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Gene therapy for the inner ear

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Abstract

Animal studies on inner ear development, repair and regeneration provide understanding of molecular pathways that can be harnessed for treating inner ear disease. Use of transgenic mouse technology, in particular, has contributed knowledge of genes that regulate development of hair cells and innervation, and of molecular players that can induce regeneration, but this technology is not applicable for human treatment, for practical and ethical reasons. Therefore other means for influencing gene expression in the inner ear are needed. We describe several gene vectors useful for inner ear gene therapy and the practical aspects of introducing these vectors into the ear. We then review the progress toward using gene transfer for therapies in both auditory and balance systems, and discuss the technological milestones needed to advance to clinical application of these methods.

1. Introduction

In this review we describe different aspects of work related to inner ear gene therapy. We consider vectors for gene delivery, routes of introducing reagents to the inner ear, challenges that remain to clinical application and first major successes in applying gene therapy in the lab. We also discuss the future of gene therapy and its potential for treating both environmental and hereditary disease, and list the main hurdles that need to be overcome before clinical applicability can be contemplated.

Hearing loss is a critical and common communication disorder in our society. When severe, it changes the patient's lifestyle significantly. Inner ear disease also can cause balance dysfunctions and these, too, can be devastating. The most common causes of inner ear disease are hereditary, environmental or a combination of the two. Among the common environmental causes are over-stimulation (loud volume), ototoxicity, mechanical trauma, infections and aging. Current therapies for severe to profound hearing loss are limited to the cochlear implant prosthesis (Wilson and Dorman, 2008). For severe loss of balance, there is currently no efficient remedy in clinical use, although progress is being made (Dai et al., 2011; Rubinstein et al., 2012). Thus, biological interventions for protection and repair of the inner ear are needed. Gene transfer technology has a potential to prevent inner ear disease and to help a large number of patients whose ears are damaged and dysfunctional. Specifically, the goals for gene therapy are phenotypic rescue of hereditary disease, prevention or moderation of environmental trauma and repair of injured, missing or

dysfunctional elements in the inner ear. Regeneration and preservation of the cochlear substrates may also be used for enhancing outcomes of cochlear implantation.

Therapies in current practice generally influence cells by pharmacological means, which typically are not cell-specific. Furthermore, the medications are given systemically or even topically and are insufficient for changing gene expression in cells or for inducing repair and regeneration. In contrast, gene therapy offers the potential for more direct manipulation of gene expression in the target cells, by directly inhibiting expression of a deleterious allele, or by inserting and forcing expression of a missing or down-regulated gene. These tasks can be accomplished by gene transfer technology.

Function of specific genes can be studied in vitro by using several methods of inserting them into cells. However, to fully understand the genes' effects in a complex organ or system, animal studies are needed, as they facilitate long-term experiments that can evaluate parameters such as interactions with other organ systems, influences of epigenetic interaction and environmental conditions, as well as provide data on physiological function. Studies of gene transfer for hearing and balance research have used several animal models, including zebrafish, birds, and mammals (chinchilla, gerbil, rat, guinea pig and mouse). Zebrafish are a useful model for genetic studies due to their short lifecycle, availability of their complete genome sequence (Broughton et al., 2001), ease of inducing and screening mutations, and readily visualized structure. Although transgenesis has been widely used in fish, gene transfer experiments are not common. Similarly, birds have a widely-studied regenerative capacity in the inner ear sensory epithelium (Cotanche et al., 2010), and the pathways activated during regeneration are shared with normal development (Daudet et al., 2009). Although it would be interesting to apply gene transfer research in the basilar papilla, this method is not commonly reported. Retrovirus vectors do have a potential for use in studies that manipulate expression of specific genes in the avian inner ear (Sienknecht et al., 2011).

