Published in final edited form as:

J Commun Disord. 2010 ; 43(4): 295–310. doi:10.1016/j.jcomdis.2010.04.001.

Future approaches for inner ear protection and repair

Seiji B. Shibata^a and Yehoash Raphael^{a,*}

^a Kresge Hearing Research Institute, Department of Otolaryngology, The University of Michigan, Ann Arbor, MI, 48109-5648, USA

Abstract

Health care professionals tending to patients with inner ear disease face inquiries about therapy options, including treatments that are being developed for future use but not yet available. The devastating outcome of sensorineural hearing loss, combined with the permanent nature of the symptoms, make these inquiries demanding and frequent. The vast information accessible online and the publicity for breakthroughs in research add to patient requests for access to advanced and innovative therapies, even before these are available for clinical use. This can sometimes be taxing on the health care provider who is in contact with the patients. Here we aim to equip the provider with information about some of the progress made for protective and reparative approaches for treating inner ears.

Learning outcomes—(1) Readers will be able to explain why hearing loss is irreversible and common, (2) Readers will be able to explain the importance of protective measures and the progress made in discovery and design of novel biological protective molecules, (3) Readers will be able to describe reparative approaches currently under investigation (such as tissue engineering), the main difficulties in the design of such therapies and the major hurdles that remain for making novel technologies clinically viable, and (4) readers will be able to explain to their patients some of the progress in developing new treatments without making the promise of imminent clinical use. With this information, readers will be able to guide patients to make better choices for their treatment and to guide students toward research in this exciting field.

Keywords

Deafness; Hair cell; Spiral ganglion; Gene therapy; Regeneration; Protection; Neurotrophin

1. Introduction

A large portion of the population requiring therapy for communication disorders is hearing impaired. Hearing loss is one of the most common disabilities in our society. Although it is not directly a life threatening impairment, hearing loss can have a significant effect on the patient's life, both emotionally and financially. Causes of this impairment can be hereditary, environmental, or a combination of the two. The effects are often cumulative, causing us to lose our hearing as we age. Among the more common environmental causes are over-stimulation, ototoxic medications, mechanical trauma and infections.

*Tel.: +1 734 763 4444; fax: +1 734 615 8111. yoash@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Hair cells, the sensory cells of the inner ear, are lost over time in most people. We speculate that epithelial cells such as hair cells are not “designed” to survive many dozens of years, and as means to extend life span for humans resulted in increased longevity, epithelial cells that cannot be replaced (hair cells of the cochlea) are gradually decreasing in number. In addition to the time factor, genetic and environmental issues may act independently or in tandem to accelerate the loss of hair cells. Similar to the hair cells, auditory neurons are also lost over time. In addition to hair cells and auditory neurons, there are other elements in the ear that can be damaged and may degenerate over time or in response to an insult. To mention just two examples, changes in the stria vascularis associated with aging are of utmost clinical importance and studies of blood supply and its changes with over-stimulation and aging should yield additional options for prevention and therapy. However, this review is focused on hair cells and spiral ganglion neurons.

Present clinical means to treat hearing loss are typically based on sound amplification (hearing aids) and/or cochlear implant prosthesis. While these provide much appreciated relief in many cases, their performance is often sub-optimal, especially when the underlying pathology is severe. As the aging population increases and the chronic exposure to high sound levels spreads among the general population (e.g. personal sound devices and other loud environmental signals), research for developing protective means and therapy is becoming increasingly important. Advances in preventive medicine are needed, along with improvements in function of amplification and electrical stimulation devices. The main goal of this report is to (1) survey different directions for research that may lead to protective and reparative procedures for the inner ear, including bio-engineering approaches, (2) discuss which therapeutic approaches are likely to become applicable sooner, and (3) address the expectations and goals for future therapies. This material hopefully will inspire new avenues for research, help entice talented and enthusiastic researchers into the field, and equip the clinical personnel in the communication disorders disciplines with information they can share with “online-educated” patients who are becoming ever more demanding and involved in their own options and therapy.

A general survey of the pathological changes associated with sensorineural hearing loss sets the stage for discussing future therapies. Therefore, we first discuss inner ear pathology in some detail, focusing on the loss of hair cells and auditory nerve. We then consider recent and future advances in providing better protection and restoration of inner ear structure and function. These new techniques could be used in combination with current treatments, or independently.

2. Histopathological changes of the inner ear after an insult

2.1. Hair cell degeneration

Cochlear pathology leading to hearing loss can occur in one or more types of cells or cochlear tissues. The most common lesions involve the sensory epithelium (hair cells and supporting cells) and the auditory neurons (spiral ganglion neurons). Neither hair cells nor auditory neurons can be replaced once lost. Therefore, the two principal structures that are indispensable for hearing, hair cells and spiral ganglion neurons, are the main targets for protection and regenerative therapy.

The most common histopathological change associated with sensorineural hearing impairment is the loss of hair cells. The mechanism is death has been described as apoptotic, necrotic, or combined, depending on the method used to induce hair cell death and the analytical methods used to study it (Alam, et al., 2000; Bodmer, Brors, Pak, Bodmer, & Ryan, 2003; Eshraghi & Van de Water, 2006; Forge, 1985; Huang, et al., 2000; Lenoir, et al., 1999; Nakagawa, Yamane, Takayama, Sunami, & Nakai, 1998; Nicotera, Hu, & Henderson, 2003). Understanding the

mechanism of hair cell death may help us develop protective means to preserve these cells. We discuss such means below, along with other protective approaches.

Hair cells are normally surrounded by highly differentiated non-sensory cells called supporting cells. Once hair cells are lost, supporting cells expand and invade the space formerly occupied by hair cells. This expansion helps maintain integrity of the apical surface of the epithelium, which is important for preventing leaks and mixing of cochlear fluids, and therefore helps maintain the remaining hair cells and hearing (Forge, 1985; Raphael & Altschuler, 1991a, 1991b). Severe lesions may involve temporary disruption in this barrier, which likely leads to progressive lesions (Bohne & Rabbitt, 1983). The scarring activity of supporting cells is extremely important for continued survival and function of cells adjacent to the site of hair cell loss. We speculate that delayed or incomplete scarring could lead to rapidly progressing hearing loss. In contrast, premature scarring activity could eliminate hair cells that are not necessarily doomed, leading to unnecessary hearing loss. Research on the regulation of supporting cell scarring could shed light on an important route of protective intervention.

In areas of hair cell loss, supporting cells that expand and fill the hair cells' places change their shape to some extent, but their overall degree of differentiation, molecular profile and organization remain relatively unaltered (Webster & Webster, 1981). However, in severely traumatized cochleae and/or in cochleae that have been damaged for a long period of time, the scarring supporting cells are replaced by a monolayer of simple epithelial cells, forming a "flat epithelium" (Kim & Raphael, 2007; Raphael, Kim, Osumi, & Izumikawa, 2007). The flat epithelium can be seen in temporal bone studies of human ears, both in patients who have been deaf for years and in ears with genetic based pathology (Nadol & Eddington, 2006). Because the substrate for future hair cell replacement therapies in deaf ears is the non-sensory epithelium that remains in the tissue, it is necessary to better understand the biology of this tissue. For instance, knowledge of the molecular profile of these cells, their surface receptors, the way they connect to each other with cell junctions, and their proliferative capability should help advance technologies for inserting stem cells or inducing transdifferentiation of these cells into new hair cells (Fig. 1). Better understanding of the flat epithelium is especially important because ears with this profound degeneration of the auditory epithelium will likely be the main candidates for novel treatments. At this point, little is known about the source and molecular composition of cells of the flat epithelium (Kim & Raphael, 2007).

In most cases of hair cell loss, the hair cells are the primary target of the insult, and any changes in supporting cells that may occur are secondary. However, in some cases supporting cells can be the primary victims of the degenerative process, especially in hereditary diseases such as DFNB1A where the mutation influences supporting cells primarily (Lustig, et al., 2004; Zelante, et al., 1997). For this and other reasons (details below) therapies aimed at preserving or regenerating supporting cells are also important.

2.2. SGN degeneration

Auditory neurons can degenerate due to several reasons, only some of which have been defined. Nerve degeneration is associated with hereditary diseases such as neurofibromatosis 2 (NF2) (Colletti, 2006). In older patients with hearing loss, auditory nerve degeneration may be involved as part of the pathology. Degeneration of hair cells can lead to subsequent loss of peripheral nerve fibers from the area of the organ of Corti, presumably because the neurons lack either stimulation or a growth factor normally provided by the hair cell. However, in many human cases, spiral ganglion neurons survive for many years, despite the loss of most or all hair cells and the withdrawal of peripheral fibers from the auditory epithelium (Bichler, Spoendlin, & Rauegger, 1983; Spoendlin & Schrott, 1989). An ideal animal model for ears with hair cell loss but no auditory nerve loss is not currently available. Rather, animal models for hair cell loss typically involve degeneration of spiral ganglion cells (Leake & Hradek,

1988; Otte, Schunknecht, & Kerr, 1978; Webster & Webster, 1981). It is not clear if the difference between humans and lab animals is a species-dependent factor or if it reflects difference in the severity of the lesion induced at the level of the hair cells. When devoid of neurons, Rosenthal's canal appears to contain a lightly stained material with poor organization and few cellular components, possibly including Schwann cells and other non-neuronal cells. The loose organization is important for attempts to replace lost spiral ganglion neurons with transplanted stem cells that can differentiate into new neurons (see below), because of relative ease of inserting the cells into the modiolus. In contrast, inner ear disease involving ossification of the cochlea, as is sometimes seen in NF2 or labyrinthitis cases, would pose additional challenges for treatment.

