Strategies to Restore Hearing

Sydney N. Sheltz-Kempf
Western Michigan University

Follow this and additional works at: https://scholarworks.wmich.edu/hilltopreview

Part of the Cell and Developmental Biology Commons

Preferred Citation Style (e.g. APA, MLA, Chicago, etc.)
Harvard (name, date)

This Article is brought to you for free and open access by the Graduate College at ScholarWorks at WMU. It has been accepted for inclusion in The Hilltop Review by an authorized editor of ScholarWorks at WMU. For more information, please contact maira.bundza@wmich.edu.
Strategies to Restore Hearing

By Sydney N. Sheltz-Kempf

Abstract: We discuss strategies within the field to restore hearing in the context of a flat epithelia model. This could assist in avoiding the limitations of current treatment options along with the obstacles associated with cellular restoration attempts. A review of the important genes required for the development, differentiation, and long-term maintenance of the organ of Corti (OC) demonstrates that any future direction to regenerate hair cells necessitates a better understanding of the gene expression in addition to the cells present during the phalangeal scarring process and the flat epithelia environment. This understanding could be achieved through the development of a characterized flat epithelia, followed by complete regeneration of various sensory cell types in the correct location that respond appropriately to noise stimuli. Of course, this strategy would have to be modified for the different types and cellular manifestations of hearing loss. The characterization of the flat epithelia model and the context of the genes can be further manipulated for precise regeneration of a functional OC based on the cellular environment within the specific patient’s cochlea.
Background

Neurosensory hearing loss is one of the most prevalent sensory disorders, with over 5% of the world’s population living with disabling hearing loss [40, 45]. In the United States alone, one in eight people over the age of 12 experience hearing loss in both ears [35], and by age 60, approximately one-third of the population has difficulty hearing [40]. Additionally, hearing loss at birth, known as congenital hearing loss, is one of the most common chronic disorders in children [27]. The hearing loss in these pediatric patients is due to genetic factors. However, other causes of hearing loss include noise-induced hearing loss, ototoxic drugs, and other environmental insults. In many cases of hearing loss, the mechanosensory cells of the inner ear responsible for transforming sound waves into electrical impulses are lost. The remaining tissue is characterized by the ‘flat epithelia’ leftover when these sensory cells have died [20, 21, 50, 56, 57]. This review will discuss leading strategies in the field to restore hearing, including the limitations of the current treatment options. We will also discuss previous attempts at cellular restoration within the inner ear, and the generation of genetic tools in a mouse model that could be vital to design novel treatment options for patients.

Anatomy of the Ear

The inner ear is split into two regions: the vestibular region and the cochlea. While the vestibular system provides a sense of balance, the cochlea is responsible for the sense of hearing as a result of a highly organized arrangement of specific cell types. These cell types can be split into three different categories:
the mechanosensory cells responsible for hearing, the supporting cells that help maintain these mechanosensory cells, and the spiral ganglion neurons (SGNs) that transmit the electrical signal produced by the mechanosensory cells to the hindbrain [11, 12, 13, 22, 56]. These mechanosensory cells are called hair cells. While these cells are not like the hair found on top of one’s head, they do have small tufts of stereocilia on their apical surface that resemble small hairs.

The hair cells and the supporting cells run the entire length of the cochlea in a region called the organ of Corti (OC) (Figure 1). The cochlea is a long, coiled tube with two ends: the apex and the base. During development, the OC is formed as a result of two opposing gradients of gene expression within the cochlea. In mice, around embryonic day 12 (E12), the cells that will eventually become the hair cells and supporting cells, collectively called prosensory cells, exit the cell cycle in the apex of the cochlea. These prosensory cells will continue to progressively exit the cell cycle towards the base until around E14. At E13.5, hair cells start to differentiate within the mid-base, which spreads to the apex and the far basal region over the next 3-4 days [14, 19, 30, 60].

In addition to these developmental gradients, an important distinction between the basal and apical sections of the cochlea is that the hair cells in these regions respond to different frequencies of sound. The cochlear base responds best to high-frequency waves, while the cochlear apex optimally responds to low-frequency waves [19, 60]. The SGNs that synapse onto hair cells in the base, mid-base, and apex help establish a tonotopic map in the hindbrain that directly corresponds to this gradient of frequencies [21, 22, 38, 71]. In cases of age-related hearing loss, most people first lose their sense of hearing in the region of higher frequencies before the hearing loss eventually progresses to the lower frequencies [28]. As such, the main function of hair cells is to respond to the physical movement of the stereocilia on their surface in order to release neurotransmitters to SGNs for sound transduction.