Among mammals, the most natural choice for inner ear gene transfer research is the mouse, due to the large genetic database and availability of a variety of different useful strains. The mouse is small, relatively inexpensive to obtain and maintain, and has a high reproductive rate, all of which make large samples fairly economical to produce. However, its small size also poses several limitations for inner ear gene transfer research due the corresponding difficulty of surgical procedures and histological preparations. For example, there is no easy way to use a cochlear implant in the mouse. Consequently, experiments examining effects of combining electrical stimulation via an implanted electrode with gene transfer protocols are not feasible. In addition, the mouse has an extremely sturdy auditory epithelium, especially the inner hair cells, highly resistant to most insults, which presents challenges in producing adequate lesions in this animal model. Still, for most studies on phenotypic rescue of a mutation, the mouse is the only mammal available, because mutant mice mimicking human genetic inner ear disease are readily available.

In order to efficiently apply gene transfer technology for protection and repair in the inner ear, it is necessary to identify and characterize the roles of specific genes and signaling pathways. This is usually accomplished by using transgenic mice, which allow for several ways to manipulate gene expression (Cox et al., 2012). For instance, work on transgenic mice helped characterize Atonal homolog1 (*Atoh1*) as a gene that is essential for hair cell (HC) differentiation (Bermingham et al., 1999), thereby providing a useful tool for inducing HC regeneration in deaf ears. Other knowledge generated by studies of transgenic mice includes the role of specific signaling pathways in auditory nerve development. The role of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin3 (NT3) in cochlear development was characterized in mice with null mutations of these genes

(Fritzsche et al., 2006, 2004; Giraldez and Fritzsche, 2007) and produced tools for enhancing spiral ganglion neuron (SGN) survival in deaf ears.

The examples above represent just a few of the many genes known to participate in inner ear development and maintenance, which cause disease when mutated, and thus have potential to serve as therapeutic genes via gene therapy. Despite a large and growing understanding of the role and function of many of these genes, gene therapy is not yet ready to make the transition from basic research to medical practice. Among the issues that remain to be addressed are the safety, reliability and consistency of results obtained from inserting exogenous genes into living ears.

2. Gene transfer vehicles applicable for inner ear therapy

Therapy based on changing gene expression can be accomplished in some cases by over-expression and in others by inhibition or down-regulation of gene expression. Over-expression is usually done by inserting genes into cells, which is most efficiently done by packing a gene in a shuttle system using viruses or other transfer agents capable of penetrating cells. Non-viral vectors for shuttling genes into cells include non-packaged plasmids or genes packaged within lipids or dendrimers (Majoros et al., 2008). These vectors exhibit reduced toxicity and inflammation compared to viral vectors, but their efficiency of inner ear gene transfer also appears to be lower (Staecker et al., 2001).

Various viral vectors are available for delivery of genes to target cells in the inner ear, with some specificity of the vector to a target cell type. Several viral vectors have been used to deliver genes into the inner ear, including adenovirus (Hussemann and Raphael, 2009), adeno-associated virus (Bedrosian et al., 2006; Iizuka et al., 2008; Lalwani and Mhatre, 2003), helper-dependent adenovirus (Wenzel et al., 2007), bovine adeno-associated virus (Shibata et al., 2009), herpes simplex virus (Chen et al., 2001; Derby et al., 1999), vaccinia virus (Derby et al., 1999) and retrovirus (Bedrosian et al., 2006). Adenovirus (AdV) has a relatively fast onset of gene expression, with transgene expression detected as early as 2 days following the infection, making these vectors appropriate for a wide variety of targets. Limitations associated with the use of AdV include transient expression due to failure of the virus to integrate into the target genome and side effects due to toxicity and immuneresponse (Boviatsis et al., 1994). Unlike AdV, adeno-associated virus (AAV) permits long-term expression of the transgene, shown to last for years in other systems (Bedrosian et al., 2006) and the duration for inner ear gene expression is likely similar. The relatively small packaging capacity of AAVs often limits their use to relatively small genes. Lentivirus (LV) also provides long-term gene expression and a large packaging capacity as well as broad host range. The disadvantages to the use of LV in the inner ear include immunogenicity and ototoxicity (Bedrosian et al., 2006). Future improvements in vector design are likely to provide better vectors that will offer enhanced cell specificity, controlled duration of gene expression, reduced toxicity and the ability to carry a large gene insert.