3. Protecting the inner ear against trauma

3.1. Protection everyone can do

Awareness of the risk of hearing loss and its consequences, along with simple rules and basic accessories can reduce the incidence of hearing loss, especially in cases of acoustic trauma. For protection against acoustic trauma, avoiding exposure to extreme levels of stimulation is currently the only option. Such protection often involves both equipment and awareness, the latter involving pro-active action, considerate behavior and some social activism. In some cases, the level of acoustic exposure cannot be easily controlled. Military personnel, construction workers, aviation staff and others who are exposed to very high levels of acoustic signals should wear ear protection. In other cases, awareness can reduce the risks of hearing loss. Music or speech amplification in discos, parties and even movie theaters are often extremely loud, requiring use of ear protection. Sports events are notoriously loud, especially when conducted indoors, making the use of ear plugs essential. In many settings, an excessive sound level is not absolutely necessary and often times it can be reduced below damaging levels when patrons request it. When levels of amplified sound in a party or a disco are loud enough to kill conversations, some hair cells are probably victims too.

The need to protect ears is not exclusively relevant to normal ears. Rather, protection of ears that already have a threshold shift, distortion and/or tinnitus is even more important. The reason for this is that ears with pathology are more vulnerable. In other words, it is likely that the more hair cells we lose, the more susceptible remaining hair cells are to further trauma. Thus, patients who already have sensorineural hearing loss are in many cases more sensitive to over-stimulation than normal hearing individuals.

3.2. Molecular substrates for protection of hair cells and auditory nerve

Understanding of the mechanism of cell death can potentially aid in designing ways to prevent degeneration of cells. In some cases, an extremely severe mechanical trauma can lead to a rapid and uncontrolled degeneration, typically referred to as necrosis. In other cases, specific biochemical activities which occur in a typical sequence lead to a programmed cell death, apoptosis. Dying hair cells after ototoxic and acoustic trauma often exhibit typical apoptotic morphologies such as condensed nuclei and fragmented DNA, suggesting that apoptosis is involved in hair cell death (Forge & Li, 2000; Lim & Melnick, 1971; Matsui, Ogilvie, & Warchol, 2002; Nakagawa, et al., 1998; Nicotera, et al., 2003; Ping Yang, Henderson, Hua Hu, & Nicotera, 2004; Usami, Takumi, Fujita, Shinkawa, & Hosokawa, 1997; Zheng & Gao, 1997; Zheng, Ikeda, Nakamura, & Takasaka, 1998). The initial step for the activation of apoptotic cascades induced by various insults in the inner ear is the formation of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Forge & Li, 2000; Ohlemiller & Dugan, 1999; Rybak, Whitworth, & Somani, 1999; Schacht, 1999). These cascades include activation of enzymes such as mitogen-activated protein (MAP) kinase and c-Jun N-terminal kinases (JNK) (Zine & van de Water, 2004) or intracellular proteins such as

caspase, p53, and Bcl-2. Identifying regulatory factors and understanding their role in the process of hair cell fate is important for development of novel pharmaceutical treatments that target specific cascades of hair cell death.

Spiral ganglion cell death is usually slower in pace compared to post-trauma hair cell loss. Degenerating spiral ganglion neurons usually undergo apoptotic cell death (Roehm & Hansen, 2005; Sekiya, et al., 2003). Causes for nerve loss may include primary (direct) influence of an ototoxic medication such as aminoglycosides, or indirect effects secondary to degeneration of hair cells, such as loss of activity or loss of neurotrophic factors (Hansen, Zha, Bok, & Green, 2001). Because neural degeneration is slower than hair cell loss, there is usually more opportunity to prevent it in human ears. The ultimate goal of the protection is to prevent nerve death, but additional goals could be to enhance maintenance of the peripheral dendrites. It appears that the degree of differentiation of the hair cell-depleted organ of Corti may have a complex and reciprocal relationship with the surviving neurons (Sugawara, Corfas, & Liberman, 2005). Enhancement of survival of differentiated supporting cells in a deaf organ of Corti that lacks hair cells may promote survival of the auditory nerve and vice-versa.

Most hair cell and auditory nerve protection studies in animals were done using anti-apoptotic agents and neurotrophic factors. Because cell death pathways and mechanisms are often shared regardless of the initial etiology, hair cell protection against ototoxic drugs or over-stimulation may be attained by similar means. For instance, hair cell protection can be accomplished by preventing formation of ROS or by inhibiting the apoptosis pathway, whether the cause of degeneration is ototoxic antibiotics, anti-tumoral agents or acoustic over-stimulation. Several families of molecules have been shown to have anti-apoptotic activity in animal deafness models (Cheng, et al., 1999; Coleman, et al., 2007; Eshraghi, et al., 2007; Huang, et al., 2000; Pirvola, et al., 2000).

ROS are produced when aminoglycosides form a complex with iron, suggesting that iron chelators or antioxidants could suppress their formation. Indeed, formation of ROS can be prevented in the inner ear by the administration of antioxidants such as D-methionine (an amino acid with chelating properties) (Schacht, 1999; Sha & Schacht, 1999, 2000), L-methionine and N-acetylcysteine (precursor of glutathione) (Garetz, Altschuler, & Schacht, 1994; Lautermann, McLaren, & Schacht, 1995) and low dose salicylate (Cho, Harada, & Yamashita, 1997; Excoffon, et al., 2006). Each has been shown to protect against aminoglycoside and acoustic trauma-induced hair cell death.

Inhibition of apoptotic hair cell death can be manipulated by blocking the caspase family, and the MAPK-JNK pathway. Caspase inhibitors are able to promote hair cell survival in the face of aminoglycosides (Cheng, Cunningham, & Rubel, 2003; Matsui, et al., 2002, 2003) and cisplatin (Liu, et al., 1998) in vitro. Inhibition of the MAPK-JNK pathway protects hair cells against ototoxicity and acoustic trauma in guinea pigs (Wang, et al., 2003).

Another group of proteins, with both developmental and protective roles, are growth factors. One family of growth factors, the neurotrophins, plays diverse roles in the mammalian CNS and PNS, such as promotion of neurogenesis, neuronal differentiation, synaptogenesis and cell survival (Chao, Rajagopal, & Lee, 2006; Zampieri & Chao, 2006). Neurotrophins include nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin 4 (NT-4). The neurotrophic function is mediated by intracellular signaling pathways activated by tyrosine kinase receptors (TrkA, B, and C) or p75 neurotrophin receptors. Neurotrophins have been used in reparative studies of the CNS and PNS (Bellamkonda, 2006; Di Polo, Aigner, Dunn, Bray, & Aguayo, 1998; Tuszynski, et al., 2005).

During development of the inner ear, BDNF and NT-3 as well as their receptors, TrkB and C, are known to be essential for the differentiation and survival of cochlear neurons. Hair cells are thought to secrete BDNF and/or NT-3 and attract nerve fibers to form synapses (Despres, Leger, Dahl, & Romand, 1994; Medd & Bianchi, 2000). Several lines of evidence have indeed shown that BDNF or NT-3 null mice exhibit partial or complete loss of afferent and efferent neurons in the cochlea (Ernfors, Kucera, Lee, Loring, & Jaenisch, 1995; Fritsch, Silos-Santiago, Bianchi, & Farinas, 1997; Fritsch, Tessarollo, Coppola, & Reichardt, 2004). It was only logical to test the potential for protective influence of neurotrophins (and other growth factors) against trauma both at the level of the hair cell and the auditory nerve. NGF was the first neurotrophin determined to have a protective capacity in the inner ear (Shah, Gladstone, Williams, Hradek, & Schindler, 1995), and the list later expanded to include other molecules. Over the years, neurotrophins and other growth factors have been delivered into the inner ear and demonstrated robust protective effects on hair cells and spiral ganglion neurons against ototoxic or acoustic trauma (Bowers, et al., 2002; Duan, Agerman, Ernfors, & Canlon, 2000; Feghali, Liu, & Van De Water, 2001; Gao, 1999; Glueckert, et al., 2008; Kanzaki, et al., 2002; Nakaizumi, Kawamoto, Minoda, & Raphael, 2004; Shepherd, Coco, & Epp, 2008; Staecker, Kopke, Malgrange, Lefebvre, & Van de Water, 1996; Wise, Richardson, Hardman, Clark, & O'Leary, 2005; Yagi, et al., 2000).

The difficulty in delivering a large molecule into the inner ear (discussed later) has restricted the development of practical clinical applicability for this approach. Clearly, protective applications for a normal ear should not have a potential for being destructive, and invasive treatments cannot be justified for protection. As such, orally given compounds, and possibly ear drops, may be the most applicable means for pharmacological inner ear protection in non-traumatized ears. Some common and readily available medications such as aspirin can significantly reduce the risk of hearing loss caused by aminoglycosides (Chen, et al., 2007).

In addition to existing medications, some of the protective molecules are present in foods or occur naturally, and it is conceivable that concentrated doses of protective compounds such as anti-oxidants could be ingested regularly and safely for preserving cochlear health (Seidman & Van De Water, 2003). Research on the protective potential of several compounds has enabled design of several formulations that are commercially available. Approaches for protection of hearing in aging ears have been recently reviewed in detail and the findings apply to other etiologies of deafness (Bielefeld, Tanaka, Chen, & Henderson, in press).

