory cell (approximately 120 mmol), causing intracellular depolarization and an increase in the frequency of the action potentials in vestibular nerve fibres. Conversely, deflection of the cilia away from the kinocilium results in intracellular hyperpolarization and a decrease in the vestibular nerve action potentials. The HCs release a neurotransmitter (glutamate) that is excitatory to the HC afferents with which they connect. At rest, there is a baseline release of the neurotransmitter. This release is important because not only does deflection of the hair cell bundle towards the kinocilium increase transmitter release, but deflection of the hair cell bundle away from the kinocilium reduces transmitter release. Thus, one hair cell detects both acceleration and deceleration along the axis of the morphological polarization vector [17].

Experimental models of vestibular protection and regeneration

Renewal of mammalian vestibular HC does not occur spontaneously, but may be induced to occur by a variety of approaches [18]. Avian vestibular receptors have been shown to continuously produce new hair cells and to spontaneously renew HCs following injury [19]. In contrast, the vestibular sensory epithelium of mammals has been shown to possess a limited restorative capacity [20, 21]. Growth factors may affect vestibular HC renewal in birds and mammals following injury to the vestibule [22, 23]. For example, transforming growth factor α (TGFα) increases cell proliferation in adult murine macula and crista explants following ototoxic damage [24]. Insulin and insulin-like growth factor type one (IGF-1) have also been demonstrated to have a mitogenic effect on HC in cultures from the avian utricular macula [23]. Insulin was found to enhance proliferation of undamaged macula by interacting with either TGFα or epithelial growth factor [23]. Retinoic acid (RA) is another powerful morphogenetic agent of the inner ear. A change in RA concentration initiates precocious differentiation of the chick otocyst, HC, and formation of supernumerary HCs in the developing mouse organ of Corti. Perilymphatic infusion of TGFα and insulin induced cell proliferation in both the utricular sensory and extrasensory epithelia of adult rats [25]. However, neither TGFα nor insulin alone resulted in cell proliferation. Brain-derived neurotrophic factor (BDNF) is essential for the survival of vestibular ganglion neurons [26]. BDNF has been shown to protect vestibular neurons from ototoxic drugs and has also been suggested to play a

role in the development of type 1 hair cells [27]. Another study has demonstrated that transotic BDNF administration promotes an increase in hair cell number in ototoxic-damaged vestibules of chinchillas [28]. Kopke *et al.* [25] demonstrated that infusion of a mixture of growth factors into ototoxic-damaged inner ears affects HC renewal, maturation of stereociliary bundles and recovery of horizontal vestibulo-ocular reflex performance. These results represented the first reports of both return of HC number and improvement in vestibular function following ototoxic damage in the mammalian vestibule. The authors concluded that infusion of a mixture of exogenous growth factors may be clinically appropriate for the treatment of some balance disorders [25].

Therapy for hair cell regeneration

Gene delivery is a relatively well-established research tool in the field of auditory neuroscience [29], with the first reports of molecular genetic therapy for inner ear pathology published slightly more than a decade ago [30]. These early efforts were prompted by the successful use of gene transfer in several other organ systems, including the central nervous system. An exciting goal for gene therapy of the inner ear is to regenerate HCs in the organ of Corti and in the vestibular epithelium [14, 30]. HC regeneration would have a remarkable impact on millions of individuals suffering from sensorineural hearing loss and balance disorders secondary to hair cell loss [13, 14]. Recent scientific and medical advances have brought us much closer to realizing the certainty of HC regeneration.

Localized delivery of molecules that induce cellular regeneration *via* gene transfer has the advantage of requiring only single vector application to achieve HC renewal rather than continuous infusion of growth factors into the ear [14]. Delivery of a vector bolus may also be performed without injuring residual hearing, which would be important when considering treatment for a patient with purely vestibular disease and normal cochlear function [14].

Studies examining fish and bird labyrinths first brought attention to the prospect of hair cell restoration [31, 32]. Several papers have documented that both fish and bird labyrinths are capable of regenerating lost hair cells following acoustic trauma [33]. However, a stem-cell population has not yet been identified in the labyrinth of either animal [34]. Instead, SCs have been found to undergo mitosis in response to the loss of hair cells in the avian basilar papilla and in the fish ear [35]. The process of phenotypic conversion from one cell type (supporting cell) to another (hair cell) is termed transdifferentiation [36]. This is a rare event in nature, although such events have been confirmed in other organs such as the eye [37]. Although these cell types are only distantly related after differentiation, very early in the developmental process, retinal cells maintain the ability to transdifferentiate into lens epithelial cells [37]. HCs and SCs arise from common progenitors, suggesting a capacity for supporting cells to transdifferentiate into HCs [38, 39].

