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Practical Aspects of Inner Ear Gene Delivery for Research and Clinical Applications

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Abstract

The application of gene therapy is widely expanding in research and continuously improving in preparation for clinical applications. The inner ear is an attractive target for gene therapy for treating environmental and genetic diseases in both the auditory and vestibular systems. With the lack of spontaneous cochlear hair cell replacement, hair cell regeneration in adult mammals is among the most important goals of gene therapy. In addition, correcting gene defects can open up a new era for treating inner ear diseases. The relative isolation and small size of the inner ear dictate local administration routes and carefully calculated small volumes of reagents. In the current review, we will cover effective timing, injection routes and types of vectors for successful gene delivery to specific target cells within the inner ear. Differences between research purposes and clinical applications are also discussed.

1. Introduction

The use of gene therapy in Otology may potentially address both genetic hearing loss and conditions that require regeneration of hair cells or neurons. To achieve these goals, we have to consider “where”, “when” and “how” to deliver specific gene(s) to the ear, and to select a therapeutic gene with the required efficacy. Several other chapters in this Special Issue of Hearing Research deal with specific diseases and targets for therapy. The main goal of this Review is to discuss practical aspect of HOW that can be accomplished. We first introduce target cells and tissues (WHERE), proceed to discussing timing for therapy (WHEN), and continue with a detailed consideration of the delivery routes, current outcomes with different types of vectors for each of these routes, side effect and complications and applicability to the human ear (HOW). Because our focus is on future clinical applications, we also attempt

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to summarize limitations and side effects, mostly covering work in mammals, primarily in mature ears, in vivo.

2. Where: Target cells or regions

Both hair cells and non-sensory cells are important targets for gene transfer (Figure 1). “Supporting cells” is a general term for non-sensory cells of the otocyst-derived epithelium that lines the scala media. Supporting cells in and around the organ of Corti are targeted because of their involvement in genetic deafness and their potential use for regenerative therapy. The most common gene related to non-syndromic hearing loss is *GJB2*, expressed in cochlear supporting cells (Del Castillo et al., 2017; Kelsell et al., 1997). In humans, the highest expression of GJB2 in normal ears is in sub-populations of supporting cells flanking the organ of Corti whereas pillar and Deiters cells appear devoid of immunoreactivity (Liu et al., 2017). Strong expression of CX26 was also found in the lateral wall area (Liu et al., 2009). However, in mice the level of CX26 in Deiters and pillar cells is higher than that found in humans, implicating a role in the active cochlea mechanism and making them a potential target for therapy (Zhu et al., 2015; Zong et al., 2017).

Supporting cells are also a target for hair cell regeneration via transdifferentiation, a phenotypic conversion that occurs spontaneously in the vestibular sensory epithelia but needs to be induced in the cochlea. Using only one gene, results can be inefficient (Kawamoto et al., 2003); but forced expression of combinatorial genes is being attempted for increasing the number of new hair cells (Minoda et al., 2007; Walters et al., 2017). As discussed below, adenovirus (AdV) vectors appear to be the most efficient gene transfer vehicles for transgene delivery into most types of supporting cells (Excoffon et al., 2006; Ishimoto et al., 2002; Venail et al., 2007).

Stria vascularis marginal cells are another important target. For instance, marginal cells are involved in cases of KCNQ1/KCNE1 mutations causing hearing loss (Knipper et al., 2006; Rivas et al., 2005). The presence of gap junctions in the stria vascularis, and the influence of mutations in these genes on endocochlear potential (EP) and hearing, make the strial cells important targets for gene therapy (Mei et al., 2017). Pannexin genes expressed in basal cells of the stria vascularis have also been linked to genetic deafness and may be a target for interventions via gene therapy (Zhao, 2016).

Hair cells are targets for gene transfer aimed at both genetic and environmental diseases. There are numerous genetic defects that affect hair cells, making them important targets for therapy (Table 1). Myosin VIIa is a motor protein in hair cells, essential for developing and arranging stereocilia bundles (Lefevre et al., 2008; Self et al., 1998) and related to Usher syndrome 1B. A mouse Myosin VIIa model showed disorganized stereocilia (Holme et al., 2002). Mutations in other myosins present in hair cells have been found in human families and modeled in mice, as reviewed by Rehman et al. (Rehman et al., 2016). The myosin genes are typically too large to load on AAV vectors, the choice vector for hair cell transduction, therefore gene therapy protocols will have to await future design of improved delivery vectors.

Several other hair cell genes involved in hearing loss are small enough to load into vectors for gene therapy approaches. The mutated transmembrane channel like 1 (TMC1) gene was identified in mammalian hereditary deafness. The TMC1 protein is a part of the mechanotransduction complex in inner hair cells and is essential for hearing (Corey et al., 2019). The feasibility of AAV mediated expression of exogenous Tmc genes for treating such mutations was shown by the Holt group (Askew et al., 2015) (Nist-Lund et al., 2019).

Mice lacking the vesicular glutamate transporter-3 (VGLUT3) are congenitally deaf due to loss of glutamate release at the inner hair cell afferent synapse. Cochlear delivery of VGLUT3 using adeno-associated virus type 1 (AAV1) leads to transgene expression in only inner hair cells (IHCs), despite broader viral uptake. Within 2 weeks of AAV1-VGLUT3 delivery, auditory brainstem response (ABR) thresholds normalize, along with partial rescue of the startle response. Lastly, partial reversal of the morphologic changes was seen within the afferent IHC ribbon synapse. These findings represent a successful restoration of hearing by gene replacement in mice, which is a significant advance toward gene therapy of human deafness (Akil et al., 2012). Other examples of gene transfer into the models for genetic deafness include mutations in whirlin (Chien et al., 2016; Isgrig et al., 2017), and harmonin (Pan et al., 2017).