3.3. Protecting supporting cells in the deaf ear

Once hair cells degenerate, hearing is lost and the basilar membrane is lined with remaining non-sensory cells. These cells often appear as differentiated supporting cells or, in more severely degenerated ears, as a simple layer of flat epithelial cells. It appears that the more differentiated the non-sensory cells are, the more likely they are to respond to regenerative therapies based on forced expression of developmental genes (see below). It has also been shown that some reciprocal influence exists between auditory nerve and the remaining non-sensory cells, and that maintaining differentiated supporting cells can enhance spiral ganglion survival (Sugawara, et al., 2005). Therefore, it is important to develop protective therapies in deaf ears that will prevent the transition of remaining non-sensory cells to the least differentiated status, the flat epithelium.

3.4. Clinical feasibility of protective approaches

Many of the pharmacological/molecular approaches described above for protecting hair cells and spiral ganglion cells were developed in animal models but have not found their way into clinical use. The reasons for the difficulty in clinical applying protective measures is that (a) the risks involved with the treatment are higher than the potential benefits, or (b) the approach

is altogether clinically unfeasible. The practical aspect of delivering therapeutic agents to the inner ear is discussed further below.

3.5. Predicting sensitivity to sensorineural hearing loss

As discussed above, sensorineural hearing loss has a multifactorial etiology, which explains why several studies have demonstrated that the same injurious exposure can produce a range of effects. Preventative methods and public awareness campaigns could be more effectively targeted if a single, easily evaluated phenotypic trait could be used to recognize populations at relatively greater risk of hearing loss. For instance, several studies have explored the predictive potential of iris or skin pigmentation, reasoning that melanocytes in the ear help maintain homeostasis, and may be more abundant or more active in individuals with greater pigmentation in other tissues (Bluestone, et al., 1986; Carlin & McCroskey, 1980; Cullington, 2001). However, small studies (< 50 patients) often are unable to reach definitive conclusions and studies that do find a significant effect, also find that the magnitude of the effect is small and has low predictive power (Bailey, Collins, Gordon, Zuur, & Priede, 2009; Bluestone, et al., 1986; Da Costa, Castro, & Macedo, 2008). More intriguing, dark pigmentation may be associated with decreased risk of hearing loss due to acoustic trauma (Da Costa, et al., 2008) or radiation (Zuur, et al., 2009), but increased risk of chemical induced hearing loss (Todd, Alvarado, & Brewer, 1995). Several factors could account for the disparity of results cited above. Iris, hair and skin pigmentation can vary independently, in color and intensity, and level of pigmentation in the inner ear may also be independent of these other tissues. The association between pigmentation and risk of hearing loss could be due to chemical properties of the melanin, other activities of the melanocytes, or other physiological traits that happen to segregate with pigmentation traits. Thus there may be an association between pigmentation in the skin or iris and risk of hearing loss, but further work is needed to characterize the mechanism and explain the variability. While pigmentation did not prove a reliable predictor, the increased power of genetic testing may reveal other markers that can be established relatively easily and help identify populations and individuals more susceptible to hearing loss, especially due to acoustic trauma.

Predicting sensitivity to ototoxicity, especially that caused by aminoglycosides, would be particularly valuable to help balance the life-saving effects of the drugs with their potential side effects. Medical decisions could be better made with information on personal sensitivity. Although tests to pre-determine hyper-sensitivity to ototoxins before they are used in the clinic would be valuable, they are not currently available. The exception is a set of mutations in mitochondrial genes which render persons with these mutations over-sensitive to ototoxic drugs (Tono, et al., 2001).

3.6. Strategies to enhance performance with the cochlear implant

In most patients who receive cochlear implants, spiral ganglion density is sufficient to produce satisfactory results for speech understanding with current standards and expectations (Roehm & Hansen, 2005). In some cases, however, performance with the cochlear implant is poor. The histological substrate correlating with poor performance is not well characterized. Several researchers in the field have speculated on the factors that may dictate the level of success and include factors such as the number and condition of the surviving neurons, the condition of their central connections in the cochlear nucleus, and the proximity of the electrodes to the neurons. Possible approaches to enhance performance, especially in cases where benefit from the electrical stimulation is poor, include enhancing the physiological state of the neurons and decreasing the distance between the electrode and the stimulated part of the neuron. Attempts to increase auditory nerve survival, enhance its physiological condition, and decrease the distance between stimulating electrodes and nerve fibers, have been made using elevated levels of neurotrophins. Neurotrophin (or other growth factor) elevation was accomplished using

infusion with mini-osmotic pumps, gene transfer technologies or pre-soaked gels (Hendricks, Chikar, Crumling, Raphael, & Martin, 2008; Pettingill, Richardson, Wise, O'Leary, & Shepherd, 2007; Wise, et al., 2005). Clear positive outcome was noted for auditory neuron survival and morphological appearance (likely reflecting better health), as well as sprouting and growth of peripheral fibers within Rosenthal's canal and beyond (Glueckert, et al., 2008; Staecker, et al., 1996; Wise, et al., 2005). However, the short term nature of the methods did not allow long-term psychophysical studies to assess performance with the implants.

The neurotrophic and protective approaches described above are not currently feasible for use in the clinic. However, more recent progress in gene transfer vectors, such as the availability of adeno-associated virus (AAV) vectors that are long acting and are not human pathogens, may lead to further development of means to improve cochlear implant function. One approach would be to induce growth of auditory nerve fibers into the basilar membrane area of the deaf ear, so as to provide nerve endings in close proximity to the electrode. This would diminish the physical barrier between the electrodes and the neurons and likely enhance survival of the neurons. We speculate that cochlear implant patients who perform poorly with the prosthesis may be the first candidates for a novel therapy that will include gene transfer technology or protein therapy. Some diseases such as NF2 involve primary lesions to the auditory nerve. For these patients, and for those who lose auditory neurons due to other causes, therapies for replacing lost neurons are being developed.

3.7. Protecting remaining hair cells from trauma caused by cochlear implant

In earlier days of cochlear implant use it was widely believed that the prosthesis and the electrical stimulation it provides are incompatible with preservation of hair cells. More recently, it became clear that residual apical (low-frequency area) hair cells can survive in implanted ears, such that many patients have been combining the use of electrical stimulation with hearing aids. Moreover, it has been found that severely deaf patients with a small number of surviving hair cells and some residual acoustic hearing, do better with their cochlear implant than do profoundly deaf patients with no hair cells. These findings, along with advances made in inducing hair cell regeneration, demonstrate the need for studies to determine the influence of cochlear implant insertion and activation on hair cell survival.

A recent animal study was designed to determine the long term effects of cochlear implantation and electrical stimulation on cochlear histology and to investigate whether the structures that provide acoustic hearing affect the perception of electrical stimuli (Kang, et al., in press). Guinea pigs with normal ears and hearing received a cochlear implant and electrical stimulation for a period up to a year and a half. Histological analysis showed that in most animals, both hair cells and spiral ganglion neurons remained intact after the lengthy period of stimulation. In a small number of animals there were lesions adjacent to the implant, in the basal turn, but the rest of the cochlea appeared normal. In addition, preserving hearing affected the psychophysical perception of an electrical stimulus. The effect of hearing preservation on the perception of an electrical stimulus likely depended on the temporal properties (pulse rate, phase duration, etc.) of the electrical stimulus. These data demonstrate the ability to preserve hair cells in the presence of a cochlear implant and electrical stimulation and start to address the psychophysical benefits of combining acoustic and electrical stimulation.

4. Cell transplantation for replacing lost hair cells and spiral ganglion cells

One approach to placing new hair cells in a cochlea that is devoid of them is to utilize stem cell technology, a relatively new and promising biotechnology (Li, Corrales, Edge, & Heller, 2004). Stem cells are capable of continued self-renewal while maintaining the ability to provide progeny cells for producing differentiated functional cells (McKay, 1997). True stem cells are pluripotent cells which, in addition to their long-term self-renewal, are able to give rise to many

somatic cell types, including neurons and hair cells. Several sources for deriving stem cells exist, with varying degrees of technological difficulties and ethical acceptance. In addition to pluripotent stem cells, several organs have been found to include organ-specific stem cells, capable of replacing cells within that organ. Relevant to this review, cells with stem cell features were identified in the vestibular sensory epithelium in mature mammals (Li, Liu, & Heller, 2003), and in the developing cochlea (Lopez, Zhao, Yamaguchi, de Vellis, & Espinosa-Jeffrey, 2004). The technology for inducing differentiation of these and other cells into the hair cell phenotype is constantly progressing, and it is very possible that soon researchers will be able to guide their differentiation into the variety of needed phenotypes such as inner and outer hair cells and even specific supporting cells.