Transdifferentiation appears to occur in the inner ear epithelium of all vertebrates with the exception of mammals. In mammals, transdifferentiation of SCs to HCs does not occur spontaneously following cochlear HC loss. Understanding the molecular signalling directing hair cell differentiation has helped researchers design ways to induce transdifferentiation in the mammalian auditory epithelium [38, 39].

Supporting cells in non-mammalian vertebrates give rise to new HCs through two distinct mechanisms: cell division and transdifferentiation [40]. Throughout cell division, SC re-enters the cell cycle forming two cells. In contrast, direct transdifferentiation occurs without cell cycle re-entry. The first new HCs to emerge after HC damage are produced by direct transdifferentiation, whereas new HCs generated by mitosis appear later and eventually comprise a substantial proportion of the new sensory cells. Once mitosis is initiated, direct transdifferentiation is down-regulated, so that later, most HCs present in the epithelium have been derived through cell division [41]. However, signals that regulate the initiation of direct transdifferentiation and stimulate the switch to mitosis are not yet identified. Mechanisms for halting regenerative behaviour in SCs have received considerably less attention than those initiating it and are therefore poorly understood [42]. One attractive control that has been documented in other sensory epithelia is a negative feedback mechanism, whereby regenerated HCs and/or SCs inhibit nearby SCs from further division or transdifferentiation [42]. As transdifferentiation occurs without entering the cell cycle and is a strictly controlled process, excessive proliferation leading to tumours is less likely to occur.

Sensory epithelia of the inner ear are highly specialized and their function depends upon the precise anatomical arrangement of HCs and SCs [40]. Direct transdifferentiation poses a specific problem, because each time a new HC is formed using this mechanism, an SC is lost from the epithelium. Tight regulation of direct transdifferentiation is essential, because, at a ratio of only 2–4 SCs per HC, excessive direct transdifferentiation would lead to SC depletion [41]. As direct transdifferentiation is initiated early, it is tempting to hypothesize that SC conversion into HCs has evolved as an early, rapid way to make new HCs if only a few are lost [43]. The selective differentiation of post-mitotic precursors into SCs would counteract SC depletion to direct transdifferentiation. However, specific temporospatial patterns of mitotic regeneration have not been characterized at different periods of regeneration, so it is not clear at this time whether mitotic regeneration is sufficient to compensate for direct transdifferentiation or if other mechanisms (*e.g.* cell death, cell rearrangement, or immigration of cells from outside the epithelium) are also involved [44].

The transcription factor *Atoh1* (formerly Math1) is critical in the differentiation of HCs [45]. *Atoh1* is a mouse homolog of the *Drosophila* gene *atonal* (the human homolog is *Hath1*) [46]. As development progresses and HCs are generated, *Atoh1* expression is down-regulated [46]. Thus, this transcription factor serves as an excellent candidate for inducing transdifferentiation of HCs from SCs. Overexpression of *Atoh1* in cultured rat organ of Corti has been shown to produce HCs in immature explants as well as in explants of mature tissues [47]. Furthermore, mice carrying a homozygous knockout of *Atoh1* fail to develop auditory or vestibular HCs [14].

Delivery of a plasmid vector expressing *Atoh1* to neonatal organ of Corti cultures produced supernumerary HCs *in vitro* [48]. These results were repeated using the human homolog of *Atoh1* (hath1) and an adenovector delivery vehicle [49]. In the human homolog study,

HCs regenerated in adult mammalian vestibular neuroepithelium *in vitro*. This demonstrated that both different atonal homologues and various delivery methods are effective in restoring HC. Notably, the *Atoh1* knockout mouse phenotype could also be rescued by delivery of the drosophila atonal gene, demonstrating the degree of conservation in sequence and function in this gene family [49].

Recently, an attempt was made to induce new hair cell development in an *in vivo* model [50]. To do this, the *Atoh1* gene was first inserted into a replication-deficient adenoviral vector (Ad-*Atoh1*), and this construct was then inoculated into 4- to 5-week-old guinea pigs *via* cochleostomy. Immunohistochemistry 4 days after inoculation confirmed the presence of green fluorescent protein in the non-sensory epithelium and primarily in the third turn near the cochleostomy. Animals were sacrificed 30 or 60 days after injection, and ectopic HCs were detected adjacent to the organ of Corti, where HCs are not typically found. These new cells were found to express a hair cell-specific marker, myosin VIIa. In addition, neurofilament labelling confirmed nerve fibres growing towards the new ectopic HCs. None of these phenomena was observed in control ears. These striking results documented the potential to induce HC generation in the adult cochlea from the supporting cell population *via* adenoviral-induced *Atoh1* expression. Neurofilament labelling suggested that these new HCs possessed the functional capability to attract neurite ingrowth [50, 51].

