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Development of gene therapy for inner ear disease: Using bilateral vestibular hypofunction as a vehicle for translational research

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Abstract

Despite the significant impact of hearing and balance disorders on the general population there are currently no dedicated pharmaceuticals that target the inner ear. Advances in molecular biology and neuroscience have improved our understanding of the inner ear allowing the development of a range of molecular targets that have the potential to treat both hearing and balance disorders. One of the principal advantages of the inner ear is that it is accessible through a variety of approaches that would allow a potential to be delivered locally rather than systemically. This significantly broadens the potential medications that can be developed and opens the possibility of local gene delivery as a therapeutic intervention. Several potential clinical targets have been identified including delivery of neurotrophin expressing genes as an adjunct to cochlear implantation, delivery of protective genes to prevent trauma and the development of strategies for regenerating inner ear sensory cells. In order to translate these potential therapeutics into humans we will want to optimize the gene delivery methodology, dosing and activity of the drug for therapeutic value. To this end we have developed a series of adenovectors that efficiently transduce the inner ear. The use of these gene delivery approaches are attractive for the potential of hair cell regeneration after loss induced by trauma or ototoxins. This approach is particularly suited for the development of molecular therapies targeted at the vestibular system given that no device based therapeutic such a cochlear implant available for vestibular loss.

Keywords

Gene therapy; atoh1; adenovector

Introduction

Currently there are no pharmaceuticals designed specifically for the inner ear. Development of therapies to treat hearing loss and balance disorders is complicated by the diversity of disease processes leading to functional loss and the current approaches available for

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treatment. Hearing loss affects approximately 31 million in the U.S. alone. Treatment of hearing loss with hearing aids has only a 30% penetration and a low rate of patient satisfaction. The incidence of peripheral vestibular disease is largely undefined although problems with balance are thought to be in the top 5 reasons for primary care visits. Part of the challenge in developing drugs for the inner ear is physical. It is separated from the general circulation by a blood cochlea barrier with similar properties as the blood brain barrier making systemic delivery of medications less efficient. There is however a significant opportunity for the development of local delivery. The inner ear is easily accessible via the round window membrane. Additionally the inner ear can also be accessed via a cochleostomy, through the stapes footplate, via a canalostomy or through the endolymphatic sac (Staecker et al., 2004). Animal models have been developed for all of these approaches. In humans we have evidence that the inner ear can be accessed without damage via all of these approaches, yielding the opportunity to develop local therapy to a variety of regions of the inner ear. One of the significant advantages of the inner ear is that it is a relatively closed space. Thus delivery of a drug or a molecular therapeutic such as a viral vector into this space would have significant advantages over systemic delivery in which large doses would be needed in order to distribute the drug to the inner ear.

Development of a vector for use in the inner ear

An ideal vector for the delivery of genes to the inner ear needs to be specific and concentrated in a low volume to avoid hydraulic trauma when delivering to the perilymph or endolymph. Specificity can be conferred by designing a vector that contains either a tissue specific promoter or a surface component of the vector that ensures tissue specific binding; this would allow vector dose to be minimized and the gene of interest to be expressed exclusively in the targeted cell (Fig 1). An ideal vector would additionally have a low incidence of eliciting immune responses from the targeted host. Development of a novel gene delivery based therapy would need to ensure that residual function of the inner ear is not compromised and that there are no deleterious effects at distant sites from the inner ear. Several different types of vectors are available to deliver a molecular therapeutic to the inner ear. They differ in complexity of construction, expression time, potential side effects and carrying capacity. Picking the right vector for different disease targets is a key factor in developing a molecular therapeutic. A variety of other viral and non viral vectors have been tested in the inner ear most current studies are focusing on optimizing adenovirus (Ad) and adeno-associated virus (AAV) based vector systems.