There are four rows of hair cells in the OC: one row of inner hair cells and three rows of outer hair cells (Figure 2).
The two types of hair cells are connected to two categories of SGNs, Type I SGNs and Type II SGNs, in order to serve two specific functions. Inner hair cells are connected to the Type I SGNs and are responsible for the transduction of sound information that will be consciously perceived, while the Type II SGNs synapse on the outer hair cells and are involved in a modulatory feedback loop [21, 22,]
Regardless of their function, both hair cell types are braced by five different types of supporting cells: inner and outer pillar cells, inner and outer phalangeal cells, and border cells. The inner and outer pillar cells delineate respectively the inner and outer hair cells and form a cavity within the OC, called the tunnel of Corti, filled with a sodium-rich and potassium-poor extracellular fluid known as perilymph [19, 73]. Perilymph is different from the potassium-rich and sodium-poor extracellular fluid that comes into direct contact with the stereocilia of the hair cell, which is known as endolymph. The ion composition of endolymph is so unique that it is not found elsewhere in the body, while the ion composition of perilymph is very similar to other extracellular fluids [73]. The strict segregation of these two fluids between the stereocilia and the body of both hair cell types is important for establishing the sensitive ion gradient required for both types of SGNs to fire electrical signals to the hindbrain. The bodies of inner hair cells come into contact with the perilymph because they are supported within the inner phalangeal cells. Likewise, the bodies of outer hair cells sit within the outer phalangeal cells. However, the stereocilia on the apical surface of both hair cell types come into contact with endolymph [19, 73].

The OC is sandwiched between two nonsensory regions called the greater epithelial ridge (GER) and the lesser epithelial ridge (LER). The inner and outer border cells delineate the sensory cells in the OC from the nonsensory GER and LER, respectively. The GER contains inner sulcus cells, while the LER contains the outer sulcus cells. As the name suggests, the single row of inner hair cells (IHCs) lies closest to the GER, while the three rows of outer hair cells (OHCs) lay closest to the LER. Together, the hair cells and supporting cells comprise the sensory region and are grouped as sensory cells, while the cells within the GER and LER are considered non-sensory (Figure 2).

The specific organization of the cells within the OC can be further visualized by immunohistochemistry for cell-specific proteins (Figure 2). The single row of IHCs and the three rows of OHCs can be seen via the Myosin VIIA antibody, which only labels hair cells. The actin stain Phalloidin binds to the stereocilia on the top of the hair cells. The merged image demonstrates how the hair cells are organized by type and stereocilia arrangement. Furthermore, the Sox2 antibody will bind to all supporting cells underneath the hair cells in order to visualize the bottom supporting layer of the OC. Together, these immunohistochemistry images establish a complete picture of the sensory cell types in the OC.

**Histological Effects During Hearing Loss**
Complete hearing loss is characterized by the presence of a flat epithelia absent of all sensory cells (Figure 3). After the sensory hair cells die, the inner and outer phalangeal cells expand to replace them. This process is called phalangeal scarring [21, 56, 57]. After phalangeal scar formation, all the sensory supporting cells are replaced by a flat environment caused when the GER and LER come together to fill this gap. The name ‘flat epithelia’ is inspired by the cellular morphology of the flat inner and outer sulcus cells from these regions joining together. Previous studies have shown that the process of creating a flat epithelium can range from a few days to several months [26, 57, 70]. This cellular

**Figure 3: Flat Epithelia via Phalangeal Scarring** (A) Cartoon depiction of normal cell types in OC. (B) Phalangeal scarring occurs when the hair cells die and the inner and outer phalangeal cells expand to fill the gap. (C) Between a few days to a few months, the rest of the sensory cells die after phalangeal scarring. They are replaced by the inner and outer sulcus cells in the GER and LER joining together to fill the gap.
environment has not been well-characterized, but it could be pivotal in hearing restoration studies since it is the phenotype seen in many hearing loss patients [14, 20, 21, 56, 57, 70]. Gene expression is strictly maintained in the GER and LER and can be visualized via in situ hybridization for the genes Fgf10 and Bmp4, respectively (Figure 3) [34, 52]. Previous studies have attempted to regenerate hair cells by converting supporting cells or nearby non-sensory GER cells into hair cells through the ectopic expression of genes known to be important for hair cell formation [1, 5, 24, 36, 42]. However, as shown in Figure 3, there are no differentiated supporting cell types within the flat epithelia, and because of this, these approaches would not easily translate into an effective treatment option for hearing loss.