As far as environmental hearing loss is concerned, hair cells can be targets for gene transfer for protection. For instance, patients who receive aminoglycosides therapy for cystic fibrosis were found, in some cases, to develop hearing loss (Zettner et al., 2018). Protective effects against hair cell loss due to aminoglycosides were demonstrated in animal models (Kawamoto et al., 2004) and may be advanced for use in cystic fibrosis patients. Similar approaches have been tried on animal models for cisplatinum ototoxicity (Chen et al., 2018a).

Other regions of the cochlea are also important., for instance, GJB2 is expressed not only in supporting cells in and around the organ of Corti, but also in fibrocytes and mesenchymal cells of the lateral wall, in basal and intermediate cells of the stria vascularis, and in spiral ganglion neurons (Iizuka et al., 2015; Lee et al., 2015; Liu et al., 2009; Liu et al., 2016; Mei et al., 2017; Yu et al., 2014). OtoGL is also expressed in multiple regions, such as interdental cell, tectorial membrane, hair cells, Claudius cells and spiral prominence (Yariz et al., 2012). Col4A3/4A4/4A5, which are genes related to Alport syndrome, are another example, being expressed at interdental cell, inner sulcus cell, basilar membrane and spiral ligament (Cosgrove et al., 1996).

As indicated by the data summarized above, several animal models are being employed for developing technology that would eventually be used in patient ears. The most commonly used animals are mice, because they serve as models for genetic deafness. However, mice ears are small and surgical procedures are complex. Some of the morphological analyses used for outcome analysis are also more challenging in the mouse ear compared to larger rodents. As such, guinea pigs (Komeda et al., 1999) rats (Venail et al., 2007) and other larger rodents serve as good models for gene therapy procedures for treating non-hereditary disease. Primates are also emerging as an important step prior to applying the therapy in the clinic (Maguire et al., in press, Hearing Research).

3. When: Time points for gene delivery

For hereditary hearing loss, the disease can be congenital or develop at a later time point, depending on the mutation. Early intervention will be best for most cases because the likelihood of rescue of cells and function is highest. Providing or preserving hearing is especially critical for early onset genetic hearing loss and genes involved in early development of the inner ear such as EYA1, CHD7, SIX1, ROR1 (Table 1), because development of language and other social and cognitive functions benefits from hearing. Progress in technology for *in utero* gene therapy may become applicable for treating early onset hereditary deafness. In mice, the cochlea is still developing and maturing after birth, facilitating rescue studies in immature ears (Akil et al., 2012; Akil et al., 2019; Askew et al., 2015; Iizuka et al., 2015; Nist-Lund et al., 2019)). Unlike mice, humans are born with mature cochleae and will likely require *in utero* gene therapy for treating developmental gene mutations (Gubbels et al., 2008; Wang et al., 2018a). Late onset hearing loss has longer time window for intervention. In general, diagnostic ability needs to improve to provide not only the identification of the mutated gene, but also an assessment of the condition and survival of the affected cells.

For acquired deafness due to environmental (non-genetic) factors, similar considerations apply as far as timing for the treatment. Because loss of hair cells due to overstimulation or aminoglycoside antibiotic treatment may lead to a flat epithelium, which is refractory to transdifferentiation (Izumikawa et al., 2008), early treatment would be preferable. However, the diagnostic tools currently available are insufficient for unequivocal determination of hair cell loss, its extent and location. In addition, some forms of hearing loss tend to improve over the first weeks after a trauma, and the expectation for this to occur is a contraindication for early gene transfer therapy.

4. How – Administration Routes

Choosing the right type of vector and right route of delivery will be important for both effective gene delivery and avoiding any off-target effects. Comparison between routes of gene delivery in human cochlea is summarized in table 2. Schematic representation of injection routes in rodent animal models and in humans is illustrated in figure 2.

Systemic injection will be the least invasive delivery method possible. However, the blood-labyrinth barrier would reduce or restrict transfer into the cochlea (Nyberg et al., 2019). This barrier can be modulated somewhat by reagents such as diuretics, mannitol or by noise (Nyberg et al., 2019; Wu et al., 2014) but the safety of such manipulations and their utility for gene delivery are not yet established. In rats, the blood labyrinth barrier is not fully matured until postnatal day 14 (Suzuki et al., 1999), facilitating research on developing ears. rAAV2/9 injected intravenously to P1 neonatal mouse resulted in transgene expression in inner hair cells, spiral ganglion neurons and vestibular hair cells (Shibata et al., 2017). Shibata and colleagues (Shibata et al., 2017) experimented with systemic delivery of viruses to the wild type murine model and reported that the successful transduction of inner ear sensory epithelium depends on the dose, tropism of the viral serotype and the age of the recipient.