Vestibular and cochlear HCs express neurotrophic factors that play an important, although not exclusive, role in guiding sensory neuronal peripheral projections, particularly for vestibular neurons [52]. BDNF and NT-3 are two of the four members of the neurotrophin family of neurotrophic factors and do promote neural ganglion cell survival, evidenced by experiments showing that neuronal cell survival *in vitro* is supported by either BDNF or NT-3 [53]. Also, it should be noted that neurotrophic factors have roles other than survival, including synaptic maintenance and function and control of the mature neuronal phenotype. Glial cell-line-derived neurotrophic factor is another paracrine factor shown to be important in the survival of inner ear neurons. During development, *Atoh1* is also required for the expression of BDNF by vestibular HCs [52, 53].

In another series of experiments, Staecker *et al.* [13] demonstrated that adenoviruses (Ad) may be used to effectively deliver *Atoh1* to the adult mammalian vestibular system, resulting in regeneration of HCs *in vitro*. Ad-*Atoh1* delivery to macular cultures promoted robust HC regeneration consistent with previous studies [13, 44–48]. The use of myosin VIIa staining *in vitro* demonstrated that hair cells had actually been destroyed after aminoglycoside exposure rather than just undergoing loss of stereocilia after injury as previously suggested. Bromo-deoxyuridine (BrdUrd) labelling was used to analyse whether the regenerated vestibular HCs were a result of cell division or differentiation of a SC as discussed by others [13]. BrdUrd-labelled myosin VII-positive cells were observed to be exceedingly rare [13], suggesting that the HCs observed in *Atoh1*-treated explants were the product of transdifferentiation rather than mitosis. Cultures treated only with aminoglycoside did not demonstrate spontaneous HC recovery [13].

To validate the efficacy of adenovector-mediated gene delivery to damaged vestibular neuroepithelium, Baker *et al.* [14] treated mouse macular organ cultures with aminoglycoside. These explants were then challenged with increasing doses of adenovector expressing green fluorescent protein (GFP) driven by a human cytomegalovirus (hCMV) promoter. GFP expression in culture was found to be similar to expression in untreated explants that were challenged with a similar dose of adenovector [14]. These experiments demonstrated that delivery of increasing vector doses to damaged macular organ cultures resulted in expanding numbers of cells expressing GFP. Delivery of the vector to macular organs also resulted in GFP expression in a larger percentage of damaged macular epithelium, suggesting that adenovector may be concentrated to a sufficient degree to saturate the targeted epithelium [14].

Methods of gene delivery to the inner ear

Several properties of the inner ear suggest that this might be a hospitable environment for gene therapy intervention [54]. First, the organ is surrounded by the temporal bone and isolated within the otic capsule, thus reducing the risk of inoculating adjacent tissues. Second, the inner ear anatomy consists of fluid-filled spaces that permit widespread diffusion of a locally introduced vector [55]. Finally, the inner ear is composed of several distinctive cell types including spiral ganglion neurons, SCs and HCs. Thus, the impact of genetic manipulation on each of these cell types may be studied using quantitative, structural and physiological analyses [14, 54].

Efficient vector delivery to the inner ear requires a technique that delivers an appropriate volume of vector with equal distribution throughout the cochlea and vestibular labyrinth while preserving hearing function [56]. For humans, the technique must be safe and pose minimal risk to the patient. Other variables of particular importance when considering gene transfer to the inner ear are means and route of delivery [14, 56]. Vector introduction is complicated by the fact that the cochlea and vestibular organs are isolated by the bony otic capsule, and fluid spaces of the ear are divided into individually isolated endolymphatic and perilymphatic compartments. Furthermore, the structures of the inner ear, and particularly hair cells, are quite sensitive to trauma [56]. One of the least invasive means of vector delivery is topical application to the round window, which would allow for diffusion across the membrane and into the scala tympani [57]. This method has resulted in some success in previous studies, although it has not been proven to be highly effective for viral vectors. Thus, most studies employ techniques that allow direct inoculation of vector-containing fluid into one of the fluid spaces of the inner ear, and this technique is typically performed with a micropipette [57].