One important hurdle blocking the application of stem cell therapy is accomplishing successful and functionally meaningful insertion and integration of these cells into the cochlea. In this arena, it seems that transplanting neural stem cells into the modiolus has been advancing faster than transplanting cells into the cochlear epithelium (Coleman, et al., 2006; Corrales, et al., 2006; Hu, Ulfendahl, Prieskorn, Olivius, & Miller, 2009; Martinez-Monedero, Yi, Oshima, Glowatzki, & Edge, 2008; Matsuoka, Kondo, Miyamoto, & Hashino, 2007; Okano, et al., 2005; Reyes, et al., 2008). Several groups have shown that cells can be introduced into the modiolus, survive, migrate in the modiolar area, express neural markers and extend both peripheral and central processes towards the appropriate targets. These results are truly exciting in their promise for improving the hearing of cochlear implant patients whose original population of spiral ganglion neurons has degenerated. Further into the future, replacement of auditory nerve cells along with hair cells could provide acoustic hearing to profoundly deaf individuals, but this awaits a solution to the problem of integrating new hair cells into the cochlear epithelium.

In the case of using stem cells to replace missing hair cells, the challenge of integration is complicated by several factors. The human ear does not allow easy surgical access to scala media (endolymph), which is the most plausible way to attempt integration into the auditory epithelium. Even if a surgical approach to scala media were developed, the chemical environment in endolymph (high potassium) is hostile to most cells, and survival until full integration may be difficult to accomplish. Last, and most important, the auditory epithelium in the deaf ear maintains a robust set of apical cell-cell junctions that help prevent fluid mixing across barriers between endolymph and perilymph (Kim & Raphael, 2007). These same junctions will also resist insertion of objects as large as cells into the epithelium. General progress in the field of cell “homing” will likely assist in the task of stem cell integration in the auditory epithelium (Penn & Mangi, 2008). This area of research is truly fascinating and promising, yet clinical implementation is clearly far into the future.

5. Regeneration of hair cells

5.1. Transdifferentiation therapy via gene transfer

The cochlear epithelium in mammals is unique among epithelia in its inability to replace lost cells. Potential future therapies could either rely on remaining non-sensory cells in the cochlea via inducing transdifferentiation (change of phenotype) to become new hair cells, or implanting stem cells or other exogenous cells, as discussed above. Progress has been made with both approaches but clinical applicability is not yet on the horizon.

In the absence of nascent stem cells or undifferentiated cells in the organ of Corti region of a deaf ear, and with the lack of spontaneous transdifferentiation of non-sensory cells into the hair cell phenotype, the option of inducing such transdifferentiation in the remaining non-sensory cells appears to be the only alternative for regeneration (Fig. 1b). The concept of transdifferentiation is fascinating because it is uncommon for differentiated cells to (a) return

to a mitotic phase or (b) change to a different phenotype, with or without a mitotic cycle. One example of spontaneous transdifferentiation is Barrett's metaplasia, a pathological transdifferentiation of the esophageal lining into intestine-like epithelium (Barbera & Fitzgerald, 2009). The molecular changes that govern this transdifferentiation are becoming more clearly understood (Colleypriest, Palmer, Ward, & Tosh, 2009; Rajendra, et al., 2006; Wong, et al., 2006), and it is possible that the knowledge can be used for inducing beneficial transdifferentiation in organs where it is needed. The other example of spontaneous transdifferentiation in mammals is of more relevance, because it occurs in the inner ear proper, in the vestibular sensory epithelium. Data generated in guinea pigs, chinchillas and mice show that after induction of an experimental lesion that eliminates vestibular hair cells, non-sensory supporting cells can transdifferentiate into hair cell phenotypes (Forge, Li, Corwin, & Nevill, 1993; Kawamoto, Izumikawa, Beyer, Atkin, & Raphael, 2009; Lopez, Honrubia, Lee, Schoeman, & Beykirch, 1997). The majority of the new hair cells in the regenerating vestibular epithelium arise by direct transdifferentiation without cell division. These findings, along with the robust transdifferentiation seen in regenerating inner ear sensory epithelia in non-mammalian vertebrates (Brignull, Raible, & Stone, 2009), have inspired research into the potential of regenerating cochlear hair cells by inducing transdifferentiation of supporting cells that remain in deaf cochleae.

One approach for inducing transdifferentiation is to force expression of genes that activate the hair cell phenotype during development of the inner ear. Initial success was accomplished using over-expression of the *Atoh1* gene, which encodes the basic helix-loop-helix (bHLH) transcription factor necessary for hair cell differentiation. (Bermingham, et al., 1999). Overexpression of *Atoh1* in cultured explants of developing cochleae resulted in robust production of ectopic hair cells (Zheng & Gao, 2000). Similar results were obtained in cultures of mature cochleae (Shou, Zheng, & Gao, 2003), demonstrating that non-sensory cells in the mature ear retain the capacity to transdifferentiate to the hair cell phenotype by forced expression of *Atoh1*. These results were extended to the living ear where ectopic hair cells were generated by viral mediated over-expression of *Atoh1*, and nerve fibers were shown to grow towards these cells (Kawamoto, Ishimoto, Minoda, Brough, & Raphael, 2003). Transdifferentiation of non-sensory cells was induced in ears deafened with kanamycin and ethacrynic acid, accompanied by improvement of hearing thresholds (Izumikawa, et al., 2005).

Although these results are exciting and provide a proof for the principle that transdifferentiation therapy may succeed, several problems need to be resolved before clinical application can be contemplated. One problem is that the regenerated hair cells did not appear normal and exhibited a mixed phenotype intermediate between hair cell and supporting cell. Other problems are that the surgical access for performing such therapy in the human ear is not clinically feasible, and the adenoviral vector may cause an immune response. Another complication is that ears that have a more severe lesion (Fig. 1c) do not respond to the transdifferentiation therapy (Izumikawa, Batts, Miyazawa, Swiderski, & Raphael, 2008). Along with a high variability in the outcome, these difficulties underline the need for much more basic research work to further develop the technology of hair cell regeneration in the mammalian cochlea.

5.2. Transdifferentiation induced by small molecules

Atoh1 is a gene acting downstream of the Notch signaling pathway. This pathway determines the fate of differentiating cells in many tissues (Baron, 2003). In the inner ear, progenitor cells differentiate into both hair cells and supporting cells (Brooker, Hozumi, & Lewis, 2006; Kelley, 2007; Kiernan, Cordes, Kopan, Gossler, & Gridley, 2005; Lanford, et al., 1999). Activation of Notch intracellular domain by attachment of Jagged1 or Delta1 ligands to Notch receptors

results in the production of bHLH transcription factors such as Hes1 and Hes5 which are negative regulators of hair cell differentiation. Hes1 and Hes5 activation prohibits the activity of Atoh1. Hes1 and Hes5 null mice exhibit increased hair cell formation in culture (Zheng, Shou, Guillemot, Kageyama, & Gao, 2000; Zine, et al., 2001). With this in mind, it is tempting to design a way to block Notch signaling in the mature traumatized cochlea in order to induce a phenotypic transdifferentiation of non-sensory cells to hair cells.

Gamma secretase is associated with the proteolytic process of the Notch intracellular domain activation. Pharmacological inhibition of gamma secretase has been shown to lead to suppression of the Notch signaling pathway (Mizutani, Taniguchi, Aoki, Hashimoto, & Honjo, 2001). The suppression of Notch subsequently leads to inhibition of Hes1 and Hes5, which is thought to increase Atoh1 expression. Indeed gamma secretase inhibitors have been shown to produce ectopic hair cells in vitro (Takebayashi, et al., 2007) and in vivo (Hori, et al., 2007). While the number of hair cell regenerated with this method was modest and clinically insignificant, the proof for the principle is truly exciting because the Notch inhibitor is a small molecule that can be delivered to the target in human ears and is likely to have little or no side effects. Moreover, it is possible that blocking Notch signaling molecules one or two steps downstream of gamma secretase could induce more robust production of new hair cells. This could potentially be accomplished by use of siRNA for blocking Hes gene products formation. The feasibility of delivering siRNA in clinically applicable ways has been shown (Maeda, Fukushima, Kawasaki, Nishizaki, & Smith, 2007).

5.3. Cell cycle regulation

The mature organ of Corti is composed of post-mitotic cells. Experimental (and eventually clinical) ability to induce proliferation is exciting and potentially important for two main reasons. First, there is a chance that dividing cells will yield progeny that will spontaneously become new hair cells. Second, supporting cells have important functions and should transdifferentiation therapy be used for hair cell regeneration, the population of supporting cells would be depleted and in need of replenishing. For that purpose, several studies have looked at ways to induce proliferation in the auditory epithelium and the influence of cell division on hair cell regeneration.

Knowledge of the basic biology of cell cycle regulation has assisted in developing experimental approaches for inducing proliferation. The transition between cell cycle phases is regulated by cyclins and cyclin-dependent kinases (Murray, 2004). The activity of these complexes is controlled by several factors including the Cip/Kip and Ink4 cyclin dependent kinase inhibitor (CKI) family (Sherr & Roberts, 1999). Several CKIs have been studied in the auditory system, including p27kip1 p21Cip1 and p19Ink4d. In the organ of Corti, p27kip1 is highly expressed in the terminally differentiated supporting cells and not in the hair cells. Presence of p27kip1 is thought to maintain the structure of the organ of the Corti and possibly be responsible for the lack of regenerative response when hair cells die. Mutant mice that lack p27kip1 exhibit supernumerary cochlear hair cells (Chen & Segil, 1999; Kanzaki, et al., 2006; Lowenheim, et al., 1999), but their hearing is degraded. Inhibition of p27kip1 allowed cells in the auditory epithelium to divide, but these divisions did not lead to hair cell production (Minoda, Izumikawa, Kawamoto, Zhang, & Raphael, 2007). Therefore, it appears that inducing cell division in the auditory epithelium, by itself, does not lead to generation of new hair cells.