The major disadvantage of the systemically delivered virus-based genetic treatment is the potential for off-target effects. That said, a systemic route of administration for a viral-mediated genetic therapy has great promise for some types of disease processes. For example, a current clinical trial focus on the use of adeno-associated virus (AAV) serotypes and systemic intravenous injections to obtain global transduction of muscle tissue in an attempt to treat spinal muscle atrophy (SMA) (ClinicalTrials.gov Identifier: NCT02122952), and Duchenne muscular dystrophy (Duan, 2018) (ClinicalTrials.gov Identifiers: NCT03368742; NCT03375164; NCT03362502) as well as the AAV-mediated gene therapy for hemophilia A (Pasi et al., 2020) (ClinicalTrials.gov Identifier: NCT02576795). The success of the viral-based treatment depends among other factors on the optimal dose of the viral particles injected. The importance of this is recognized in clinical studies that use the determination of the viral dose as the primary outcome measure. So far, no serious adverse effects have been noted at various stages of these trials.

The systemic delivery of viral vectors to specifically target inner ear will require increased targeting specificity. This might be achieved by identification of specific serotypes whose natural, or genetically modified tropism will narrow the range of the target organs.

Improved viral targeting specificity for cochlear treatment will not address other limitations of systemic delivery. The blood-labyrinth barrier, neutralizing antibodies, blood clearance of viral particles, and the recipient's immune-response will also have to be addressed, as reviewed in-depth by Duan (Duan, 2016).

Intracochlear delivery involves injecting materials into the cochlear fluids, endolymph or perilymph. The potential risks and negative effect of some of these procedures is summarized in Table 3 and explained in further detail below.

The oval window (OW) is readily visualized with a transcanal or transmastoid approach in humans; but in mice, the shape of the bulla and its position relative to the cochlea make the round window (RW) easier to visualize than the oval window. In addition, the RW is membraneous and thus is more easily penetrated than the bony stapes footplate in the oval window. Consequently, the RW approach is used to perform intracochlear surgery in many in vivo animal models. For instance, AAVs injected into perilymph via the RW transfected hair cells and effectively treated mutations of *Vglut3* (Akil et al., 2012) and *TMC1* (Nist-Lund et al., 2019). AAVs also can reach and transfect other cell types including supporting cells via RW injection (Isgrig et al., 2019), (Tan et al., 2019). Moreover, RW injection can preserve residual hearing with both AdV and AAV (Iizuka et al., 2008).

One consideration for RW injection is that virus can disseminate to the cerebellum and contralateral inner ear through the cochlear aqueduct which connects the perilymph with the CSF (Kho et al., 2000; Stover et al., 2000). This may be less of a problem in humans where the flow in the aqueduct is likely restricted or absent (Gopen et al., 1997; Rask-Andersen et al., 1977). Vector can also disseminate systematically through temporal bone marrow or via hematogenous spread (Kho et al., 2000).

Intracochlear gene delivery in humans can be achieved using an approach through the ear canal similar to that commonly used in stapedotomy for otosclerosis. The procedure is

performed using a microscope with the patient under a general anesthetic, though potential use of a local and/or sedative type anesthetic could be considered. After the patient is anesthetized, the tympanic membrane is elevated allowing access to the middle ear space and lateral face of the otic capsule. A small hole can then be made in the footplate of the stapes using a drill or laser to enable access to the perilymph of the vestibule which is close to and in direct communication with the perilymph of the scala vestibuli. The only current gene therapy clinical trial in humans utilizes this approach to achieve intra-labyrinthine infusion of a virus carrying the basic helix-loop-helix transcription factor Atonal 1 (Atoh1), which speaks to the feasibility of this route for gene delivery. This trial aims to evaluate the safety, and tolerability of the tested agent, and its potential ability to improve hearing in humans (ClinicalTrials.gov Identifier: NCT02132130) however the results thereof have not yet been reported.

As an alternative to stapes footplate injection, the same transcanal surgical approach could be taken to allow access to the scala tympani through incision of the round window membrane. Occasionally overhanging round window niche bone and/or membranous duplications can present minor though manageable impediments to this approach. Alternatively, the scala tympani or scala vestibuli can be approached through a cochleostomy where precise drilling of otic capsule bone adjacent to the round window membrane would enable injection into these spaces. Surgical placement of a cochleostomy can be challenging and potentially traumatic, particularly for the scala vestibuli, therefore making a cochleostomy approach less ideal for intracochlear gene delivery.

Intracochlear delivery in humans, whether into the scala vestibuli or scala tympani, might be complicated by a number of factors. First, the fluids of the cochlea do not flow at any significant rate, although they may contribute to drug distribution if drug application can be extended to days (Salt et al., 2009). Therefore, a concentration gradient of viral solution during the single injection into the basal region might remain very high locally and may never reach the apical region at therapeutic concentrations. One solution to this would be to create a second fenestration to allow for displacement of perilymph upon injection so that the length of the cochlear duct could be perfused rather than simply injected. For example, if injecting virus into the stapes footplate one could incise the round window membrane to allow for efflux of displaced perilymph upon injection. While this solution might produce more rapid and uniform distribution of gene therapy agents along the length of the cochlear duct, trauma associated with the procedure may worsen any residual hearing that may be present.

Another factor that might complicate intracochlear delivery would be the potential for spread of an injected gene therapy agent through the cochlear aqueduct. This bony channel filled with cerebrospinal fluid enters scala tympani at the basal turn and connects to the subarachnoid space of the posterior cranial cavity at its opposite end. Anatomical analysis of the normal human cochlear aqueduct performed in the temporal bones of humans at ages from zero to one hundred years concluded that significant flow of fluid through the cochlear aqueduct would be unlikely (Gopen et al., 1997). That said, in children whose cochlear aqueducts are patent (Bachor et al., 1999), and also potentially in certain cases of adults, the injected gene therapy agent might migrate through the cochlear aqueduct away from the

cochlea and transduce off-target cells in the brain and the contralateral ear, as seen in animal studies (Kho et al., 2000) (Stover et al., 2000). In addition to the dilution leading to reduced titer, flow to the CSF might result in off-target effects of the transgene on cells in the CNS.