Aside from gene therapy, an alternative approach to treat hearing loss is the injection of cells into the inner ear with the expectation that they would survive and proliferate into sensory cells after the addition of prosensory factors. The field of regenerative medicine has a history of using stem cells in order to regenerate tissues, but the cochlea poses a unique problem due to the extracellular fluid that comes into contact with the OC. First, the endolymph creates a toxic environment for any non-native cell types due to the high potassium concentration. Second, there is no stem cell niche in the inner ear. As a result, previous studies have shown that human embryonic stem cells did not survive more than one day post-injection into the inner ear [32, 46]. Other studies attempted to inject HeLa cells into the ear due to their more robust nature and potential to survive in this hostile cellular environment. While these cells survived up to a week post-injection, these studies were also unsuccessful [31, 32, 50]. Another study attempted to inject neural stem cells into the cochlea with the intention to generate functional SGNs. A small number of cells were generated that resembled satellite cells and Type I SGNs, but these were not maintained long-term [51]. These studies reiterated the specificity of the toxic micro-environment of the cochlea to non-native cells but also demonstrated that the cochlea might provide signals for the differentiation of the various cell types. Due to this unique problem, current approaches in the field to treat hearing loss predominantly focus on gene therapy and the manipulation of cell types in the cochlea.
A novel approach to treat hearing loss is the use of gene therapy in order to generate functional hair cells. Studying the development of the cochlea in a mouse model allows for the identification of genes necessary to generate hair cells normally. This is especially important due to the lack of a stem cell niche to study in the inner ear. Therefore, genes discovered in these studies can be manipulated in order to generate new hair cells in hearing loss models. Previous studies have identified several transcription factors that influence the development of different cell types in the OC. *Atoh1*, a basic helix-loop-helix (bHLH) transcription factor,
is found to be necessary for the differentiation of both inner and outer hair cells (Figure 4) [5, 6, 75]. Due to its essential role within hair cells, Atoh1 has been the primary gene of interest for several studies attempting to regenerate hair cells [5, 6, 24, 36, 47, 48, 75]. Furthermore, Atoh1 lends itself to manipulation because it has two different enhancers and auto-regulates its own expression by binding to one of its own enhancers [47, 48]. Another important transcription factor called Sox2 is expressed in all the supporting cell types discussed in Figure 2, including the inner and outer border cells, phalangeal cells, and pillar cells. It has also been shown that the SOX2 protein acts in a physical complex with EYA1 and SIX1 in order to regulate ATOH1 expression in the hair cells by physically binding to the second Atoh1 enhancer [1, 12, 55]. This suggests that Atoh1 and Sox2 may work together in order to create the specific organization of hair cells and supporting cells. However, it is not that simple. Other studies have demonstrated that Delta-Notch signaling regulates expression of Hes/Hey genes that may also play a role in determining the specific patterning between hair cells and supporting cells [4, 8, 42, 53].

In addition, previous studies have shown that there are other transcription factors required for long-term maintenance of sensory cell types. For example, the zinc-finger transcription factor called Gata3 is particularly interesting due to the variance in its expression throughout both embryonic and postnatal development [9, 10, 23, 34]. Gata3 is highly expressed alongside Sox2 in the supporting cells, but both types of hair cells have low residual levels of Gata3 expression at postnatal day 0 (P0) [34, 75]. While Gata3 is originally expressed in both the GER and LER in early embryonic development, it is important to note that Gata3 is not expressed in these regions at P0. Because Gata3 is expressed in multiple cell types at the early stages of development, but is highly restricted later on, it has been suggested that Gata3 may be modifying the expression of other transcription factors for the long-term survival of sensory cell types [21].