AAV is a small parvovirus that carries an approximately 4500 bp genome flanked by terminal repeat sequences. These sequences are needed for the initiation of DNA replication and viral packaging. The native virus can exist in a lytic life cycle or can integrate into the host cells' DNA as a provirus. A helper virus (adenovirus) is needed for the virus to complete the lytic cycle or for production of an AAV vector. This quality of a latent state allows this vector to potentially be used for treatments that require long expression periods such as the correction of a fixed genetic deficit or production of a growth factor that would be required over extended time periods. The size of the genes that can be transferred using this system is limited by the overall size of the vector particle. About 95% of the AAV native genome can be replaced. However it is not clear whether the recombinant vectors that are produced truly integrate into the host genome. However overall evidence does suggest that this vector system would be useful for long term expression applications such as the delivery of a neurotrophin gene. One of the great advantages of this packaging system is that it is not associated with any known human disease making it a very safe vector to use (Kapturczak et al., 2001; Lalwani et al., 1996; Snyder, 1999). Recent developments in AAV vectorology include production of more concentrated vector stocks which is particularly important for applications in the inner ear where there are limitations on the volume of

vector that can be delivered (Boulaiz et al., 2005). Non human AAV vectors have also been successfully developed for gene delivery in the inner ear (Shibata et al., 2009).

Adenovectors are increasingly being used in gene therapy research for the inner ear (Husseman et al., 2009). Adenovectors are non-enveloped viruses containing a linear double stranded DNA genome. They measure 90 nm in diameter and have a molecular weight of 2500 kDa. There are over myriad strains of adenovirus from human and nonhuman sources. However, human subgroup C adenovirus serotypes 2 or 5 are predominantly used as vectors. The life cycle of adenovirus does not involve integration into the host genome, rather they replicate as episomal elements in the nucleus of the host cell and consequently there is no risk of insertional mutagenesis. The carrying capacity of genes is essentially not limited and can be as much as the full genome length of the adenovirus (30–35 kb). There are four early transcriptional units (E1, E2, E3 & E4), which have regulatory functions, and a late transcript, which codes for structural proteins. Progenitor vectors have either the E1 or E3 gene inactivated, with the missing gene being supplied in trans either by a helper virus, plasmid or integrated into a helper cell genome. Second generation vectors additionally use an E2a temperature sensitive mutant or an E4 deletion. Adenoviral vectors are very efficient at transducing target cells in vitro and in vivo, and can be produced at high titres ($>10^{11}$ /ml). This is an important factor in inner ear gene therapy where the volume of delivered molecular agent can be limited by the volume of the inner ear. Delivery of excessive volume to the inner ear results in trauma and loss of function (Praetorius et al., 2003). Because of the ease of development and the ability to produce very concentrated vector stock adenovectors are ideal for use in the inner ear when short term gene delivery is needed such as delivery of a short acting transcription factor like *atoh1*.

To develop molecular therapeutics for hearing loss and balance disorders we need to be able to accurately diagnose a given disease on a molecular level in the inner ear since multiple different genetic deficits can cause similar hearing losses. The molecular pathology of vestibular disease is largely undefined. As seen in Schucknecht's Pathology of the Ear, standard audiometry does not necessarily reflect the actual state of cellular health within the inner ear. For example, a hearing loss can potentially be caused by loss of hair cells, loss of the spiral ganglion, dysfunction of the striavascularis or an undefined process, all of which may yield similar standard audiograms. Since we are unable to biopsy the living inner ear we have to rely on having an accurate clinical history in combination with appropriate physiologic testing.

Loss of vestibular hair cells results in changes in the vestibulo-ocular reflex (VOR) response. Central compensation after a unilateral fixed vestibular loss can result in recovery of VOR gain but no recovery of the phase difference between stimulus and VOR is seen, thus yielding an available measure of permanent vestibular damage. VOR testing provides a reliable measure of vestibular function and allows us to identify patients and their prognosis for recovery using standard equipment available in the clinical setting. Bilateral vestibular hypofunction (BVH) results in permanent chronic balance dysfunction and inability to fix a target on the retina while moving. Complete recovery from vestibular loss may only be possible through replacement of the missing vestibular sensory cells. Patients with bilateral vestibular disorders, such as aminoglycoside induced BVH, represent a critical patient population in need of a therapeutic approach for whom no other clinical treatment is available. Aminoglycoside induced vestibulopathy represents a population where we know the underlying pathology (vestibular hair cell loss), making the use of a molecular agent to regenerate hair cells possible. Focusing on hair cell loss as a disease process allows us to identify a target (supporting cells), pick a gene or series of genes that can be tested to regenerate cells and gives us a clinical target for translational research.