One additional method that should be mentioned for intracochlear gene delivery would be delivery through a cochlear implant electrode array. Indeed cochlear implantation along with a drug for a one-time intracochlear delivery has been described clinically (Bento et al., 2016). Scala tympani is a preferred location for the cochlear implant insertion, and the electrode is generally inserted through the round window or via a cochleostomy (O'Connell et al., 2016). Gene delivery could be paired with cochlear implantation by injecting gene vectors through the electrode array port, or by insertion of an array wrapped in a porous medium impregnated with the vector. Both methods could provide access to the perilymph in the basal and middle turns of the cochlea. However apical access would be still limited as electrode arrays are not inserted that far along the cochlear duct. Cochlear implants procedures can be combined with delivery of plasmid or other gene vectors, with electrode-derived current driving electroporation of the genetic material into target cells (Pinyon et al., 2014; Pinyon et al., 2019).

Transtympanic approaches are widely used in human for drug delivery due to relative ease of its procedure. This route of administration relies on absorption of the injected agent from the middle ear to the inner ear through the RW. However, the RW is a three-layered membrane allowing only small molecules to diffuse, and gene vectors are too large. Attempts to increase the RW permeability were partly successful (Shibata et al., 2012).

The advantage of the transtympanic method of delivery stems from the relatively short, simple procedure and local, rather than general, anesthesia needed. The procedure can be performed during an office visit and with only topical anesthetic applied to the tympanic membrane. A small volume (0.5 – 1 milliliter) of the treatment solution is injected into the middle ear through a small needle that punctures the tympanic membrane. Ultimately, the round window membrane comes to contact with the injected solution, and the patient is positioned to minimize drug loss through the Eustachian tube for some time. The three-layer round window membrane permeability, however, might create variability between patients in the rate at which a drug solution enters the perilymph. Hahn and colleagues demonstrated variability between animals in the entry rate of applied compounds (Hahn et al., 2006).

Furthermore, the round window membrane may not be permeable to certain viruses (Jero et al., 2001). The inconsistency of the transtympanic delivery may be somewhat mitigated by increasing permeability of the round window membrane with different compounds such as by local anesthetics, histamine or healon (Chandrasekhar et al., 2000; Shibata et al., 2012). Perhaps in the future of gene therapy, viral vectors could be replaced by nanoparticles, and/or liposomes as vectors which may traverse the round window membrane more readily than viral vectors after transtympanic delivery (Maeda et al., 2007).

Canalostomy refers to the delivery of an agent into a semicircular canal and is a useful approach in rodent models and feasible in humans via transmastoid surgery. Various types of AAV infected inner hair cells and outer hair cells by posterior semicircular canal injection in

mouse (Tao et al., 2018). Several serotypes of AAV could also preserve auditory functions by this way. Outcomes of combining RW and canalostomy showed enhanced gene expression (Yoshimura et al., 2018). While a mastoidectomy approach in humans allows for access to all three semicircular canals, the superiority of gene delivery via a canalostomy over round or oval window injections would need to be well established prior to this approach being realistically adopted in humans given the increased invasiveness of a transmastoid approach versus a transcanal approach and the risk of side effect such as those described following superior canal dehiscence repair (Xie et al., 2017).

Scala Media Cochleostomy:

In humans, the entire cochlea is embedded in the temporal bone and not visible to the surgeon, with the exception of the promontory, which is the most viable option for creation of a cochleostomy. In theory, access to the endolymph in the scala media could be achieved in humans through a precise cochleostomy on the promontory. That said, given the small size of the scala media, precise targeting of a cochleostomy to allow for injection of this space would likely be extremely challenging. In contrast, more of the rodent cochlea protrudes into the middle ear cavity facilitating access to perilymph as well as scala media endolymph. Thinner bone found in animal models, compared to humans, also allows visualization of the pigmented stria vascularis, providing a landmark for locating the scala media. The latter is significant for research purposes because for AdV to infect supporting cells in and around the organ of Corti, it needs to be injected into scala media (Excoffon et al., 2006; Ishimoto et al., 2002; Venail et al., 2007). Because the human ear does not provide such visible access to scala media injections, AdV cannot be easily delivered to supporting cells through the scala media, and therefore treatments focused on transdifferentiation of supporting cells to hair cells may need to await availability of appropriate vectors or sophisticated surgical technology.

5. How – Types of vectors to use

Adenovirus (AdV).

AdV can infect both dividing and non-dividing cells which makes its feasible for targeting terminally differentiated cells like HCs, supporting cells and inner ear neurons as well as epithelial progenitor cells. The most commonly used serotypes are 2 and 5 (Ahmed et al., 2017). Compared to other gene vectors, first and second generation AdV have the advantage of being able to carry larger genes (up to 14kb), but the disadvantage of the potential to provoke an immune reaction. Transgene expression peaks rapidly several days after injection and lasts for several months. Third generation AdV (gutted Ad) shows minimal immunogenicity and has an even larger cargo capacity of up to 36kb (Alba et al., 2005). Transgene expression was detected up to a year after injection in case of CNS (Soudais et al., 2004; Zou et al., 2000). AdV injection into scala media of mouse neonatal cochlea transduced mostly supporting cells however, cellular specificity depended on AdV vectors and the cochlear turn. Sometimes the inner and outer hair cells were transduced (Shu et al., 2016a) as well. Perilymphatic injection in adult cochleae showed transduction only of cells lining the perilymphatic space (mesothelial cells of scala tympani, scala vestibuli, basilar membrane and Reissner’s membrane) (Venail et al., 2007). Endolymphatic perfusion

resulted in infection of Hensen, Deiters, pillar and phalangeal cells in the auditory epithelium and the satellite cells around spiral ganglion neurons. Venail et al. also showed that when forced injection into scala tympani caused membrane rupture, some inner and outer hair cells also were infected.