There are several cases where the therapeutic goal is to inhibit gene expression. Among these cases are dominant negative genetic diseases and therapies that involve inhibition of developmental pathways. One way to accomplish inhibition of gene expression of a specific gene is RNA interference (RNAi) gene inactivation (Fire et al., 1998). Two types of small RNA molecules are central to gene silencing by RNAi: microRNA and small interfering RNA (siRNA). MicroRNA are post-transcriptional regulators that bind to complementary sequences on target messenger RNA, usually resulting in translational repression or target degradation (Ambros, 2004). In contrast, siRNA interferes with the expression of specific genes with complementary nucleotide sequence (Vickers et al., 2003).

Use of siRNA for inner ear therapy shows promise, as demonstrated by the first few publications using this approach. One example is the inhibition of *NOX3*, which serves as the primary source of reactive oxygen species generation in the cochlea. siRNA against *NOX3*, delivered via a transtympanic administration, produced significant and prolonged knockdown of *NOX3*, protected the outer HCs and SGNs from cisplatin-induced damage and attenuated hearing loss (Mukherjea et al., 2010). Another example of siRNA use in the inner ear is treatment of a mouse model for a dominant negative form of a connexin26 mutation (Maeda et al., 2005).

Use of small molecules to influence gene expression is advantageous over delivery with a viral vector since side effects to the presence of the virus are circumvented, but the effects are limited to the short term activity of the delivered reagent. One example of small molecules that interfere with expression of developmental genes is DAPT, a small molecule that inhibits gamma-secretase (Crawford and Roelink, 2007). Since reducing Notch signaling pathway may increase the number of *Atoh1* expressing cells, DAPT may lead to generation of new HC, as shown in vitro (Lin et al., 2011; Zhao et al., 2011). Using this approach in vivo, new HCs could be generated after infusing DAPT into mature mammalian ears (Hori et al., 2007). Combining a small molecule therapy with additional molecules such as growth factors may also be considered, as shown in experiments in culture (Munnamalai et al., 2012). Delivery of a small molecule such as DAPT would still be most effective and safe if it is directed into the inner ear, because most developmental pathways are likely not specific to the inner ear, and side effects in other organs should be prevented. For instance, in *Norrin* knockout mice, which display congenital blindness and progressive deafness due to severe developmental blood vessel defects in the retina and the inner ear (Zuercher et al., 2012), systemic DAPT administration led to an increased vascular density.

Another method for delivering therapy to the inner ear is nanoparticles, which are particles with diameters of less than 1000 nm (Hornyak, 2005) and typically in the size range of 200 nm or less. Several studies reported the outcome of delivering nanoparticles to perilymph through the round window membrane (Buckiova et al., 2012; Ge et al., 2007; Tamura et al., 2005). Interestingly, liposome and polymersome nanoparticles applied to the round window membrane in mice were mainly detected in the SGNs, and to a lesser extent in the organ of Corti (Buckiova et al., 2012), whereas, poly-lactic/glycolic acid nanoparticles were identified in the cochlea after systemic or local application through the round window membrane (Tamura et al., 2005). Nanoparticles are not smaller than viruses and their mechanism of entering cells is usually passive, leading to less efficient transduction as compared with viral vectors. It is likely that once the efficiency of gene delivery by nanoparticles is increased, they would become an attractive means for inserting transgenes with biological outcome for prevention or therapy in the inner ear, since the vehicle itself should have fewer side effects than a viral vector.