Despite the inability of induced proliferation in the auditory epithelium to generate new hair cells, the addition of cells to the epithelium is important. Induced proliferation is valuable for replenishing supporting cells both for providing more cells for transdifferentiation therapy and for maintaining a sufficient number of functional differentiated supporting cells. In addition, ability to initiate the cell cycle in the auditory epithelium opens the way for inserting large genes using viral vectors, such as retroviruses, that can carry large DNA inserts but require

genomic integration to express their genes. Such genomic integration typically occurs during the cell cycle.

6. Delivering therapies into the inner ear

Delivery of pharmaceutical reagents is simplest when systemic routes using skin patch or oral doses are possible. These are useful for delivering small molecules, but it is less likely that a systemic application will be feasible for inducing ear-specific regenerative processes. After systemic delivery, the final concentration in the cochlea would likely be too low to be effective. The body's natural filtration system may remove many reagents before they reach the ear and many would be excluded from the ear altogether due to the blood-ear barrier. There is also the risk of side-effects from exposure of non-target organs to the reagent. It is therefore likely that delivery of cells or even genes will necessitate direct inoculation into the ear. Such invasive and complex surgical procedures would typically be justified for reparative measures but not for protection.

Ideal delivery into the ear would be via the outer ear canal, which would not necessitate any surgery. However, the ear drum is a formidable barrier, and even if its integrity is transiently disrupted, the amount of reagent likely to reach the cochlea is not very high. Still, methods should be developed to facilitate diffusion of reagents from the ear drum along the ossicles to the oval window. Delivery vehicles and routes for inner ear application have been reviewed (Patel, Mhatre, & Lalwani, 2004; Salt & Plontke, 2009) and further progress is being made (Mikulec, Plontke, Hartsock, & Salt, 2009).

7. The present and future of inner ear tissue engineering: ideal versus practical goals

Personnel in Speech & Hearing clinics regularly face patients with questions, and need to provide answers regarding future therapies and their feasibility. It is often necessary to provide to the patients a realistic assessment of the clinical potential and main hurdles to accomplishing clinical applicability.

At present only protection is a feasible intervention. However, the rapid advances in gene discovery, delivery methods and stem cell technology promise future tissue-engineering approaches for inner ear therapy. It could be argued that reconstruction/regeneration of the sensorineural elements in a cochlea from the worst degenerative state (flat epithelium, for instance) back to normal would be nearly impossible. We speculate that rebuilding of the structures that account for the active cochlea, especially the outer hair cell system and its connection to the tectorial membrane will be a more difficult task than providing basic hearing. However, inducing regeneration of inner hair cells that can pull auditory nerve fibers (Kawamoto, et al., 2003) and reform a synapse with nerve endings is more feasible, especially considering that inner hair cell depolarization can occur even without contact with the tectorial membrane. We argue that there is nothing wrong in setting a utopian ideal of perfect regeneration as a goal, while being ready to accept a less than perfect restoration as an acceptable realization of that ideal. Thus, for a person with no hearing at all, a modest number of regenerated hair cells that are placed in the cochlea by transdifferentiation or stem cell therapy and provide usable hearing would be a very welcome outcome. It is in this spirit that our research is advancing, aiming for the best restoration and acknowledging that any improvement would be valuable.

We are frequently asked to provide predictions of the time it might take for regenerative therapies to become clinically available. In our opinion, a realistic estimate of the number of years this will take is impossible and therefore irresponsible. Rather, it should be noted that

several technologies need to be advanced and enhanced, and some breakthroughs are necessary, before any of the reparative techniques can be applied. It seems reasonable to expect that the technology to combine tissue engineering with cochlear implant therapy will be applicable before hair cell regeneration therapies become practical. As for hair cell regeneration therapies, it is likely that ears traumatized by environmental causes will be better candidates than those with hereditary disease, because inducing differentiation of new hair cells with the same mutation as the original cells may be futile. Stem cell therapy or phenotypic rescue approaches by insertion of a wild-type gene or a gene down-stream of the mutated gene may be the approaches applicable for genetic based hearing loss. While none of these technologies are close to being clinically applicable in the near future, constant improvement in technology and on-going efforts in many laboratories provides hope for continued advances towards eventual therapies.

Acknowledgments

We thank Hiu Tung Wong for generating the schematic image, and Mark Crumling and Donald Swiderski for helpful comments. Our work is supported by the A. Alfred Taubman Medical Research Institute, the Berte and Alan Hirschfield Foundation, the R. Jamison and Betty Williams Professorship, and NIH/NIDCD Grants R01-DC01634, R01-DC007634, 5R21-DC009293, T32-DC005356 and P30-DC05188.

References

- Alam SA, Ikeda K, Oshima T, Suzuki M, Kawase T, Kikuchi T, et al. Cisplatin-induced apoptotic cell death in Mongolian gerbil cochlea. *Hearing Research* 2000;141:28–38. [PubMed: 10713493]
- Bailey DM, Collins MA, Gordon JD, Zuur AF, Priede IG. Long-term changes in deep-water fish populations in the northeast Atlantic: A deeper reaching effect of fisheries? *Proceedings of the Royal Society B: Biological Sciences* 2009;276:1965–1969.
- Barbera M, Fitzgerald RC. Cellular mechanisms of Barrett's esophagus development. *Surgical Oncology Clinics of North America* 2009;18:393–410. [PubMed: 19500732]
- Baron M. An overview of the Notch signaling pathway. *Seminars in Cell and Developmental Biology* 2003;14:113–119. [PubMed: 12651094]
- Bellamkonda RV. Peripheral nerve regeneration: An opinion on channels, scaffolds and anisotropy. *Biomaterials* 2006;27:3515–3518. [PubMed: 16533522]
- Bermingham NA, Hassan BA, Price SD, Vollrath MA, Ben-Arie N, Eatock RA, et al. *Math1*: An essential gene for the generation of inner ear hair cells. *Science* 1999;284:1837–1841. [PubMed: 10364557]
- Bichler E, Spoendlin H, Rauchegger H. Degeneration of cochlear neurons after amikacin intoxication in the rat. *Archives of Oto-Rhino-Laryngology* 1983;237:201–208. [PubMed: 6870653]
- Bielefeld EC, Tanaka C, Chen GD, Henderson D. Age-related hearing loss: Is it a preventable condition? *Hearing Research*. (in press).
- Bluestone CD, Fria TJ, Arjona SK, Casselbrant ML, Schwartz DM, Ruben RJ, et al. Controversies in screening for middle ear disease and hearing loss in children. *Pediatrics* 1986;77:57–70. [PubMed: 3940361]
- Bodmer D, Brors D, Pak K, Bodmer M, Ryan AF. Gentamicin-induced hair cell death is not dependent on the apoptosis receptor Fas. *Laryngoscope* 2003;113:452–455. [PubMed: 12616196]
- Bohne BA, Rabbitt KD. Holes in the reticular lamina after noise exposure: Implication for continuing damage in the organ of Corti. *Hearing Research* 1983;11:41–53. [PubMed: 6350251]
- Bowers WJ, Chen X, Guo H, Frisina DR, Federoff HJ, Frisina RD. Neurotrophin-3 transduction attenuates cisplatin spiral ganglion neuron ototoxicity in the cochlea. *Molecular Therapy* 2002;6:12–18. [PubMed: 12095298]
- Brignull HR, Raible DW, Stone JS. Feathers and fins: Non-mammalian models for hair cell regeneration. *Brain Research* 2009;1277:12–23. [PubMed: 19245801]
- Brooker R, Hozumi K, Lewis J. Notch ligands with contrasting functions: Jagged1 and Delta1 in the mouse inner ear. *Development* 2006;133:1277–1286. [PubMed: 16495313]