AAV has several advantages over AdV including longer gene expression and lower immune response and toxicity, and is becoming the vector of choice for gene therapy in several fields. After penetrating the host cell, AAV can either persist in a stable episome form or integrate into the host genome (Yang et al., 1999). One disadvantage of AAV is its small size: with a capsid diameter of 20-25 nm, it has a limited gene cargo capacity on only 4.8kb (Ahmed et al., 2017). For delivering larger genes, additional techniques such as a dual AAV strategy are needed (Akil et al., 2019). Inner ear transduction efficiency of several serotypes was evaluated in multiple studies, especially those targeting hair cells, as described above. AAV1, 2, 4, 5, 6, 7, and 8 can infect spiral limbus, spiral ligament and spiral ganglion cells. AAV1, 2, 3, 5, 6, and 8 are able to transduce genes into inner hair cells (Ahmed et al., 2017). AAV5 and 6.2 were able to infect supporting cells (Iizuka et al., 2015; Shu et al., 2016b). Recently, viral engineering techniques have been used to develop highly efficient AAVs: Exo-AAV, Anc80L65, AAV9-PHP.B, AAV2.7m8 and AAV-ie were reported to show high efficiency transduction into inner and outer hair cells. These vectors were also able to transfect some supporting cells, especially after injection to immature cochleae (Gyorgy et al., 2019; Isgrig et al., 2019; Landegger et al., 2017; Tan et al., 2019). So far, transfection restricted supporting cell has not been reported with any AAV, as recently reviewed (Martin et al., 2017). The ability to transfect supporting cells exclusively can only be accomplished using AdV vectors, but scala media delivery is needed.

Other viruses.

Lentivirus can also infect non-dividing cells and carry foreign DNA up to 8kb. After injection into scala media, it could infect marginal cells of stria vascularis and organ of Corti, and spiral ganglion neurons (Wei et al., 2013); however, its efficacy for transfecting hair cells was very limited (Pietola et al., 2008; Wang et al., 2013)). There were also trials with Sendai virus and Herpes virus vectors for inner ear gene delivery (Derby et al., 1999; Kanzaki et al., 2007). Safety of using these vectors remains to be addressed.

Other approaches for gene delivery.

Cationic lipid was used for Cas9-guide RNA-lipid complexes targeting the Tmc1 delivery by scala media injection. It successfully edited the genome and reduced progressive hearing loss (Gao et al., 2018). Electroporation can be used for gene transfer into cells of embryonic otocysts (Brigande et al., 2009). Plasmid electroporation driven by cochlear implant electrodes was used to deliver BDNF and GFP in guinea pig and stimulated survival and sprouting of spiral ganglion neurons (Pinyon et al., 2014).

6. Conclusions

The increasing interest in developing gene based therapy for hearing loss, combined with advances in genetic engineering, seems poised to deliver viable treatments in the future, with

real potential for the pursuit of clinical trials. Our review of the delivery routes indicates the limits to which animal studies can improve surgical methods of delivery in a human patient. These limits are based on the anatomical differences between rodent and human ears, and the level of acceptable invasiveness of a surgical procedure. Nonetheless, animal models continue to be useful in the search for cell type specific genetic vectors, optimization of viruses to decrease their immunogenicity, increase transduction efficiency and regulate duration of gene expression. Better understanding of the differences between species will streamline the process of translation from lab to clinic, including customization of the chosen delivery vectors, specific cell types to be targeted, surgical route and the gene load to meet clinical standards for efficacy and safety.