3. Practical routes for delivering therapeutic agents

The method and route for gene transfer protocols (see schematic Fig. 1) need to be considered based on feasibility, risk, side effects and the actual outcome measured by efficiency and specificity of gene expression. The most economical and practical methods for future human therapy would be minimally invasive, but due to the anatomy of the inner ear, access to target cells may be compromised or impossible unless an invasive surgical approach is used. However, procedures such as opening a cochleostomy in the lateral wall of the cochlea, canalostomy of semicircular canals or penetrating the round window membrane, pose risks of perilymphatic fistula and other mechanical damage to the cochlea (Fig. 1a). In order to use gene transfer for protective purposes, it is imperative to preserve hearing and balance functions during surgery. In cases where gene transfer is used for repair and

regeneration in the inner ear, it may be acceptable to tolerate a slightly more invasive procedure since lesions are already present and therefore the risk of inflicting more damage may be less important than the potential benefit of the therapy.

The main approaches that are available for gene delivery are systemic, transtympanic (into the outer or middle ear), or into the cochlea (perilymph or endolymph). Systemic administration is attractive because it does not involve a surgical procedure. However, the systemic approach has severe limitations for delivering substances specifically into the inner ear. One is the variable penetration into the inner ear due to the presence of a blood-cochlea barrier which separates the stria vascularis from peripheral circulation (Juhn et al., 2001; Zhang et al., 2012). The other limitation is the potential for undesirable systemic side effects such as systemic inflammatory response syndrome (SIRS), which can have extreme consequences (Raper et al., 2003).

The transtympanic approach is a safe and effective drug treatment route commonly used by otologists to apply medications for treating the middle ear. This route also could be used for gene transfer into the inner ear. The goal would be to place the gene therapy reagent (viral vector, siRNA or a small molecule) on the round window membrane that separates the middle-ear cavity and the scala tympani. The round window membrane serves as a barrier providing protection for the inner ear by limiting transfer of molecules as a function of factors such as size, electrical charge and concentration (Goycoolea, 2001). Because most therapeutic agents are likely to be relatively large molecules it will be necessary to transiently increase the permeability of the round window membrane to permit their transport to the inner ear. One option is to soak a vector solution into a Gelfoam (gelatin) sponge that is placed in the middle ear cavity on the round window. This technique is easy to perform, produces little or no permanent damage to the round window, and shows no symptoms of systemic or inner ear inflammation. Experiments using gels absorbed with liposomes or AdV, showed success in mediating transgene expression (Jero et al., 2001). Further enhancement of gene delivery through the round window was accomplished by a novel method that employed a partial digestion of the membrane with collagenase, resulting in increased permeability of round window membrane to recombinant AAV vectors (Wang et al., 2012). Another study showed that placing hyaluronic acid (a nontoxic and biodegradable reagent) on the round window membrane enhanced delivery of an AdV carrying the eGFP reporter gene to cochlear cells (Shibata et al., 2012).

A direct intracochlear delivery of therapeutic agents permits more control over the site of delivery and the amount delivered. Sites to which vectors can be injected include the cochlear fluid spaces (perilymph or endolymph) and the fluid in the vestibular system. Both osmotic pump and trans-round window injection deliver the reagent into scala tympani perilymph (Fig. 1a–c). In most cases, this route can be used for delivering gene products that are secreted and can diffuse to reach their target cells. However, scala media inoculation (Fig. 1d) is usually needed for delivering viral vectors to cells of the auditory epithelium (epithelial cells of the membranous labyrinth), which are not transduced after a perilymph inoculation in mature animals (Ishimoto et al., 2002; Stover et al., 1999; Venail et al., 2007).

An important disadvantage of intracochlear delivery is that it involves a more invasive procedure requiring drilling in the bone to access the cochlea, for both research animal models and human ears. However, cochleae microinjected through the round window (Fig. 1a) demonstrated intact cochlear architecture and no permanent hearing dysfunction (Lalwani et al., 2002; Stover et al., 1999; Xia et al., 2012). When there is a need to deliver into scala media, it is necessary to open a cochleostomy, which involves a significant threshold shift and loss of HC (Ishimoto et al., 2002; Wise et al., 2010). This may be permissible for procedures such as HC regeneration in ears with no HCs, but not for any

protective protocols or procedures in ears with significant residual function. In humans, access to the scala media is more difficult than in animals, making this option less attractive.