Successful adenoviral gene therapy in the inner ear is dependent upon the ability to target the vector to the appropriate tissue. To examine this question, the transfection patterns of various inoculation techniques have been assessed. Techniques that have been considered include directly piercing the round window, performing a cochleostomy to access the scala tympani, scala vestibuli, or scala media, or injecting the vector into the endolymphatic sac

[57–63]. For potential human application, gene delivery techniques that do not violate the round window or cochlea have been emphasized [14, 64].

Adenoviral transfection *via* cochleostomy into the basal turn of the scala tympani was found to be more efficient than an approach through the round window [54, 62]. In both approaches, transfection was most efficient in the mesothelial cells lining the fluid spaces, and particularly in the scala tympani [54, 61, 62]. However, the cochleostomy group demonstrated greater and more widespread labelling, with labelling occasionally reaching all turns of the cochlea. Possible explanations for this difference in expression include deeper entry into the scala tympani with cochleostomy, mechanical differences in injection between the two techniques, and altered cochlear homeostasis induced by cochleostomy.

Of note, both delivery techniques failed to result in transfection within the membranous labyrinth (lining the endolymphatic space), including clinically important targets such as the marginal cells of the stria vascularis and the organ of Corti [63].

However, other approaches have resulted in successful transfection of target cell populations in the endolymphatic space. One technique described inoculation into the endolymphatic sac of healthy guinea pigs [58]. In this study, transfected cells were identified in the endolymphatic sac and duct of all animals. Within the vestibular system, expression was greatest in the transitional epithelium of the utricle and saccule, and expression was present to a lesser extent in the semicircular canals. During injection, some animals demonstrated swelling of the endolymphatic sac, and these animals were found to have transfected cells in the endolymphatic space of the cochlea. Specifically, infection was noted in the marginal cells of the stria vascularis, Hensen's cells in the organ of Corti, and occasionally in the spiral ligament, connective tissue and Reissner's membrane. HCs in the vestibular system and organ of Corti were not affected. Praetorius *et al.* [64] described a technique of herpes simplex virus-1 vector injection through a small opening in the utricle, which achieved hearing preservation as well as efficient reporter gene expression.

Baker *et al.* [14] also developed a surgical technique that models a human stapedotomy and is a method that could potentially be used for human gene delivery. Using a postauricular approach, the middle ear of adult mice was exposed and the stapedial artery was followed until the stapes and oval window could be identified [14]. Using an argon laser, a fenestration was created into the vestibule at the edge of the footplate, thereby avoiding injury to the stapedial artery. Injection of an advanced-generation GFP-expressing adenovector resulted in broad distribution of GFP signal throughout the inner ear, including expression in the vestibular neuroepithelium [14].

An important variable in gene therapy is the choice of vector [65]. As large nucleic acid molecules do not readily penetrate the plasma membrane, they require packaging into a vector that is readily taken up by target cells. Non-viral vectors such as liposomes and naked plasmids have been used in previous studies and are advantageous in that they are associated with fewer side effects than virally derived vectors [62].

However, the transduction efficiency of these alternatives is quite low, and they are thus largely limited to *in vitro* use where cells may be exposed to high vector concentrations. Viral vectors have proven to be much more efficient but also to have the potential to produce cytotoxicity or immune responses [66]. A number of different viral vectors have been used to treat the inner ear, including adeno-associated virus, herpes simplex virus, vaccinia virus, retrovirus, helper-dependent adenovirus and adenovirus vectors [62, 65–72]. Each of these vectors offers distinct advantages and disadvantages [68–72]. Advanced-generation adenoviral vectors have become among the most frequently used viral vectors in the inner ear [73–76]. Adenoviral vectors are associated with a minimal side-effect profile, may be prepared at high titres, and may enable transgene expression for up to several months [72–75]. On the other hand, adenoviral vectors are advantageous over retroviruses in that they are not dependent on cell replication and therefore may be used to transfect quiescent cochlear cells. Over 40 adenoviral serotype strains have been identified, most of which cause benign respiratory tract infections in humans [72–75]. However, subgroup C serotypes 2 and 5 are predominantly used as vectors. The life cycle of this virus does not normally involve integration into the host genome; rather, these serotypes replicate as episomal elements in the nucleus of the host cell, and consequently, there is no risk of insertional mutagenesis [73, 75]. Adenoviral (Ad) vectors are very efficient in transducing target cells *in vitro* and *in vivo* and may be efficiently produced at high titres (>1011/ml) [72–75]. Titre concentration is an important factor in vector delivery, where the volume of delivered agent may be limited by the size of the inner ear. Delivery of excess volume into the inner ear may result in trauma and loss of function [23, 72].