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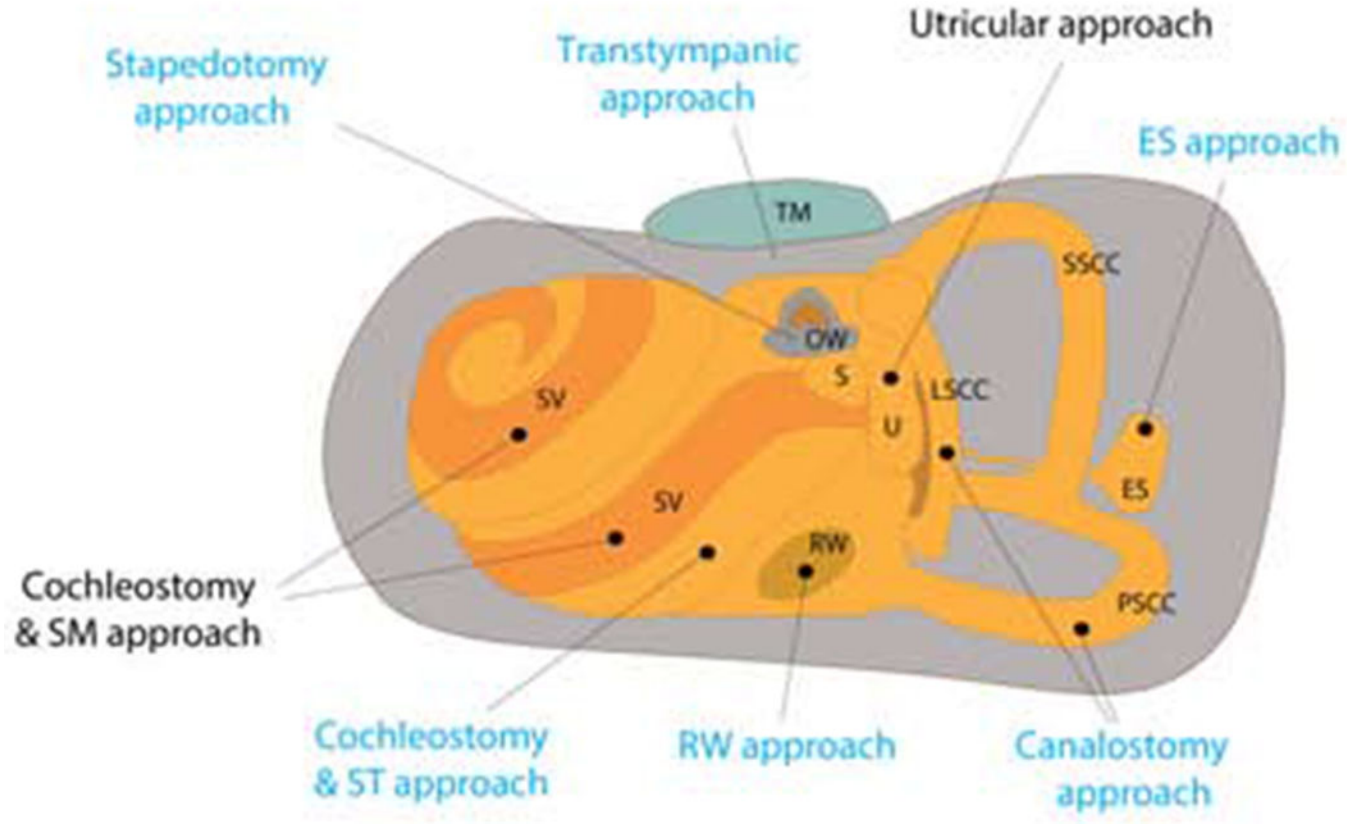

Figure 1. Target cells for the inner ear gene therapy.

IDC, Interdental cell; ISC, Inner sulcus cell; IHC, Inner hair cell; OHC, Outer hair cell; SV, Stria vacularis; MC, Marginal cell; IC, Intermediate cell; BC, Basal cell; EC, Endothelial cell; SL, Spiral ligament; FC, Fibrocyte; SGN, Spiral ganglion neuron; SchC, Schwann cell; IBC, Inner border cell; IPhC, Inner phalangeal cell; IPC, Inner pillar cell; OPC, Outer pillar cell; DC, Deiters cell; Hensen cell; CC, Claudius cell; SC, Supporting cell; TBC, tympanic border cells.