One method for intracochlear delivery of fluids commonly used in research animals is a mini-osmotic pump (Prieskorn and Miller, 2000; Sly et al., 2012; Tan et al., 2008). For short term, sustained release of a therapeutic compound, slow release via a mini-osmotic pump is an attractive option. For delivering viral vectors, the pump may be less useful because many of the viral vectors do not survive long at body temperature unless they infect a host cell. Consequently, as soon as the pump reservoir is loaded with the virus, titer would start declining as viruses disintegrate.

While delivering the gene proper into cells of the auditory epithelium may require a scala media inoculation (Fig. 1a and d), therapies that involve diffusible factors such as neurotrophins can be applied using a scala tympani inoculation. In this scenario, AAV or AdV vectors infect the mesothelial cells that line the perilymphatic space (Fig. 1c). The infected cells secrete the gene products, which become available in a paracrine fashion to cells in the vicinity. Because the basilar membrane is permeable to relatively large molecules, the fluid space between cells of the auditory epithelium is practically continuous with scala tympani perilymph. The true practical barrier between the two fluids is the reticular lamina, made of the apical membrane of the auditory epithelium and the apical junctions that connect them with each other.

A canalostomy through the posterior semicircular canal can be used to introduce vectors with a preference to the vestibular organs (Fig. 1a). Canalostomy gene transfer also leads to transfection of some cells in the cochlea, causing much less threshold shift than cochleostomy (Kawamoto et al., 2001). The main disadvantage of this procedure in lab animals is the inability to ensure whether the reagent is inoculated into the endolymphatic space or into the perilymph, due to the small diameter of the canal (Kawamoto et al., 2001). Nevertheless, there is access for a canalostomy in the human ear and, since the diameter is larger than in small lab animals, the procedure holds promise for gene delivery with accuracy and minimal side effects.

In selecting a site and route for inoculation, another consideration is the diffusion in the cochlea. Cochlear fluid flows as well as passive diffusion influence the spread of chemicals away from the site of inoculation. Pharmacokinetics studies for reagents other than viral vectors show that inoculation through the round window into perilymph leads to some spread towards the apical cochlea, but the concentrations are reduced gradually toward cochlear apex. This was shown for both gentamicin (Plontke et al., 2007) and dexamethasone (Plontke et al., 2008). These concentration gradients were stable over time (Hahn et al., 2012). Inoculation of viral vectors with a reporter gene into scala tympani indeed demonstrated most efficient gene expression in the vicinity of the inoculation site and gradually reduced expression levels away from the site (Stover et al., 1999).

4. Gene therapy experiments with biologically relevant genes

4.1. Auditory nerve preservation and fiber regeneration

Current therapy for profound deafness is limited to the cochlear implant prosthesis, a surgically implanted electronic device that transduces sound to electrical signals that stimulate the auditory nerve directly. For the implant to function optimally, it is necessary to maximize the number of surviving SGNs and to enhance their biological functionality in the deaf ear. These two tasks have been accomplished in lab animals by using growth factors such as neurotrophins. Neurotrophins and their receptors have been shown to be expressed in the developing and mature cochlea and to play important roles in the development of the

auditory system (Fritzsche et al., 1999; Pirvola et al., 1994). Beyond development, neurotrophins have been shown to preserve SGNs in deaf ears that were infused via mini-osmotic pumps (Wise et al., 2005). Similar results were obtained by using gene therapy techniques. AdV-mediated gene transfer of glial cell line-derived neurotrophic factor (GDNF) prevented SGN degeneration for up to 4 weeks after systemic deafening of guinea pigs (Yagi et al., 2000). The combination of GDNF and electrical stimulation was more effective in protecting the spiral ganglion than was each component alone (Kanzaki et al., 2002). Spiral ganglion protection was also shown in mice in experiments using cisplatin lesions and NT-3 gene transfer (Bowers et al., 2002; Chen et al., 2001).