In a recent set of experiments, an Ad-*Atoh1* vector was used to successfully regenerate vestibular HCs after chemical ablation [13, 14]. The vector was administered through a scala tympani cochleostomy in adult mice 2 days after intracochlear aminoglycoside treatment. Mice were chosen as the experimental model because of the availability of multiple mutant strains with vestibular disorders that could be potentially tested as animal models of human vestibular disease [13, 14]. Vestibular recovery was evaluated both functionally and histologically 8 weeks following vector delivery. Swim testing was chosen as the testing modality to evaluate rodents' balance [13, 14]. Intracochlear injection of neomycin caused complete histological ablation of hair cells and resulted in measurable abnormalities on functional testing, demonstrating a significant prolongation in swim time in aminoglygoside-only-treated animals. HC counts in the saccule, utricle and lateral canal ampula showed significant regeneration in Ad-*Atoh1*-treated animals compared with aminoglycoside-only-treated animals [13]. Despite a significant recovery of sensory cell number *in vivo*, the regenerated vestibular neuroepithelium showed some clear abnormalities [14]. The regenerated epithelium was characterized by both a decrease in overall epithelial thickness and a decrease in the total number of SCs [14]. These data suggest that *Atoh1* gene transfer induced replacement of vestibular hair cells through conversion of SCs to sensory cells [13]. At 8 weeks post-delivery, the mice also demonstrated functional vestibular recovery based on swim testing, with no significant differences from untreated animals [13, 14]. Aminoglycoside-only-treated animals demonstrated significantly increased swim times. Of note, there was no evidence of cochlear hair cell regeneration or hearing threshold recovery in this

study. These findings are consistent with previous experiments suggesting that scala media inoculation is necessary to achieve effects on the auditory epithelium [13, 14]. These data on HC regeneration in the cochlea and the vestibular epithelia highlight the potential for adenovirus-mediated inner ear therapy [14].

Studies in human tissue

To examine the feasibility of gene therapy in humans, Kesser *et al.* [76, 77] developed a novel experimental model. Vestibular epithelia (ampullae from the semicircular canals and maculae from the utricle and saccule) were harvested from patients undergoing removal of vestibular schwannoma or labyrinthectomy for Meniere's disease [67]. In this study, both first-generation and second-generation adenoviral vectors were shown to transfect HCs and SCs in cultured human vestibular tissue [76]. By demonstrating that human vestibular epithelium remains viable in culture for as long as 5 days, these experiments establish a model for the study of therapeutic agents to treat inner ear disease. Transfection rates were found to be both titre- and time-dependent [77]. Adenovirus drove expression not only of the GFP reporter gene but also of the deafness gene, KCNQ4, as demonstrated by significantly higher expression rates of KCNQ4 in GFP-positive cells. Human vestibular explants harvested from mature human vestibular organs provide a unique opportunity to study gene transfer agents as well as other pharmacological therapeutics designed to treat inner ear disorders. This *in vivo* model may be instrumental in the future translation of agents from the laboratory to clinical trials for patients with vestibular disease [76, 77].

Conclusions

While the results of viral-mediated hair cell regeneration are promising and pave the road for further research, much work remains to be done. Viral-mediated gene transfer into the inner ear has significantly evolved over the last several years. Adenovirus is a promising vehicle for gene transfer, and experiments have demonstrated the effectiveness of hair cell regeneration using *Atoh1* gene transfer. Challenges as inner ear gene therapy continues to progress will probably include trouble-shooting viral vectors, refining the method of delivery and discovering new genes whose replacement may restore inner ear function. The adenovirus vector should be optimized for greater transfection efficiency and localized targeting of specific cell subpopulations. Surgical technique should also be improved to minimize the traumatic impact of cochleostomy on perilymphatic and especially endolymphatic spaces. Experiments demonstrating that *Atoh1* expression after aminoglycoside injury enhances vestibular hair cell recovery and may aid in the recovery of vestibular disorders shed light on the potential impact of the viral delivery approach. Experiments demonstrating gene transfer in the human vestibular epithelium have also revealed that the human inner ear is a suitable target for gene therapy. While much work remains to be accomplished, the prospect of applying viral delivery techniques to clinical scenarios is becoming more feasible.

Conflict of interest

The authors confirm that there are no conflicts of interest.

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