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CLINICAL UPDATE: GENE THERAPY FOR
HEARING LOSS AND ADVANCEMENTS IN
COCHLEAR HAIR CELL REGENERATION

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GENSKA TERAPIJA GUBITKA SLUHA I
NAPREDAK NA POLJU REGENERACIJE
KOHLEARNIH VLASASTIH ČELIJA

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Apstrakt

Dugo je bilo poznato da kohlearne i vestibularne vlasaste ćelije mogu da regenerišu u nekih vrsta kao što su ribe, ptice i ajkule. Naučnici širom sveta su neumorno pokušavali da nađu genetske mehanizme koji uzrokuju ćelijsku transformaciju i transdiferencijaciju u tih navedenih vrsta. Još uvek je velika misterija zašto to nije generalna osobina sisara. Proučavanja na nivou genske terapije i regeneracije senzorijskih ćelija uveliko je ubrzala potencijal za identifikaciju i alteraciju gena koji uslovljavaju gubitak sluha. Istraživanja su identifikovala gene kao što su *Atoh 1*, sa potencijalom injiciranja transdiferencijacije potpornih ćelija u funkcionalne, vlasaste ćelije. Neurotrojni faktori kao što su neurotrofin-3 (NT-3), bili su veoma uspešni u zaštiti vlasastih ćelija protiv ototoksičnog oštećenja. Primena ovih tehnika je od kritičnog značaja za bolesnike s oštećenjem i gubitkom sluha.

INTRODUCTION

There are more than 250 million people, globally speaking, who suffer from hearing and/or balance disorders (Tang, Montemayor & Pereira, 2006; Kesser, Hashisaki, Fletcher, Eppard, & Holt, 2007). These disorders are primarily due to damage to sensory hair cells within the auditory and vestibular systems housed in labyrinth. There are two types of hair cells in the cochlea which are referred to as Inner Hair Cells (IHC) and Outer Hair Cells (OHC). The superior surface of these cells is covered by delicate fibers referred to as stereocilia (Figure 1).

Damage to these hair cells in humans is irreversible. The most common causes of damage to sensory hair cells are genetic factors, infections, and environmental factors. Environmental factors can include ototoxic medications and exposure to loud sounds. Advancement in hearing aid technology, as well as cochlear implants, can help in the improvement of the quality of life for individuals with hearing loss; however, at this time there is no absolute cure for hearing loss secondary to sensory hair cell damage. There has been extensive research on possible cures for hearing

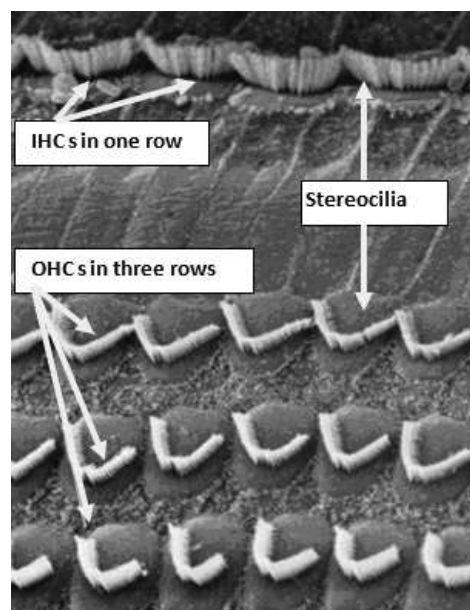


Figure 1. Electron microscopic image of the mammalian cochlea. Note the organization of the inner and outer hair cells in one and three to five rows, respectively.

loss including possible gene therapies, as well as the phenomenon of cochlear hair cell regeneration. No studies have been completed on humans at this time. However, there have been numerous studies, as referenced in the current update, completed *in vitro* and *in vivo* using animal models that provide a great deal of insight in the potential development for a cure for hearing loss in humans. This clinical update will provide an overview of advances in research for gene therapy and hair cell regeneration for the auditory and vestibular systems.

GENE THERAPY

Gene therapy is a procedure where a desired gene is inserted into involved subjects' cells with the hope of treating or preventing a variety of diseases and/or disorders (National library of medicine, 2009). The gene therapy protocols for the management of hearing loss have been utilized by injecting genetic material directly or indirectly into the cochlea. There are two common ways in which this can occur. The first method is diffusion through the round window into the perilymph. In this method genetic material is injected to the middle ear through a small incision on the tympanic membrane. The second route is through a cochleostomy. A cochleostomy is a surgical procedure where an opening is generated in the temporal bone to access the scala tympani. The advantage of using a cochleostomy is that the gene is injected directly into the perilymph and therefore, it facilitates gene transfer to the target area (Duan, Venail, Spencer, & Mezzina, 2004).

In order to transfer genetic material into the body of the recipients, viral vectors have been employed (Figure 2). There are numerous ways gene therapy can travel through the inner ear including: adenoviral, adeno-associated viral, herpes simplex viral, and lentiviral vectors. The adenoviral vector is the most commonly used gene therapy vector in the inner ear (Duan et al., 2004). An adenoviral vector is a virus that is changed to carry the new gene into the cell. Adenoviral vectors are effective in transducing cells *in vitro* and *in vivo* (Genetic engineering and biotechnology news, 2009; MicrobiologyBytes, 2004).