In addition to preservation of the cell body of auditory neurons, more recent work has shown that the presence of neurotrophins can induce nerve fiber extension. The fiber growth was robust but not restricted to the basilar membrane area when growth factors were infused with a mini-osmotic pump (Glueckert et al., 2008; Wise et al., 2005). However, fiber growth was more preferentially targeted to the area of the basilar membrane by use of gene transfer technology, where the highest concentration of neurotrophin is near the cells that secrete it. With this method, neurotrophin gene therapy was shown to induce auditory nerve fiber growth to the basilar membrane area in animals that had no remaining HCs (Shibata et al., 2010; Wise et al., 2011, 2010). The regenerative capability can be used to reduce the distance between the implant electrode and the nerve ending which should enhance the performance of cochlear implants by reducing impedance and current spread.

4.2. HC preservation

HCs are an attractive target for gene and cell therapy approaches, both for research purposes and for future therapies. Because cochlear HCs in the mammals cannot be replaced spontaneously once lost, it is important to protect these cells, when possible, or to design ways to regenerate them after they are lost. There are several examples of situations where protection of HCs is needed. Workers in extremely loud environments could use protection, especially when use of sound attenuation devices is not feasible. Similarly, people who are put on aminoglycosides may need protection against inner ear side effects. Gene transfer methods can be used for protecting the inner ear by over-expression of several types of molecules. Transgene expression of GDNF prevented HC degeneration using AdV (Kawamoto et al., 2003b; Yagi et al., 1999) and AAV (Liu et al., 2008). Also AAV-mediated delivery of the X-linked inhibitor of apoptosis protein prevented cisplatin-induced elevation of hearing thresholds and HC loss (Cooper et al., 2006). Silencing of the transient receptor potential vanilloid 1, which was shown to be up-regulated in cisplatin treatment, decreased damage to outer HCs and reduced hearing loss from cisplatin ototoxicity (Mukherjee et al., 2008). Most protection studies used neurotrophins, for both HC protection (auditory and vestibular) and for preventing loss of the neurons in ears with missing HCs (Budenz et al., 2012). So long as the gene vector or the injection process is harmless, these protective measures may be harnessed for clinical use, especially for cases where the likelihood for morbidity in the absence of protection is high or certain.

4.3. HC regeneration

Once the ear has pathology involving HC loss, the therapeutic focus shifts from protection to reparative/regenerative efforts. Experiments with transgenic mice have revealed and characterized molecular components regulating HC development and regeneration, thereby pointing to potential avenues for repair by gene transfer. For instance, production of new HCs has been accomplished by manipulating cell proliferation control (Chen and Segil, 1999; Lowenheim et al., 1999), or by influencing expression of genes that specify HC differentiation. The latter studies mostly involved regulating expression of *Atoh1*. In *Atoh1* inducible transgenic mice, the new ectopic cells are densely clustered in distinct regions

representing the population of cells that are responsive to *Atoh1* (Kelly et al., 2012). Work with a conditional knockout mouse model with self-terminating *Atoh1* expression showed that the duration of expression is critical for HC survival and for the type of HC that is generated (Pan et al., 2012). The supernumerary HCs that are generated by induced *Atoh1* expression are a result of transdifferentiation of non-sensory cells in the organ of Corti and its flanking areas. Several studies have demonstrated that the competence of the non-sensory cells to undergo trans-differentiation is gradually reduced as the cochlea matures (Kelly et al., 2012; Liu et al., 2012). These and other studies using transgenic mice were seminal in disclosing the molecular pathways and specific gene(s) that need to be influenced for inducing HC regeneration in a clinically-feasible manner, by gene transfer or other means of altering gene expression patterns in the mature ear.