Identifying molecular strategies for regeneration

The effort to determine the molecular basis for hair cell generation has led to examination of factors controlling hair cell differentiation (Kelley et al., 2009; Warchol, 2010). Two main types of molecular strategies have evolved. One of the key differences between mammalian and non mammalian vertebrates is the capacity for spontaneous hair cell regeneration. Non mammalian vertebrates can functionally regenerate both the vestibular and auditory system (Cotanche, 1999; Cotanche et al., 2010). The capacity for this roughly correlates with the neuro-epithelium's capacity for mitosis (Warchol, 2010). A potential approach to this problem is to alter cell cycle control, allowing division of supporting cells and differentiation of these cells into hair cells. Ablation of retinoblastoma protein in neonatal mice results in proliferation of supporting cells (Liu et al., 2008; Weber et al., 2008; Yu et al., 2010). Other cell cycle control genes such as p27kip1 and cyclinD have also been implicated in maintaining the quiescent state of the mammalian inner ear (Laine et al., 2010; Lowenheim et al., 1999). Although pharmacologic inhibitors of different components of the cell cycle are available, use of a gene therapy approach would allow individual cells to be targeted so that for example, cell cycle control is only interrupted in supporting cells. An interesting observation in the p27kip1 studies was that animals that produced supernumerary hair cells did not have normal hearing, suggesting that the complex structure of the auditory neuroepithelium needs to be maintained for optimal function.

A second approach is to take advantage of the observation that non mammalian hair cells can be generated from supporting cells by transdifferentiation (Stone et al., 2007). This could potentially be achieved by over-expressing the genes that control hair cell genesis or altering the expression of genes controlling patterning of the neuro-epithelium. One of the genes involved in the development of hair cells is the mammalian homolog of the drosophila helix loop helix gene atonal (math1/atoh1). Mice carrying a homozygous knockout of math1 fail to develop auditory or vestibular hair cells (Bermingham et al., 1999; Shailam et al., 1999; Woods et al., 2004). Further investigation of this pathway has demonstrated that a complex regulatory pathway specifies the differentiation of hair cells and supporting cells and that suppression of atonal effects is what specifies a cell as a supporting cell (Lanford et al., 2000; Qian et al., 2006; Zine et al., 2002; Zine et al., 2001). Recent studies have also begun to identify further downstream regulators that are dependent on math1 function.

Conceptually, altering this pathway either by changing expression of the lateral inhibitory pathway or over-expressing atoh1 should produce new hair cells via transdifferentiation of supporting cells (Murata et al., 2006; Zine et al., 2002). Pharmacological inhibitors of notch signaling have also been shown to help induce ectopic hair cell regeneration (Hori et al., 2007; Yamamoto et al., 2006). Delivery of a plasmid vector expressing math1 to neonatal organ of Corti cultures produced supernumerary hair cells in vitro (Zheng et al., 2000). These results were repeated using the human homolog of math1 (hath1) and an adenovector as the delivery vehicle. In this study hair cells were shown to be regenerated in adult mammalian vestibular neuroepithelium in vitro (Shou et al., 2003). Most subsequent work has focused on the auditory system. Based on transduction studies in the guinea pig, it was demonstrated that optimal transfection of supporting cells was achieved by delivering adenovector to the scala media (Ishimoto et al., 2002). Using injection of a math1 expressing adenovector, Kawamoto demonstrated that delivery of math1 to the inner ear of mature guinea pigs. This resulted in the production of ectopic hair cells that could attract innervation (Kawamoto et al., 2003). The hair cells produced could therefore have both the physical characteristics of a hair cell (stereocilia, staining for myosin VII) as well as produce neurotrophins and therefore undergo the critical needed step for hearing, which is innervation of a newly produced hair cell. A follow up study from this group treated mature guinea pigs with systemic administered aminoglycosides and a diuretic resulting in severe to profound hearing loss in both ears (Izumikawa et al., 2005). Administration of math1 via a

scala media approach resulted in the generation of inner hair cells and a cell in the outer hair cell region that spanned from the luminal surface of corti's organ to the basement membrane. It was speculated that this represented a cell with both supporting cell and hair cell characteristics. Animals treated with the *math1* gene also recovered hearing. Although stereocilia bearing cells could already be seen at 4 weeks after gene transfer, recovery of hearing was not observed until 8 weeks post gene transfer. Cells generated through *math1* transfection have also been characterized by patch clamping and have been found to have the functional mechanotransduction (Gubbels et al., 2008). Transfer of *atoh1* is therefore potentially a viable method for restoring damaged auditory/vestibular neuroepithelium and could be considered for a translational therapy. Interestingly, potentially combining both proliferation (forced expression of *skp2*) and forced transdifferentiation (overexpression of *atoh1*) may yield optimal generation (Minoda et al., 2007).