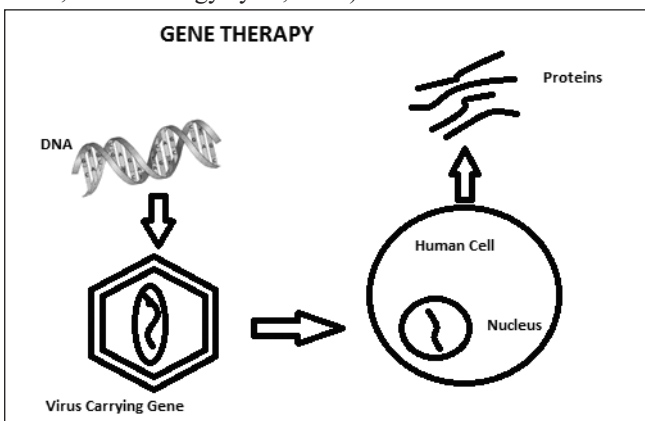


Figure 2. Illustration of gene therapy. Genetic material is injected to the viral vectors which carry them to the target cells which in turn make appropriate proteins.

Kessler et al. (2007) studied inner ear gene therapy using an *in vitro* model. The researchers used an adenoviral vector with the gene for green fluorescent protein (GFP). This was referred to as Ad2-GFP. GFP is used as a marker in genetic

research because it will turn green when examined under a blue light. GFP can be used to show when a specific gene has entered a cell (Yang, Moss, & Phillips, n.d., p.1). Ad2-GFP was injected into cultured human utricle cells and stained in order to identify the presence of myosin VIIa. Myosin VIIa is a dimeric protein complex that is expressed only in hair cells and not in the supporting cells (Kessler, et al, 2007). Myosin VIIa mutations can cause both dominant forms of deafness as well as recessive forms of deafness including Usher's syndrome Type 1B (Kessler et al., 2007; University of Edinburg, 2003, p.1). Kessler et al. (2007) found hair cells that were positive for myosin VIIa as well as for GFP. This indicated that the adenovirus can enter myosin VIIa positive hair cells. This could lead to myosin VIIa therapy of inner ear hair cells for those suffering from Type 1B Usher's syndrome to hopefully restore hearing and balance function. Kessler et al. (2007) also studied the adenovirus carrying GFP and KCNQ4. The KCNQ4 is a potassium channel gene. KCNQ4 allows potassium to travel between cells, which can generate electrical signals (National library of medicine, 2009). The locus of gene KCNQ4 is at 1p34 (i.e., chromosome one, short arm, region three, band four). Autosomal dominant progressive deafness, DFNA2, is caused by mutations in KCNQ4 (Kessler et al., 2007; National library of medicine, 2009). Kessler et al. (2007) found GFP positive cells had higher levels of KCNQ4 within the cell, indicating successful adenoviral entrance into the cell. These researchers suggested that gene therapy with KCNQ4 can be used to restore potassium ion channels in patients that suffer from DFNA2.

During embryological development the cellular precursors are similar between both hair cells and supporting cells. There are different genes that trigger differentiation of these precursor cells into either hair cells or supporting cells. One of these genes is *Atoh1*, also called *Math1* (Izumikawa et al., 2005). *Atoh1* is a "basic helix-loop-helix protein transcription factor" (Izumikawa et al., 2005). By introducing *Atoh1/Math1* into the inner ear, supporting hair cells can change into the hair cell phenotype through a process called transdifferentiation. Transdifferentiation is a process that occurs when a change is induced in one cell resulting in an entirely new cell. Transdifferentiation mostly occurs in cells that are closely related (Batts & Raphael, 2007). Transdifferentiation occurs naturally in non-mammals such as birds and fish (Batts & Raphael, 2007; Brignull, Raible, & Stone, 2009; Walshe, Walsh & Mcconn, 2003).

Kawamoto, Ishimoto, Minoda, Brough, & Raphael (2003) studied *Math1* in guinea pigs *in vivo*. An adenoviral vector carrying the *Math1* gene was surgically injected into cochlear endolymph. Results showed that non-sensory cells were able to switch into myosin VIIa positive (hair cell marker) hair cells. In addition, results also showed that neurons also grew toward the new hair cells positive for myosin VIIa, providing innervations for them. In another study, Izumikawa et al. (2005) studied *Atoh1* gene in young adult guinea pigs. The guinea pigs were completely deafened using ototoxic drugs and lack of hair cells and myosin VIIa was confirmed through clinical and electrophysiologic testing. After administration of *Atoh1* new myosin VIIa positive hair cells were detected. Two months after *Atoh1* injection there was a significant increase in the number of outer and inner hair cells as well as a mixed phenotype cells, which

expressed properties of both outer hair cell and supporting hair cell. Auditory brainstem testing was completed at eight weeks and ten weeks after *Atoh1* injections which showed improved hearing thresholds from prior to *Atoh1* injection. These studies showed that *Atoh1/Math1* gene therapy can cause transdifferentiation to occur and can improve auditory function after therapy.