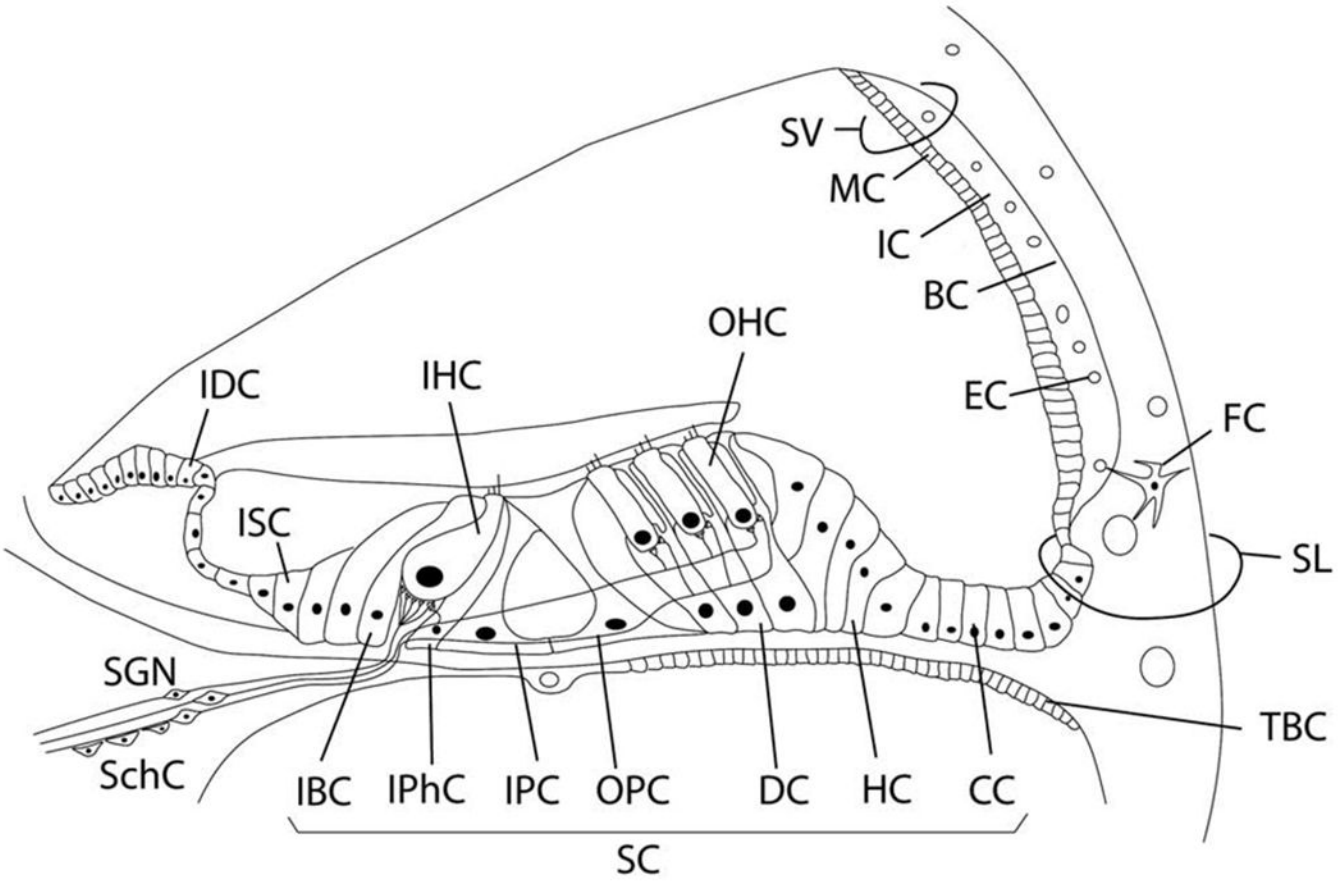

Figure 2. Routes for gene delivery in rodent animal models and the human cochlea.

Sites in black font are currently only possible in rodents. OW, Oval window; ES, Endolymphatic sac; SM, Scala media; RW, Round window; ST, scala tympani; SV, Stria vascularis; S, Saccule; TM, tympanic membrane; U, Utricle; LSCC, Lateral semicircular canal; SSCC, Superior semicircular canal; PSCC, Posterior semicircular canal.

Table 1.

Target cells (or regions) for gene therapy related to human inner ear mutation. Genes that are not localized at present are excluded.

Cell or area	Potential target genes (Ref)
Embryonic Development Period	
Otocyst	EYA1 (Abdelhak et al., 1997), CHD7 (Yao et al., 2018), (Durruthy-Durruthy et al., 2018).
Sensory epithelium	Six1 (Zheng et al., 2003)
Non-sensory epithelium	ROR1,SGN & SV (Diaz-Horta et al., 2016).
Postnatal to Adult Period	
Supporting cells	GJB2 (Qu et al., 2012), (Chen et al., 2018b), GJB6 (Qu et al., 2012), (Locher et al., 2015), MYH14 (Donaudy et al., 2004), WFS1 (Cryns et al., 2003), EYA4 (Matsuzaki et al., 2018), TMPRSS3 (Liu et al., 2018), DCDC2 (Grati et al., 2015), GPSM2 (Walsh et al., 2010), KARS (Santos-Cortez et al., 2013).
Inner hair cell	KCNQ4 (Beisel et al., 2000), WFS1 (Cryns et al., 2003), Myo7a (Liu et al., 1997), (Holme et al., 2002), MYH9 (Lalwani et al., 2000), (Mhatre et al., 2004), Myo6 (Mochizuki et al., 2010), REST (Nakano et al., 2018), TMC1/2 (Askew et al., 2015), (Kawashima et al., 2011), TBC1D24 (Zhang et al., 2014), OSBPL2 (Thoenes et al., 2015), HOMER2 (Azaiez et al., 2015), MCM2 (Gao et al., 2015), DMXL2 (Chen et al., 2017), Myo3a (Schneider et al., 2006), PDE1C (Wang et al., 2018b), TMIE (Shin et al., 2010), TMPRSS3 (Shin et al., 2010), (Molina et al., 2013), PCDH15 (Webb et al., 2011), (Maeda et al., 2014), RDX (Khan et al., 2007), GRXCR1 (Odeh et al., 2010), TRIOBP (Shahin et al., 2006), ESPN (Zheng et al., 2000b), ILDR1 (Sang et al., 2015), CIB2 (Giese et al., 2017), DCDC2 (Grati et al., 2015), LOXHD1 (Grillet et al., 2009), TPRN (Rehman et al., 2010), GPSM2 (Walsh et al., 2010), TBC1D24 (Zhang et al., 2014), ELMOD3 (Jaworek et al., 2013), SERPINB6 (Sirmaci et al., 2010) CABP2 (Yang et al., 2016) TMEM132E (Li et al., 2015), GRXCR2 (Avenarius et al., 2018) EPS8 (Olt et al., 2014) CLIC5 (Gagnon et al., 2006).
Outer hair cell	KCNQ4 (Kharkovets et al., 2000), Myo7a (Liu et al., 1997), (Holme et al., 2002), Pou4f3 (Frenz et al., 2015), MYH9 (Lalwani et al., 2000), (Mhatre et al., 2004), REST (Nakano et al., 2018), TBC1D24 (Zhang et al., 2014), OSBPL2 (Thoenes et al., 2015), HOMER2 (Azaiez et al., 2015), MCM2 (Gao et al., 2015), DMXL2 (Chen et al., 2017), Myo3a (Schneider et al., 2006), PDE1C (Wang et al., 2018b), TMIE (Shin et al., 2010), TMPRSS3 (Liu et al., 2018), OTOF (Yasunaga et al., 1999), (Akil et al., 2019), PCDH15 (Webb et al., 2011), (Maeda et al., 2014), RDX (Khan et al., 2007), GRXCR1 (Odeh et al., 2010), TRIOBP (Shahin et al., 2006), ESPN (Zheng et al., 2000b), , ILDR1 (Sang et al., 2015), CIB2 (Giese et al., 2017), SLC26A5 (Zheng et al., 2000a), DCDC2 (Grati et al., 2015), SYNE4 (Horn et al., 2013), LOXHD1 (Grillet et al., 2009), TPRN (Rehman et al., 2010), GPSM2 (Walsh et al., 2010) TBC1D24 (Zhang et al., 2014) ELMOD3 (Jaworek et al., 2013) SERPINB6 (Sirmaci et al., 2010) CABP2 (Yang et al., 2016) GRXCR2 (Avenarius et al., 2018) EPS8 (Olt et al., 2014) CLIC5 (Gagnon et al., 2006).
Interdental cell	CEACAM16 (Kammerer et al., 2012).
Stria vascularis	MYH14 (Donaudy et al., 2004), COCH (Kimura et al., 2013), BDP1 (Girotto et al., 2013), SLC22a4 (Ben Said et al., 2016), MET (Shibata et al., 2016), KCNQ1/KCNE1 (Neyroud et al., 1997), (Schulze-Bahr et al., 1997).
Spiral ligament	GJB3 (Xia et al., 2000), (Lopez-Bigas et al., 2001), COCH (Robertson et al., 2001), (Kimura et al., 2013), MYH9 (Lalwani et al., 2000), (Mhatre et al., 2004), KARS (Santos-Cortez et al., 2013).
Spiral ganglion neurons	KCNQ4 (Beisel et al., 2000), WFS1 (Cryns et al., 2003), NLRP3 (Chen et al., 2016), TBC1D24 (Zhang et al., 2014), DMXL2 (Chen et al., 2017), TBC1D24 (Zhang et al., 2014) GRAP (Li et al., 2019).
Vestibular Hair cells	KCNQ4 (Kharkovets et al., 2000), WFS1 (Cryns et al., 2003), TMC1/2 (Askew et al., 2015), (Kawashima et al., 2011), RDX (17226784), GRXCR1 (Odeh et al., 2010), ESPN (Zheng et al., 2000b), LOXHD1 (Grillet et al., 2009), TPRN (Rehman et al., 2010) CLIC5 (Gagnon et al., 2006).