Animal models of vestibular regeneration

Functional regeneration of mammalian vestibular hair cells does not occur spontaneously although there is some evidence of the presence of low regeneration after injury (Kawamoto et al., 2009; Li et al., 1997). Regeneration of vestibular hair cells can be induced in a variety of model systems including guinea pig macular organs, rodent and human macular organs, and the chinchilla crista ampullaris (Lambert, 1994; Lopez et al., 1997; Tanyeri et al., 1995; Warchol et al., 1993). A variety of factors have been identified that could increase the number of hair cells generated in an aminoglycoside injury/regeneration-repair model including retinoic acid, TGF α and IGF (Lambert, 1994). Kopke et. al. demonstrated that infusion of a cocktail of growth factors into the scala tympani after aminoglycoside injury in guinea pigs resulted in a statistically significant renewal of vestibular hair cells and recovery of vestibular function as measured by recovery of the horizontal VOR (Kopke et al., 2001). This study is particularly significant since it demonstrated recovery of VOR phase demonstrating that there was recovery of peripheral function rather than central compensation. Together, these studies suggest that the vestibular neuroepithelium has significant potential to recover and thus is a potential target for directed therapy that induces sensory cell recovery. A potential explanation for this may be differences in the expression of *Hes5* expression when comparing auditory and vestibular tissues as well as changes in *Hes5* expression after injury (Hartman et al., 2009; Wang et al., 2010).

We have evaluated the delivery of *atoh1* in a mouse vestibular injury model in vitro and in vivo. Macular organ cultures treated with an Ad5 vector that expressed *atoh1* under the control of the human cytomegalovirus (hcmv) promoter resulted in 60% regeneration of vestibular hair cells after ablation with neomycin. These cells were not the product of a mitotic event based on BRDU staining (Staecker et al., 2007). Time course studies using these cultures demonstrated that the first hair cells can be seen between 7 and 10 days post *atoh1* transduction. Delivery of *atoh1* to the perilymph of mice treated with intracochlear injection of neomycin also resulted in regeneration of vestibular hair cells. These mice also showed an improvement in swim test times after delivery of *atoh1* indicating improvement of balance. Similar to the in vitro experiments, approximately 60% of the original hair cell population recovered even though delivery of 1×10^8 particles of *atoh1* expressing vector into the inner ear should result in transfection of all available cells. A potential explanation for this is that lateral inhibition via notch signaling non transfected supporting cells limits the number of hair cells that can be produced. A potential concern from a translational research standpoint is that cells that have been generated through delivery of a transcription factor may not be stable for prolonged time periods. Delivery of an Ad5 capsid vector, that expressed *atoh1*, into the perilymph space of neomycin treated mice induced robust regeneration that were maintained over a 6 month period (Fig 2), demonstrating that hair cells produced through forced transdifferentiation of supporting cells are stable.

Effective delivery of vectors to the inner ear

Several different approaches have been evaluated for delivery of gene therapy vectors into the inner ear of animals. Most studies have used a cochleostomy or injection through the round window membrane to deliver vector to the perilymphatic space using direct injection or an osmotic pump. Kawamoto et. al. compared gene expression and cochlear function in mice who underwent vector delivery either through a canalostomy or a cochleostomy (Kawamoto et al., 2001a). Animals were injected with Ad *lacZ* through the posterior semicircular canal (PSCC) or the round window. Canalostomy delivery resulted in gene expression for up to 28 days in the perilymphatic spaces of the PSCC, utricle, saccule and cochlea. It is not clear if this represented active transcription or persistence of *lacZ*. Cochleostomy delivery similarly showed gene expression in the cochlear perilymphatic space, as well as expression in IHCs, Deiter's cells and supporting cells in the saccule. Cochlear function, measured by ABR, showed minimal threshold shifts in canalostomy animals. Cochleostomy, however, resulted in threshold shifts significantly higher than those seen in the canalostomy group for all three frequencies tested. The authors concluded that canalostomy provides a procedure for transducing both vestibular and cochlear tissues as well as preserving cochlear function. Alternate delivery modalities to the inner ear have been tried including delivery to the endolymphatic sac, delivery to the round window as well as delivery to the scala media via a cochleostomy through the striavascularis. Delivery of adenovector into the scala media appears to damage hair cells but overall improve transgene expression in the organ of Corti. This may explain why prior auditory hair cell regeneration experiments showed that *math1* delivery in the guinea pig was effective only via a scala media approach (Izumikawa et al., 2005). An in vitro approach has been developed to examine if there are differences in perilymph or endolymph delivery of *atoh1* to the vestibular system effected production of hair cells. Overall the endolymph modeled delivery appeared more effective (Huang et al., 2009). The underlying biology of this is unclear. The basal portion of supporting cells are in perilymph and should be exposed to vector delivered into this space. The receptor for Ad5 has been identified in the tight junction system at the luminal end of the auditory neuroepithelium and potentially the endolymph based approaches yield better access to this area. As discussed above, perilymph based delivery of adenovectors expressing *atoh1* is effective in the mouse in vivo. Potentially delivery in these cases is mediated by non CAR receptors.