- Carlin MF, McCroskey RL. Is eye color a predictor of noise-induced hearing loss? *Ear & Hearing* 1980;1:191–196. [PubMed: 7409355]
- Chao MV, Rajagopal R, Lee FS. Neurotrophin signaling in health and disease. *Clinical Science (London)* 2006;110:167–173.
- Chen P, Segil N. p27 (Kip1) links cell proliferation to morphogenesis in the developing organ of Corti. *Development* 1999;126:1581–1590. [PubMed: 10079221]
- Chen Y, Huang WG, Zha DJ, Qiu JH, Wang JL, Sha SH, et al. Aspirin attenuates gentamicin ototoxicity: From the laboratory to the clinic. *Hearing Research* 2007;226:178–182. [PubMed: 16844331]
- Cheng AG, Cunningham LL, Rubel EW. Hair cell death in the avian basilar papilla: Characterization of the in vitro model and caspase activation. *Journal of the Association for Research in Otolaryngology* 2003;4:91–105. [PubMed: 12417974]
- Cheng AG, Huang T, Stracher A, Kim A, Liu W, Malgrange B, et al. Calpain inhibitors protect auditory sensory cells from hypoxia and neurotrophin-withdrawal induced apoptosis. *Brain Research* 1999;850:234–243. [PubMed: 10629769]
- Cho H, Harada N, Yamashita T. Extracellular ATP-induced Ca²⁺ mobilization of type I spiral ganglion cells from the guinea pig cochlea. *Acta Oto-Laryngologica* 1997;117:545–552. [PubMed: 9288210]
- Coleman B, Hardman J, Coco A, Epp S, de Silva M, Crook J, et al. Fate of embryonic stem cells transplanted into the deafened mammalian cochlea. *Cell Transplant* 2006;15:369–380. [PubMed: 16970279]
- Coleman JK, Kopke RD, Liu J, Ge X, Harper EA, Jones GE, et al. Pharmacological rescue of noise induced hearing loss using N-acetylcysteine and acetyl-L-carnitine. *Hearing Research* 2007;226:104–113. [PubMed: 17023129]
- Colletti V. Auditory outcomes in tumor vs. nontumor patients fitted with auditory brainstem implants. *Advances in Oto-Rhino-Laryngology* 2006;64:167–185. [PubMed: 16891842]
- Collepriest BJ, Palmer RM, Ward SG, Tosh D. Cdx genes, inflammation and the pathogenesis of Barrett's metaplasia. *Trends in Molecular Medicine* 2009;15:313–322. [PubMed: 19564133]
- Corrales CE, Pan L, Li H, Liberman MC, Heller S, Edge AS. Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: Growth of processes into the organ of Corti. *Journal of Neurobiology* 2006;66:1489–1500. [PubMed: 17013931]
- Cullington HE. Light eye colour linked to deafness after meningitis. *British Medical Journal* 2001;322:587. [PubMed: 11238154]
- Da Costa EA, Castro JC, Macedo ME. Iris pigmentation and susceptibility to noise-induced hearing loss. *International Journal of Audiology* 2008;47:115–118. [PubMed: 18307091]
- Despres G, Leger GP, Dahl D, Romand R. Distribution of cytoskeletal proteins (neurofilaments, peripherin and MAP-tau) in the cochlea of the human fetus. *Acta Oto-Laryngologica* 1994;114:377–381. [PubMed: 7976309]
- Di Polo A, Aigner LJ, Dunn RJ, Bray GM, Aguayo AJ. Prolonged delivery of brain-derived neurotrophic factor by adenovirus-infected Muller cells temporarily rescues injured retinal ganglion cells. *Proceedings of the National Academy of Sciences USA* 1998;95:3978–3983.
- Duan M, Agerman K, Ernfors P, Canlon B. Complementary roles of neurotrophin 3 and a N-methyl-D-aspartate antagonist in the protection of noise and aminoglycoside-induced ototoxicity. *Proceedings of the National Academy of Sciences USA* 2000;97:7597–7602.
- Ernfors P, Kucera J, Lee KF, Loring J, Jaenisch R. Studies on the physiological role of brain-derived neurotrophic factor and neurotrophin-3 in knockout mice. *International Journal of Developmental Biology* 1995;39:799–807. [PubMed: 8645564]
- Eshraghi AA, Van de Water TR. Cochlear implantation trauma and noise-induced hearing loss: Apoptosis and therapeutic strategies. *The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology* 2006;288:473–481.
- Eshraghi AA, Wang J, Adil E, He J, Zine A, Bublik M, et al. Blocking c-Jun-N-terminal kinase signaling can prevent hearing loss induced by both electrode insertion trauma and neomycin ototoxicity. *Hearing Research* 2007;226:168–177. [PubMed: 17098385]
- Excoffon KJ, Avenarius MR, Hansen MR, Kimberling WJ, Najmabadi H, Smith RJ, et al. The Coxsackievirus and Adenovirus receptor: A new adhesion protein in cochlear development. *Hearing Research* 2006;215:1–9. [PubMed: 16678988]

- Feghali JG, Liu W, Van De Water TR. L-n-acetyl-cysteine protection against cisplatin-induced auditory neuronal and hair cell toxicity. *Laryngoscope* 2001;111:1147–1155. [PubMed: 11568534]
- Forge A. Outer hair cell loss and supporting cell expansion following chronic gentamicin treatment. *Hearing Research* 1985;19:171–182. [PubMed: 4055536]
- Forge A, Li L. Apoptotic death of hair cells in mammalian vestibular sensory epithelia. *Hearing Research* 2000;139:97–115. [PubMed: 10601716]
- Forge A, Li L, Corwin JT, Nevill G. Ultrastructural evidence for hair cell regeneration in the mammalian inner ear. *Science* 1993;259:1616–1619. [PubMed: 8456284]
- Fritzsch B, Silos-Santiago II, Bianchi LM, Farinas II. Effects of neurotrophin and neurotrophin receptor disruption on the afferent inner ear innervation. *Seminars in Cell and Developmental Biology* 1997;8:277–284.
- Fritzsch B, Tessarollo L, Coppola E, Reichardt LF. Neurotrophins in the ear: their roles in sensory neuron survival and fiber guidance. *Progress in Brain Research* 2004;146:265–278. [PubMed: 14699969]
- Gao WQ. Role of neurotrophins and lectins in prevention of ototoxicity. *Annals of the New York Academy of Sciences* 1999;884:312–327. [PubMed: 10842603]
- Garetz SL, Altschuler RA, Schacht J. Attenuation of gentamicin ototoxicity by glutathione in the guinea pig in vivo. *Hearing Research* 1994;77:81–87. [PubMed: 7928740]
- Glueckert R, Bitsche M, Miller JM, Zhu Y, Prieskorn DM, Altschuler RA, et al. Deafferentation-associated changes in afferent and efferent processes in the guinea pig cochlea and afferent regeneration with chronic intrascalar brain-derived neurotrophic factor and acidic fibroblast growth factor. *Journal of Comparative Neurology* 2008;507:1602–1621. [PubMed: 18220258]
- Hansen MR, Zha XM, Bok J, Green SH. Multiple distinct signal pathways, including an autocrine neurotrophic mechanism, contribute to the survival-promoting effect of depolarization on spiral ganglion neurons in vitro. *Journal of Neuroscience* 2001;21:2256–2267. [PubMed: 11264301]
- Hendricks JL, Chikar JA, Crumling MA, Raphael Y, Martin DC. Localized cell and drug delivery for auditory prostheses. *Hearing Research* 2008;242:117–131. [PubMed: 18573323]
- Hori R, Nakagawa T, Sakamoto T, Matsuoka Y, Takebayashi S, Ito J. Pharmacological inhibition of Notch signaling in the mature guinea pig cochlea. *Neuroreport* 2007;18:1911–1914. [PubMed: 18007185]
- Hu Z, Ulfendahl M, Prieskorn DM, Olivius P, Miller JM. Functional evaluation of a cell replacement therapy in the inner ear. *Otology and Neurotology* 2009;30:551–558. [PubMed: 19395986]
- Huang T, Cheng AG, Stupak H, Liu W, Kim A, Staecker H, et al. Oxidative stress-induced apoptosis of cochlear sensory cells: otoprotective strategies. *International Journal of Developmental Neuroscience* 2000;18:259–270. [PubMed: 10715580]
- Izumikawa M, Batts SA, Miyazawa T, Swiderski DL, Raphael Y. Response of the flat cochlear epithelium to forced expression of Atoh1. *Hearing Research* 2008;240:52–56. [PubMed: 18430530]
- Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, et al. Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. *Nature Medicine* 2005;11:271–276.
- Kang SY, Colesa DJ, Swiderski DL, Su GL, Raphael Y, Pfingst BE. Effects of hearing preservation on psychophysical responses to cochlear implant stimulation. *Journal of the Association for Research in Otolaryngology*. (in press).
- Kanzaki S, Beyer LA, Swiderski DL, Izumikawa M, Stover T, Kawamoto K, et al. p27 (Kip1) deficiency causes organ of Corti pathology and hearing loss. *Hearing Research* 2006;214:28–36. [PubMed: 16513305]
- Kanzaki S, Stover T, Kawamoto K, Prieskorn DM, Altschuler RA, Miller JM, et al. Glial cell line-derived neurotrophic factor and chronic electrical stimulation prevent VIII cranial nerve degeneration following denervation. *Journal of Comparative Neurology* 2002;454:350–360. [PubMed: 12442325]
- Kawamoto K, Ishimoto S, Minoda R, Brough DE, Raphael Y. Math1 gene transfer generates new cochlear hair cells in mature guinea pigs in vivo. *Journal of Neuroscience* 2003;23:4395–4400. [PubMed: 12805278]
- Kawamoto K, Izumikawa M, Beyer LA, Atkin GM, Raphael Y. Spontaneous hair cell regeneration in the mouse utricle following gentamicin ototoxicity. *Hearing Research* 2009;247:17–26. [PubMed: 18809482]