Gene therapy can also be used to protect hair cells from damage due to ototoxic medication specifically, aminoglycoside antibiotics. Neurotrophic factors including, neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), and ciliary neurotrophic factor (CNTF) have shown to protect hair cells and spiral ganglion cells (SGC) from aminoglycoside damage (Duan et al., 2004; Tang et al., 2006). Neurotrophic factors are growth factors that promote development and maturing neurons (Ceregene, n.d. p. 1). Miller et al (1997) as cited in (Duan et al., 2004) showed infusion of BDNF can increase the survival of SGC after kanamycin and ethacrynic-acid damage. Numerous studies also cited in Duan et al. (2004) showed NT-3 can protect and trigger re-growth of SGC after aminoglycoside ototoxicity. For instance, Bcl-2 is a gene that codes the protein 250kDa. This protein is on the surface of the mitochondria and is important in the regulation of calcium and pH voltage in the mitochondria (Pfannenstiel, Praetorius, Plinkert, Brough, & Staecker, 2009). Pfannenstiel et al. (2009) researched the protective effects of bcl-2 against aminoglycoside ototoxicity through an in vivo mouse model. Results showed that mice that were pretreated with bcl-2 gene therapy prior to administration of gentamicin had preservation of hair cells and better hearing thresholds when compared to controls. These studies suggest the potential use of neurotrophic factors and bcl-2 gene therapy for protection of ototoxic hearing loss.

Research has shown that gene therapy has great potential for finding a cure for hearing loss due to hair cell damage in humans as well as a potential to be used as an otoprotective agent in patients that need to receive ototoxic medications (Pfannenstiel et al., 2009; Duan et al., 2004; Ceregene, n.d. p. 1; Izumikawa et al., 2005; Kawamoto et al., 2003).

HAIR CELL REGENERATION

Hair cell regeneration occurs spontaneously in birds and fish (Brignull et al., 2009; Edge & Chen, 2008; Walshe et al., 2003). Numerous studies quoted in Brignull et al. (2009) found that the vestibular hair cells in birds continuously regenerate. In contrast, the auditory hair cells are regenerated, through transdifferentiation, after extreme noise exposure (Brignull et al., 2009; Walshe et al., 2003). After regeneration, birds can restore auditory and vestibular function in one to two months (Brignull et al., 2009). Fish have sensory hair cells in the inner ear as well as along the lateral line. The fish lateral line system is used to detect objects and changes in water vibrations (Walshe et al., 2003). Studies completed on the zebra fish have shown that hair cell regeneration can occur through transdifferentiation or through mitotic regeneration.

Advances have been made in hair cell regeneration in mammals using stem cells. Coleman et al. (2006) implanted mouse embryonic stem cells into adult guinea pig cochlea. The cells were successfully identified within the cochlea; however, the cells dispersed throughout a widespread area of

the cochlea and not attaching to the intended site. In addition, most of the cells did not survive in the cochlea after four weeks post-implantation. Neural stem cells have also been used in hair cell regeneration studies in guinea pigs. Like the embryonic stem cells the neural stem cells were successfully implanted, however the survival rate was extremely poor (Vlastarakos et al., 2008). Bone marrow stem cells have been shown to take on the form of brain tissue. Bone marrow stem cells have been implanted into chinchillas and initially showed neural phenotypes; however, these cells lost the neural properties with time. This research suggests that bone marrow stem cells may have potential for spiral ganglion cell (SGC) regeneration (Vlastarakos et al., 2008).

Researchers have obtained a great deal of information of hair cell regeneration and the potential uses of hair cell regeneration through non-mammalian animal studies. Much research still needs to be completed on hair cell regeneration in mammals starting with survival of stem cells after implantation.

CONCLUSION

Millions of people currently suffer from permanent hearing loss. At this time there is no absolute cure for hearing loss without technological intervention. Numerous studies have been completed to find a cure for hearing loss. Gene therapy has shown a great deal of potential in addressing genes that can cause hearing loss. *Atoh1* has shown the potential to initiate transdifferentiation of supporting cells into functional hair cells. Neurotrophic factors have been successful in protecting hair cells against ototoxic damage. Finally, hair cell regeneration using stem cells are in the beginning stages of research in mammals. More research will need to be completed before these techniques will be used for human patients. However, with all the new scientific advances, there may be a cure for hearing loss in the future.

Abstract

For many years we have learned that the cochlear and vestibular hair cells can regenerate in certain species such as fish, birds, and sharks. Scientists around the world have tirelessly attempted to find the genetic triggers which cause cell transformation and transdifferentiation in those species. Why it is not a common property in mammals still remains a mystery. Gene therapy and sensory hair cell regeneration studies have prompted a great deal of potential in identification and altering those genes that can cause hearing loss. Research has identified genes such as *Atoh 1* with the potential to initiate transdifferentiation of supporting cells into functional hair cells. Neurotrophic factors such as neurotrophin-3 (NT-3) have been successful in protecting hair cells against ototoxic damage. Application of these techniques is of significant importance for the patients affected by hearing loss.

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