Table. 2

Routes of delivery to the human cochlea

Route	Advantages	Disadvantages	Problems Mitigation
Systemic	Easy Procedure, Minimally Invasive, Procedure itself does not pose any risk to residual hearing	High risk for off-target spread, Difficult to preserve the therapeutic dose due to neutralizing antibodies, blood clearance of viral particles, and potential immune response of the recipient	Increase viral targeting specificity, reversibly modulating blood-labyrinth barrier permeability
Intra Cochlear (via Round or, Oval Windows, and via cochleostomy)	Delivery directly to the inner ear brings viruses to perilymph (scala tympani-via RW) or directly into endolymph (scala media – via OW). Viruses can transfect hair cells or supporting cells directed by their tropism.	Invasive procedures requiring full anesthesia. Risk of residual hearing loss. Risk of virus dissemination to the cerebellum and contralateral inner ear via the cochlear aqueduct or systematically through temporal bone marrow or via hematogenous spread. Viral concentration might be very high locally and not spread efficiently throughout the entire cochlea.	If the injection is made carefully residual hearing can be preserved, Virus spread via the cochlear aqueduct is less likely in adults, so the choice of the patient's age should be considered. Viral injection in the cochlear fluids has to be very slow or used only if the target is located near the injection site.
Canalostomy via transmastoid surgery	Allows access to all three semicircular canals	A Very invasive procedure without established advantages over the intracochlear surgery	Demonstrate clear superiority of transmastoid viral delivery over the intracochlear injections.
Trans Tympanic	An easy and short procedure, minimally-invasive, with local anesthesia, already performed in humans	The concentration of the virus in the inner ear is poorly controlled due to the variable entry rate or imperviousness of the RW, to different viruses.	Increase the permeability of the RW using different compounds. Replace the viral particle with nanoparticle or liposome with stable RW permeation capability.

Table 3.

Negative clinical outcomes.

Route	Potential negative outcomes	Disadvantages
Systemic	Viral dose and patient dependent Immune response. Other organs, besides the cochlea might also be targeted.	Difficult to preserve the therapeutic dose due to neutralizing antibodies, blood clearance of viral particles, and potential immune response of the recipient
Intra Cochlear (via Round or, Oval Windows, and via cochleostomy)	Loss of residual hearing, due to injection evoked mechanical damage to HCs. Full anesthesia related complications. Potential infection of the CNS.	Risk of virus dissemination to the cerebellum and contralateral inner ear via the cochlear aqueduct or systematically through temporal bone marrow or via hematogenous spread. Viral concentration might be very high locally and not spread efficiently throughout the entire cochlea.
Canalostomy via transmastoid surgery	Benign paroxysmal positional vertigo, deep vein thrombosis (Xie et al., 2017)	An invasive procedure used to treat severe Superior semicircular canal dehiscence.
Trans Tympanic	Post-injection pain, tongue numbness, transient dizziness, tinnitus, small persistent perforation of the tympanic membrane	The concentration of the virus in the inner ear is poorly controlled due to the variable entry rate or imperviousness of the RW, to different viruses.