The delivery location for human *atoh1* delivery could take several different forms. Currently, human equivalents exist for all delivery modalities. Stapedectomy is a well established surgical procedure that accesses the perilymphatic spaces of the vestibule. It has a long safety track record and is easy to perform. The scala tympani perilymph can also be accessed via a basal turn cochleostomy or through the round window. Recent advances in cochlear implantation have demonstrated that up to 28mm long electrodes can be inserted into the scala tympani without hearing loss, suggesting that injection of vector could also be achieved via this route (Prentiss et al., 2010). Recent studies of steroid delivery to the inner ear have demonstrated that the endolymphatic spaces of the human inner ear can also be accessed without loss of function making this approach possible (Colletti et al., 2010). The volume constraints of the endolymphatic spaces may ultimately constrain delivery due to the limited number of vector particles that can be delivered.

Identification of the necessary components of successful molecular therapy

The biology of *atoh1* provides an interesting approach to identify the necessary components of successful molecular based therapies. For example, genes must be efficiently delivered to appropriate target cells (in the case of *atoh1*, supporting cells) in order for the gene to have

the desired therapeutic activity. The choice of vector system is one key to building a molecular therapy since it determines whether the therapeutic gene is delivered appropriately. Adenovectors have been well characterized in the inner ear and are preferred over other vectors for clinical studies due to well defined clinical grade manufacturing protocols and greater vector purity. Adenovectors have generally been shown to have a limited expression time, making them ideal for delivering a gene where only a limited time period of delivery is needed such as *Atoh1*, which is active only during a brief time during development. One critique is the potential of immune related toxicity. This can be mitigated by controlling total vector dose and modification of the vector backbone and capsid (Kawamoto et al., 2001b; Praetorius et al., 2009a).

Adenovector modification provides a means to achieve selective delivery to specific cells within the inner ear allowing for a high percentage of the targeted supporting cell to be transduced. An important advantage for our approach is the adenovector the production system used in the proposed studies as it provides highly concentrated drug that allows delivery of a very low volume and high particle count to the inner ear. The specificity of gene delivery can be modulated by changing promoters. The bulk of inner ear gene delivery studies to date have used the hcmv promoter. Expression level of the delivered transgene can be modified by using different promoters, which along with the limitation in adenovector expression in vivo can be used to control dosage of the transgene (Praetorius et al., 2009b). Further specification of which cell type a vector is expressed in can be achieved by using tissue specific promoters that limit expression of the transgene to subsets of cells within a tissue. An inner ear supporting cell specific promoter has been identified and has been used to increase the specificity of gene delivery to supporting cells in vitro (Stone et al., 2005). Gene chip studies of laser capture dissected vestibular supporting cells have identified numerous other genes that may be specific to supporting cells, allowing the identification of other supporting cell specific promoters (Cristobal et al., 2005). Interestingly there appears to be an inverse relationship between promoter strength and success at generating hair cells with *atoh1* transduction. When looking at the effect of different strength promoter systems on hair cell regeneration in adult mouse utricular cultures, it was evident that the strongest promoter (chicken beta actin) did not provide that same level of regeneration than weaker or supporting cell specific promoters (Praetorius et al., 2009b). This suggests that there is an optimal dose for *atoh1* that is needed for hair cell regeneration. The use of a tissue specific promoter like the GFAP promoter also provides a means to limit the expression of the gene to only specific cells and in this case supporting cells (Fig 3). Use of the *gfap* promoter in conjunction with *atoh1* delivery resulted in robust regeneration of vestibular hair cells in vivo. Adult mice were treated with an intra-cochlear neomycin injection which reliably ablated greater than 90% of vestibular hair cells within 48 hours. At 2 days post aminoglycoside treatment an Ad5 vector expressing *atoh1* driven by the *gfap* promoter was injected into the scalatypmani via the round window. At 1 month post vector delivery, robust regeneration of hair cells could be seen (Fig 4). These hair cells were also innervated with clear evidence of calycealinnervation of type I hair cells (Fig 5). This demonstrates that a supporting cell specific promoter could be used to efficiently restore vestibular hair cells after aminoglycosideototoxicity.