- Kelley MW. Cellular commitment and differentiation in the organ of Corti. *International Journal of Developmental Biology* 2007;51:571–583. [PubMed: 17891718]
- Kiernan AE, Cordes R, Kopan R, Gossler A, Gridley T. The Notch ligands DLL1 and JAG2 act synergistically to regulate hair cell development in the mammalian inner ear. *Development* 2005;132:4353–4362. [PubMed: 16141228]
- Kim YH, Raphael Y. Cell division and maintenance of epithelial integrity in the deafened auditory epithelium. *Cell Cycle* 2007;6:612–619. [PubMed: 17351334]
- Lanford PJ, Lan Y, Jiang R, Lindsell C, Weinmaster G, Gridley T, et al. Notch signaling pathway mediates hair cell development in mammalian cochlea. *Nature Genetics* 1999;21:289–292. [PubMed: 10080181]
- Lautermann J, McLaren J, Schacht J. Glutathione protection against gentamicin ototoxicity depends on nutritional status. *Hearing Research* 1995;86:15–24. [PubMed: 8567412]
- Leake PA, Hradek GT. Cochlear pathology of long term neomycin induced deafness in cats. *Hearing Research* 1988;33:11–33. [PubMed: 3372368]
- Lenoir M, Daudet N, Humbert G, Renard N, Gallego M, Pujol R, et al. Morphological and molecular changes in the inner hair cell region of the rat cochlea after amikacin treatment. *Journal of Neurocytology* 1999;28:925–937. [PubMed: 10900095]
- Li H, Corrales CE, Edge A, Heller S. Stem cells as therapy for hearing loss. *Trends in Molecular Medicine* 2004;10:309–315. [PubMed: 15242678]
- Li H, Liu H, Heller S. Pluripotent stem cells from the adult mouse inner ear. *Nature Medicine* 2003;9:1293–1299.
- Lim DJ, Melnick W. Acoustic damage of the cochlea. A scanning and transmission electron microscopic observation. *Archives of Otolaryngology* 1971;94:294–305. [PubMed: 5098718]
- Liu W, Staecker H, Stupak H, Malgrange B, Lefebvre P, Van De Water TR. Caspase inhibitors prevent cisplatin-induced apoptosis of auditory sensory cells. *Neuroreport* 1998;9:2609–2614. [PubMed: 9721942]
- Lopez I, Honrubia V, Lee SC, Schoeman G, Beykirch K. Quantification of the process of hair cell loss and recovery in the chinchilla crista ampullaris after gentamicin treatment. *International Journal of Developmental Neuroscience* 1997;15:447–461. [PubMed: 9263025]
- Lopez IA, Zhao PM, Yamaguchi M, de Vellis J, Espinosa-Jeffrey A. Stem/progenitor cells in the postnatal inner ear of the GFP-nestin transgenic mouse. *International Journal of Developmental Neuroscience* 2004;22:205–213. [PubMed: 15245756]
- Lowenheim H, Furness DN, Kil J, Zinn C, Gultig K, Fero ML, et al. Gene disruption of p27 (Kip1) allows cell proliferation in the postnatal and adult organ of Corti. *Proceedings of the National Academy of Sciences USA* 1999;96:4084–4088.
- Lustig LR, Lin D, Venick H, Larky J, Yeagle J, Chinnici J, et al. GJB2 gene mutations in cochlear implant recipients: prevalence and impact on outcome. *Archives of Otolaryngology: Head Neck Surgery* 2004;130:541–546. [PubMed: 15148174]
- Maeda Y, Fukushima K, Kawasaki A, Nishizaki K, Smith RJ. Cochlear expression of a dominant-negative GJB2R75W construct delivered through the round window membrane in mice. *Neuroscience Research* 2007;58:250–254. [PubMed: 17462767]
- Martinez-Monedero R, Yi E, Oshima K, Glowatzki E, Edge AS. Differentiation of inner ear stem cells to functional sensory neurons. *Developmental Neurobiology* 2008;68:669–684. [PubMed: 18278797]
- Matsui JI, Haque A, Huss D, Messana EP, Alosi JA, Roberson DW, et al. Caspase inhibitors promote vestibular hair cell survival and function after aminoglycoside treatment in vivo. *Journal of Neuroscience* 2003;23:6111–6122. [PubMed: 12853430]
- Matsui JI, Ogilvie JM, Warchol ME. Inhibition of caspases prevents ototoxic and ongoing hair cell death. *Journal of Neuroscience* 2002;22:1218–1227. [PubMed: 11850449]
- Matsuoka AJ, Kondo T, Miyamoto RT, Hashino E. Enhanced survival of bone-marrow-derived pluripotent stem cells in an animal model of auditory neuropathy. *Laryngoscope* 2007;117:1629–1635. [PubMed: 17632425]
- McKay R. Stem cells in the central nervous system. *Science* 1997;276:66–71. [PubMed: 9082987]

- Medd AM, Bianchi LM. Analysis of BDNF production in the aging gerbil cochlea. *Experimental Neurology* 2000;162:390–393. [PubMed: 10739644]
- Mikulec AA, Plontke SK, Hartsock JJ, Salt AN. Entry of substances into perilymph through the bone of the otic capsule after intratympanic applications in guinea pigs: implications for local drug delivery in humans. *Otology and Neurotology* 2009;30:131–138. [PubMed: 19180674]
- Minoda R, Izumikawa M, Kawamoto K, Zhang H, Raphael Y. Manipulating cell cycle regulation in the mature cochlea. *Hearing Research* 2007;232:44–51. [PubMed: 17658230]
- Mizutani T, Taniguchi Y, Aoki T, Hashimoto N, Honjo T. Conservation of the biochemical mechanisms of signal transduction among mammalian Notch family members. *Proceedings of the National Academy of Sciences USA* 2001;98:9026–9031.
- Murray AW. Recycling the cell cycle: Cyclins revisited. *Cell* 2004;116:221–234. [PubMed: 14744433]
- Nadol JB Jr, Eddington DK. Histopathology of the inner ear relevant to cochlear implantation. *Advances in Oto-Rhino-Laryngology* 2006;64:31–49. [PubMed: 16891835]
- Nakagawa T, Yamane H, Takayama M, Sunami K, Nakai Y. Apoptosis of guinea pig cochlear hair cells following chronic aminoglycoside treatment. *European Archives of Oto-Rhino-Laryngology* 1998;255:127–131. [PubMed: 9561858]
- Nakaizumi T, Kawamoto K, Minoda R, Raphael Y. Adenovirus-mediated expression of brain-derived neurotrophic factor protects spiral ganglion neurons from ototoxic damage. *Audiology and Neurotology* 2004;9:135–143. [PubMed: 15084818]
- Nicotera TM, Hu BH, Henderson D. The caspase pathway in noise-induced apoptosis of the chinchilla cochlea. *Journal of the Association for Research in Otolaryngology* 2003;4:466–477. [PubMed: 14534835]
- Ohlemiller KK, Dugan LL. Elevation of reactive oxygen species following ischemia-reperfusion in mouse cochlea observed in vivo. *Audiology and Neurotology* 1999;4:219–228. [PubMed: 10436314]
- Okano T, Nakagawa T, Endo T, Kim TS, Kita T, Tamura T, et al. Engraftment of embryonic stem cell-derived neurons into the cochlear modiolus. *Neuroreport* 2005;16:1919–1922. [PubMed: 16272879]
- Otte J, Schunknecht HF, Kerr AG. Ganglion cell populations in normal and pathological human cochleae. Implications for cochlear implantation. *Laryngoscope* 1978;88:1231–1246. [PubMed: 672357]
- Patel NP, Mhatre AN, Lalwani AK. Biological therapy for the inner ear. *Expert Opinion on Biological Therapy* 2004;4:1811–1819. [PubMed: 15500409]
- Penn MS, Mangi AA. Genetic enhancement of stem cell engraftment, survival, and efficacy. *Circulation Research* 2008;102:1471–1482. [PubMed: 18566313]
- Pettingill LN, Richardson RT, Wise AK, O’Leary SJ, Shepherd RK. Neurotrophic factors and neural prostheses: potential clinical applications based upon findings in the auditory system. *IEEE Transactions on Biomedical Engineering* 2007;54:1138–1148. [PubMed: 17551571]
- Ping Yang W, Henderson D, Hua Hu B, Nicotera TM. Quantitative analysis of apoptotic and necrotic outer hair cells after exposure to different levels of continuous noise. *Hearing Research* 2004;196:69–76. [PubMed: 15464303]
- Pirvola U, Xing-Qun L, Virkkala J, Saarma M, Murakata C, Camoratto AM, et al. Rescue of hearing, auditory hair cells, and neurons by CEP-1347/KT7515, an inhibitor of c-Jun N-terminal kinase activation. *Journal of Neuroscience* 2000;20:43–50. [PubMed: 10627579]
- Rajendra S, Ackroyd R, Karim N, Mohan C, Ho JJ, Kutty KM. Loss of HLA class I and gain of class II expression are early events in carcinogenesis - Clues from a study of Barrett’s oesophagus. *Journal of Clinical Pathology* 2006;59:952–957. [PubMed: 16467164]
- Raphael Y, Altschuler RA. Reorganization of cytoskeletal and junctional proteins during cochlear hair cell degeneration. *Cell Motility and the Cytoskeleton* 1991a;18:215–227. [PubMed: 1711932]
- Raphael Y, Altschuler RA. Scar formation after drug-induced cochlear insult. *Hearing Research* 1991b;51:173–183. [PubMed: 1709631]
- Raphael Y, Kim YH, Osumi Y, Izumikawa M. Non-sensory cells in the deafened organ of Corti: Approaches for repair. *International Journal of Developmental Biology* 2007;51:649–654. [PubMed: 17891723]
- Reyes JH, O’Shea KS, Wys NL, Velkey JM, Prieskorn DM, Wesolowski K, et al. Glutamatergic neuronal differentiation of mouse embryonic stem cells after transient expression of neurogenin 1 and