For now, the success in inducing regeneration in wild-type mature ears (non-transgenic work) is limited. Kawamoto et al. (Kawamoto et al., 2003a) demonstrated that a small number of ectopic new HCs were generated in mature guinea pig cochleae after *Atoh1* gene transfer with an AdV vector. These ectopic HCs were present both medially and laterally to the native HCs, and were shown to attract neural fibers. Attempts to induce HC regeneration with *Atoh1* over-expression did not succeed in mature guinea pig ears that were deafened with neomycin (a severe insult that leaves no HCs and a flat layer of supporting cells). This indicated that a more complex treatment will be needed in mature traumatized ears, perhaps involving a multi-step approach for first rebuilding a new layer of non-sensory cells that are competent to undergo trans-differentiation, and then forcing the expression of *Atoh1*. While a severe ototoxic lesion seems to prevent *Atoh1*-induced trans-differentiation of supporting cells to HCs in the cochlea, more positive results were found in the vestibular sensory epithelium. The mammalian vestibular organs have a limited capacity to spontaneously regenerate HCs (Forge et al., 1993; Kawamoto et al., 2009) and means to augment this regenerative capacity are needed. *Atoh1* gene therapy was efficient at generating new vestibular HCs and restoring balance in mice ears that were lesioned with neomycin (Schlecker et al., 2011; Staecker et al., 2011, 2007). These data are especially important since there are no artificial devices similar to the cochlear implant that can be used to restore lost vestibular function.

4.4. Hereditary disease

The methods described above for repairing inner ear sensory epithelia via HC regeneration would not apply to cases of hereditary disease because the effects of the mutation will likely lead to the demise or dysfunction of any new cells that might arise. Instead, genetic hearing loss may be potentially treated by gene replacement or supplementation therapies, aimed at providing the wildtype gene product needed for function. Exciting and promising advances have been made towards treatment of hereditary inner ear disease by use of mice that model human genetic deafness. For instance, hearing of deaf connexin30 null mice could be restored by genetically overexpressing the connexin26 gene (Ahmad et al., 2007). More recently, a successful restoration of hearing was shown in VGLUT3 mouse mutants. AAV1-VGLUT3 gene delivery into the ears of these knockout mice restored synaptic transmission and hearing (Akil et al., 2012). The siRNA silencing was used by Maeda et al. (2005) to block expression of a dominant negative connexin26. They determined that siRNA selectively suppressed translation of the gene leading to improvement of thresholds in these mice. Another recent study showed that BDNF gene therapy enhanced auditory nerve survival and induced peripheral nerve sprouting in *Pou4f3* mouse ears (Fukui et al., 2012), an outcome that is likely to improve the benefits from cochlear implant therapy in patients with hereditary deafness. These first successes in treatment of hereditary inner ear disease may lead to the ultimate utility of gene transfer, gene replacement therapy.

5. Feature directions

The obstacles to introducing gene therapy into the otology clinic have been listed above, and include aspects of delivery, specificity to targets, side effects and regulation of quantity and duration of gene expression. Current and future research will contribute to further improvement of vector design and technology and, combined with better understanding of specific influence of genes and signaling pathways, gene therapy approaches may become an extremely useful tool for protection, repair and regeneration in the inner ear.

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Fig. 1.

A schematic diagram showing the left inner ear (a), the major cell types that typically require gene transfer for research or clinical purposes (b) and the most efficient ways to transduce mesothelial cells (c) and supporting cells (d). (a) Microtubes (yellow) inserted into the scala tympani via a cochleostomy (left), or through the round window (middle) or via a canalostomy (right) for delivering therapeutic agents to the mature left inner ear. (b) A cross section through the membranous labyrinth and surrounding tissues showing the three fluid chambers (scala vestibule, SV; scala tympani, ST; and scala media, SM; and specific cell types: inner hair cells (IHCs), outer hair cells (OHCs), supporting cells (SCs) and spiral ganglion neurons (SGNs) in Rosenthal's canal. (c) Vectors carrying transgenes inserted via

ST usually transduce the mesothelial cells. (d) When transgene vectors are inoculated into the SM, supporting cells are usually transduced.