Adenovectors can be retargeted to improve specificity

Besides using tissue specific promoters, adenovector specificity can also be changed by altering the binding of the vector to cells. Standard serotype 5 adenovectors bind to cells via interaction of the vector fiber/knob with cellular coxackie adenovirus receptor (CAR). Interaction with cell surface integrins, in particular integrin alpha V is needed for internalization. The specificity of vector binding to cells can be improved through retargeting of adenovectors (Lai et al., 2002). Different strategies can be used to modify the

adenovirus fiber knob to bind epitopes other than CAR. One of the most commonly used approach for retargeting are ligand based approaches. Biphasic antibodies that recognize the vector and a cell surface receptor can be constructed and are currently being used to screen for possible receptor targets. Other approaches include genetically altering the fiber to avoid native tropism and creating customized fibers for individual vectors that target specific cells for transduction. Currently there is evidence that retargeted vectors increase specificity of vector delivery (Lu et al., 2006). We have evaluated genetic retargeting of standard Ad5 fiber knob domains. Ad5 vectors that showed enhanced binding to heparin were able to transduce vestibular neuroepithelium more efficiently than a vector with enhanced binding to integrins or native capsid vector (Fig 6)(Praetorius et al., 2009a). If supporting cell specific epitopes could be identified, vectors could potentially be made even more specific to their intended target. Alternately different vectors that have alternate binding capacities could be identified in an attempt to retarget using a native capsid.

Immunology of adenovectors

One of the potential disadvantages of adenovectors is that they can induce inflammatory responses and be recognized by preexisting immunity. This can change the clearance characteristics of the vector and also potentially result in deleterious effects on the target tissue. Early studies of adenovector delivery defined that in the mouse damage to inner ear tissue was related to the total volume of vector delivered rather than the expression of the gene itself in mice that had not previously been immunized with the adenovector (Praetorius et al., 2003). The repeated administration of Ad5 in the guinea pig resulted in hearing loss that was reversed by steroids, suggesting that this was due to an adenovirus based inflammatory response (Ishimoto et al., 2003). Based on other systems the total dose of vector probably plays a role in the development of local inflammation. Current evidence demonstrates that vestibular hair cell regeneration is achievable with a single dose of Ad5 capsid vector that does not induce inflammation in the inner ear. Given the doses effective to elicit functional responses for gene delivery in the inner ear it will be interesting to see if these inflammatory responses will be limiting for the use of adenovectors or if the results seen in the guinea pig are dose and animal species dependent. For example in another sensory organ, the eye, it has been shown that increasing doses of adenovector reached significant inflammatory responses only at very high doses and that repeated delivery into the eye was feasible (Hamilton et al., 2006). It is currently unknown if the doses necessary for functional impact for therapeutics in the inner ear will be similar to those that were found safe in the eye. Additional pharmacological studies will be necessary for future development of these therapeutic approaches.

Several studies have suggested that severity of injury can affect the ability of a damaged neuroepithelium to regenerate (Izumikawa et al., 2008). This has been associated with what is described as “flat” epithelium that continues to have characteristics of supporting cells but no longer can be induced to become sensory cells (Oesterle et al., 2008; Taylor et al., 2008). When looking at human temporal bone specimens of patients who had aminoglycoside induced vestibulopathy, it is evident that the flat epithelium that is seen in animal models is not seen in the vestibular system of human patients. In fact even patients with loss of caloric induced nystagmus were found to have residual hair cells as well as the standard cytoarchitecture of normal neuroepithelium. Recent studies of supporting cell population of the human vestibular system have also revealed that in humans there is a large viable supporting cell population suggesting that these would allow further rounds of regeneration after loss of sensory cells.