- treatment with BDNF and GDNF: In vitro and in vivo studies. *Journal of Neuroscience* 2008;28:12622–12631. [PubMed: 19036956]
- Roehm PC, Hansen MR. Strategies to preserve or regenerate spiral ganglion neurons. *Current Opinion in Otolaryngology & Head and Neck Surgery* 2005;13:294–300. [PubMed: 16160524]
- Rybak LP, Whitworth C, Somani S. Application of antioxidants and other agents to prevent cisplatin ototoxicity. *Laryngoscope* 1999;109:1740–1744. [PubMed: 10569399]
- Salt AN, Plontke SK. Principles of local drug delivery to the inner ear. *Audiology and Neurotology* 2009;14:350–360. [PubMed: 19923805]
- Schacht J. Antioxidant therapy attenuates aminoglycoside-induced hearing loss. *Annals of the New York Academy of Sciences* 1999;884:125–130. [PubMed: 10842589]
- Seidman MD, Van De Water TR. Pharmacologic manipulation of the labyrinth with novel and traditional agents delivered to the inner ear. *Ear, Nose and Throat Journal* 2003;82:276–300.
- Sekiya T, Yagihashi A, Shimamura N, Asano K, Suzuki S, Matsubara A, et al. Apoptosis of auditory neurons following central process injury. *Experimental Neurology* 2003;184:648–658. [PubMed: 14769356]
- Sha SH, Schacht J. Salicylate attenuates gentamicin-induced ototoxicity. *Laboratory Investigation* 1999;79:807–813. [PubMed: 10418821]
- Sha SH, Schacht J. Antioxidants attenuate gentamicin-induced free radical formation in vitro and ototoxicity in vivo: D-methionine is a potential protectant. *Hearing Research* 2000;142:34–40. [PubMed: 10748326]
- Shah SB, Gladstone HB, Williams H, Hradek GT, Schindler RA. An extended study: protective effects of nerve growth factor in neomycin-induced auditory neural degeneration. *American Journal of Otolaryngology* 1995;16:310–314. [PubMed: 8588624]
- Shepherd RK, Coco A, Epp SB. Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss. *Hearing Research* 2008;242:100–109. [PubMed: 18243608]
- Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes & Development* 1999;13:1501–1512. [PubMed: 10385618]
- Shou J, Zheng JL, Gao WQ. Robust generation of new hair cells in the mature mammalian inner ear by adenoviral expression of *Hath1*. *Molecular and Cellular Neuroscience* 2003;23:169–179. [PubMed: 12812751]
- Spoendlin H, Schrott A. Analysis of the human auditory nerve. *Hearing Research* 1989;43:25–38. [PubMed: 2613564]
- Staecker H, Kopke R, Malgrange B, Lefebvre P, Van de Water TR. NT-3 and/or BDNF therapy prevents loss of auditory neurons following loss of hair cells. *Neuroreport* 1996;7:889–894. [PubMed: 8724667]
- Sugawara M, Corfas G, Liberman MC. Influence of supporting cells on neuronal degeneration after hair cell loss. *Journal of the Association for Research in Otolaryngology* 2005;6:136–147. [PubMed: 15952050]
- Takebayashi S, Yamamoto N, Yabe D, Fukuda H, Kojima K, Ito J, et al. Multiple roles of Notch signaling in cochlear development. *Developmental Biology* 2007;307:165–178. [PubMed: 17531970]
- Todd NW, Alvarado CS, Brewer DB. Cisplatin in children: Hearing loss correlates with iris and skin pigmentation. *Journal of Laryngology & Otolaryngology* 1995;109:926–929. [PubMed: 7499942]
- Tono T, Kiyomizu K, Matsuda K, Komune S, Usami S, Abe S, et al. Different clinical characteristics of aminoglycoside-induced profound deafness with and without the 1555 A→G mitochondrial mutation. *Journal for Oto-Rhino-Laryngology and Its Related Specialties* 2001;63:25–30. [PubMed: 11174059]
- Tuszynski MH, Thal L, Pay M, Salmon DP, UHS, Bakay R, et al. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nature Medicine* 2005;11:551–555.
- Usami S, Takumi Y, Fujita S, Shinkawa H, Hosokawa M. Cell death in the inner ear associated with aging is apoptosis? *Brain Research* 1997;747:147–150. [PubMed: 9042539]
- Wang J, Van De Water TR, Bonny C, de Ribaupierre F, Puel JL, Zine A. A peptide inhibitor of c-Jun N-terminal kinase protects against both aminoglycoside and acoustic trauma-induced auditory hair cell death and hearing loss. *Journal of Neuroscience* 2003;23:8596–8607. [PubMed: 13679429]

- Webster M, Webster DB. Spiral ganglion neuron loss following organ of Corti loss: a quantitative study. *Brain Research* 1981;212:17–30. [PubMed: 7225854]
- Wise AK, Richardson R, Hardman J, Clark G, O’Leary S. Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3. *Journal of Comparative Neurology* 2005;487:147–165. [PubMed: 15880560]
- Wong NA, Warren BF, Piris J, Maynard N, Marshall R, Bodmer WF. EpCAM and gpA33 are markers of Barrett’s metaplasia. *Journal of Clinical Pathology* 2006;59:260–263. [PubMed: 16473928]
- Yagi M, Kanzaki S, Kawamoto K, Shin B, Shah PP, Magal E, et al. Spiral ganglion neurons are protected from degeneration by GDNF gene therapy. *Journal of the Association for Research in Otolaryngology* 2000;1:315–325. [PubMed: 11547811]
- Zampieri N, Chao MV. Mechanisms of neurotrophin receptor signaling. *Biochemical Society Transactions* 2006;34:607–611. [PubMed: 16856873]
- Zelante L, Gasparini P, Estivill X, Melchionda S, D’Agruma L, Govea N, et al. Connexin26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. *Human Molecular Genetics* 1997;6:1605–1609. [PubMed: 9285800]
- Zheng JL, Gao WQ. Analysis of rat vestibular hair cell development and regeneration using calretinin as an early marker. *Journal of Neuroscience* 1997;17:8270–8282. [PubMed: 9334402]
- Zheng JL, Gao WQ. Overexpression of Math1 induces robust production of extra hair cells in postnatal rat inner ears. *Nature Neuroscience* 2000;3:580–586.
- Zheng JL, Shou J, Guillemot F, Kageyama R, Gao WQ. Hes1 is a negative regulator of inner ear hair cell differentiation. *Development* 2000;127:4551–4560. [PubMed: 11023859]
- Zheng Y, Ikeda K, Nakamura M, Takasaka T. Endonuclease cleavage of DNA in the aged cochlea of Mongolian gerbil. *Hearing Research* 1998;126:11–18. [PubMed: 9872129]
- Zine A, Aubert A, Qiu J, Therianos S, Guillemot F, Kageyama R, et al. Hes1 and Hes5 activities are required for the normal development of the hair cells in the mammalian inner ear. *Journal of Neuroscience* 2001;21:4712–4720. [PubMed: 11425898]
- Zine A, van de Water TR. The MAPK/JNK signaling pathway offers potential therapeutic targets for the prevention of acquired deafness. *Current Drug Targets - CNS & Neurological Disorders* 2004;3:325–332. [PubMed: 15379608]
- Zuur CL, Simis YJ, Lamers EA, Hart AA, Dreschler WA, Balm AJ, et al. Risk factors for hearing loss in patients treated with intensity-modulated radiotherapy for head-and-neck tumors. *International Journal of Radiation Oncology-Biology-Physics* 2009;74:490–496.

Appendix A. Continuing education

1. Persons with normal hearing can reduce the likelihood for acoustic trauma-induced hearing loss by
 - a. Normal ears are not sensitive to acoustic trauma
 - b. Ear plugs or protective headphones need to be worn in loud areas
 - c. Increasing awareness and avoiding exposure to loud signals
 - d. All of the above
 - e. Answers b and c
2. The identification and characterization of genes that participate in ear development and function is important because
 - a. People are curious and want to know stuff
 - b. Manipulating developmental genes may assist in regenerative therapy
 - c. Protecting hair cells and auditory nerve cells from death may be accomplished by expression of developmental genes

- d.** Future therapies for hereditary deafness may depend on such knowledge
 - e.** Answers b-d
- 3.** People with threshold shifts need louder signals for hearing and can allow themselves exposure to louder noise and sound.
 - a.** True, because their ears are less sensitive
 - b.** This relates only to noise but not to sounds and speech
 - c.** False. On the contrary, they are usually more sensitive
 - d.** The thresholds are not indicative of sensitivity to overstimulation
- 4.** The current clinical options for restoring hearing in a profound ear include
 - a.** Limited hair cell regeneration with Notch signaling inhibition
 - b.** cochlear implant prosthesis with additional hair cells regenerated by Atoh1 gene therapy
 - c.** cochlear implant with or without amplification of acoustic signals
 - d.** answers a and c
- 5.** The main problems in delivering large therapeutic molecules into the ear is
 - a.** Surgical approaches are complex
 - b.** Biological barriers prevent molecules from reaching the labyrinth
 - c.** A single application may not last and multiple deliveries are even more complex
 - d.** All the above

Answer key: 1:e; 2:b–d; 3:c; 4:d; 5:c

Figure 1.

A schematic showing the normal organ of Corti (a) and two stages of pathology with complete hair cell loss associated with profound hearing loss: supporting cells remaining in a differentiated state (b) or supporting cell replaced by a flat epithelium (c). Transdifferentiation therapy was accomplished by gene transfer or other means as long as supporting cells remained differentiated (b). Transdifferentiation therapy has so far failed in the flat epithelium, suggesting the need for transplantation of cells from exogenous source (c). Currently only a cochlear implant prosthesis is a therapeutic option in the clinic for ears with no hair cells (b and c).