Conclusion

Multiple studies in different labs have shown the potential of gene delivery and in particular atoh1 transfection to produce functional sensory hair cells in a variety of model systems. In order to translate these observations into a clinically relevant molecular based therapeutics we need to focus on easily diagnosable disease process. Bilateral aminoglycoside induced vestibulopathy fulfills many of the criteria we need for a target disease. Animal models suggest that the vestibular system may be amenable to repair and currently there is no cochlear implant equivalent for the treatment of BVH. We have an established track record for the use of Ad 5 capsid vector in a range of human applications. Development of this vector with a supporting cell specific promoter could yield a molecular therapeutic ideally suited for the treatment of vestibular hair cell loss. Challenges that need to be addressed is finding the optimal dosing of the vector and avoiding any potential vector mediated side effects.

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Figure 1.

Local delivery and modification of vectors can allow very specific delivery of a molecular therapeutic in the inner ear. This model of an Ad5 vector demonstrates that modification of the different components of the vector can result in different biological effects. Modification of the fiber-knob region can create a vector that binds specific subsets of cells within the inner ear. Modification of the capsid (hexons) can decrease immunogenicity of a vector and finally use of regulatable or tissue specific promoters would allow further specification of vector targeting.

Figure 2.

Ad5 mediated delivery of *atoh1* can result in generation of hair cells that persist over long time periods: Two month old mice were treated with an intracochlear injection of neomycin 10^{-3}M followed after 2 days with an injection of an Ad5 vector expressing *atoh1* driven by an hCMV promoter. After 6 months animals were compared to age matched untreated controls (A) or neomycin only treated animals (B). As can be seen in the neomycin treated animals there is loss of hair cells with survival of an occasional vestibular hair cells (arrow). *Atoh1* treated animals show a robust and stable population of stereocilia bearing cells (C).

Figure 3. Effect of a tissue specific promoter (GFAP promoter) on GFP deliver in vivo. Expression of GFP driven by a tissue specific promoter (GFAP promoter) results in immunolabeling of cells expressing GFAP (A). Expression of the delivered transgene is thereby limited to a smaller cell population. Supporting cells in the organ of Corti (B) and in the ampulla (D, arrow) are labeled while hair cells are not. The spiral ganglion (C) demonstrates labeling of glial cells but not neurons (arrow).

Figure 4.

To test the effect of promoter modification on hair cell regeneration in vivo, 2 month old mice were treated with an intracochlear injection of neomycin 10^{-3}M followed after 2 days with an injection of an Ad5 vector expressing atoh1 driven by an gfap promoter. After 1 month animals were immunostained for myosin VII (red) and the supporting cell marker EAAT1 (green). Neomycin only treated animals showed only occasional surviving hair cells (B, arrow) compared to control populations (A). As can be seen in (C), atoh1 treated animals showed a robust population of myosin VII positive cells compared to neomycin only treated controls (B). Interestingly, the atoh1 treated animals occasionally demonstrated cells that had a myosin VII positive extension to the basement membrane (C, arrow). This may suggest that these area cells are undergoing transdifferentiation.

Figure 5.

After restoration of hair cells by treating aminoglycoside damaged inner ears with an Ad5 vector expressing *atoh1* driven by an *gfap* promoter we found that the hair cells were also innervated. Macular organs from animals treated with either intracochlear neomycin (A,D) or gentamicin (B) showed residual neurofilament immunostaining (green, arrow in D) 40 days post original delivery of aminoglycoside. Immunostaining for the supporting cell marker EAAT1 is shown in blue and immunostaining for myosin VII is shown in red. Animals treated with neomycin or neomycin followed after 10 days by Ad.gfap.*atoh1* demonstrated hair cells that stained for myosin VII and EAAT1 (pink) (C), that appeared to be innervated (C and E, arrow) at 40 days post vector delivery.

Figure 6. Altering the binding characteristics of adenoviruses alters transfection patterns: After delivery of a standard Ad5 vector expressing green fluorescent protein (GFP) to the perilymph resulted in GFP expression in supporting cells and occasional hair cells (A). Modification of the fiber/knob region of the vector by inserting extra heparin binding sequences resulted in more uniform GFP expression (B).