As previously mentioned, without SGN presents, these cell types will not be functional. NeuroD1, a bHLH transcription factor in the same family as Atoh1, is specifically expressed in the SGNs that synapse onto these hair cells [21, 22, 38, 49]. It is vital to note that bHLH transcription factors often form complex regulatory networks in sensory systems, which is especially true in the ear [66]. Since NeuroD1 represses Atoh1 expression within SGNs, there is a negative feedback loop between these two transcription factors in the ear [49]. It has been suggested that the expression of NeuroD1 and the subsequent downregulation of Atoh1 is key in determining which prosensory cells will become SGNs instead of hair cells, or vice versa [11, 13, 43]. Expression of Atoh1 is still required for proneurosensorial cells to develop [5]. Another bHLH transcription factor,
Neurog1, has a strong regulatory role and also seems to play an overlapping role with Atoh1 in the ear. Early in development, at E 9.5, the prosensory progenitor cells that will eventually become SGNs express Neurog1, and previous studies have reported that Neurog1 expression increases the progenitor population and cell expansion [37, 67, 71]. Interestingly, in the absence of Atoh1 or Neurog1 expression, no hair cells or SGNs form [5, 37]. It has also been shown that the replacement of one Atoh1 gene with the Neurog1 gene will partially rescue this phenotype, implying that these two transcription factors play a similar role even if they are not a complete substitute for each other. While Neurog1 has a regulatory role in order to increase the proneurosensory progenitor cells, it also acts in a network with NeuroD1 and Atoh1 in order to influence which cells ultimately become SGNs or hair cells. In conclusion, hair cell formation requires a network of several transcription factors throughout development combined with different gene expression gradients that are eventually restricted to certain cell types.

Current Treatment Options

There are typically only two types of treatment available to patients who suffer from hearing loss: cochlear implants and hearing aids. Cochlear implants do not rely on the hair cells, but rather directly stimulate the SGNs that relay sound information from the ear to the hindbrain. These SGNs make up part of cranial nerve VIII [78]. In order to work, cochlear implants require at least some functional SGNs [72]. Interestingly, it has been shown in previous studies that SGNs require support from the hair for survival [3, 29, 69, 71]. As a result, SGNs may be lost long-term in patients with cochlear implants, rendering this treatment a temporary one. Another practical limitation of cochlear implants includes the lack of perception of music. While patients with cochlear implants can comprehend speech and detect simple rhythms under normal hearing conditions, this technology does not appropriately account for the perception of pitch, timbre, or melody recognition [41]. Most patients who utilize cochlear implants report that they no longer find the sound of music as enjoyable or maintain their personal genre preference [41].

In contrast to cochlear implants, hearing aids amplify the sound to assist with partial hearing loss, but they still require at least a small population of functional hair cells in addition to SGNs in order for sound to be relayed to the brain and interpreted [18, 33, 40]. While this treatment option does allow patients to listen to music, the use of hearing aids carries a heavy social stigma along with limitations in noisy environments. This prevents most people who need hearing aids from actually using them. Previous studies have reported that almost 80% of people aged 54-70 who need hearing aids intentionally choose not to use them [40]. Further investigations have shown that these patients attribute external societal pressures in the media, self-perceived judgment, and struggles with ageism and vanity as reasons for declining this treatment option [74]. The problem of hearing loss and lack of effective treatments is further compounded by the increase in average lifespan and continued rapid growth in the aging
Therefore, the regeneration of sensory hair cells would be preferable to the currently available treatment options.

**Flat Epithelial Models of Hearing Loss**

Based on previous studies and other attempts to restore hearing, gene manipulation within the flat epithelia seems to have the most potential for the successful regeneration of hair cells. All types of hearing loss are characterized by the loss of sensory cells, which results in a flat epithelium. However, in order to perform experiments within a flat epithelia model, the model itself must be characterized. There are two methods to create a flat epithelium: 1) the *Pou4f3-DTR* genetic mouse line and 2) antibiotic exposure. Uniquely, mice are immune to diphtheria because their cells do not have the receptor on the plasma membrane for the diphtheria toxin (DTX). *Pou4f3* is a gene that is expressed early in hair cell development (Figure 5). In this genetic model, the cells that express *Pou4f3* will also express the human receptor for the diphtheria toxin. After injection with DTX, only the hair cells will die because only these cells within the inner ear express the receptor [16, 62]. Using this technique to target the hair cells specifically is similar to the method in the paper that first reported phalangeal scarring [26, 50, 56, 57, 62]. The supporting cells are unharmed but are destroyed as the enlarged phalangeal scars are replaced by the inner and outer sulcus cells of the GER and LER, respectively. Characterization of the gene expression and cell
types in the flat epithelia will be possible since this model is easily repeated with a single DTX injection. However, another option to create the flat epithelia is to inject with antibiotics that have been shown to have ototoxic properties. For example, gentamicin and neomycin are commonly-prescribed antibiotics, but both have been shown to kill hair cells and sensory cells in the cochlea after high doses or prolonged use [17, 58, 64, 76, 77]. Additionally, commonly-prescribed chemotherapeutic drugs like cisplatin have also demonstrated ototoxicity at high levels and after long-term use [61, 65]. The advantage of the diphtheria injection model is that it should affect all the hair cells of the OC, while the model created through antibiotics can be patchy in the OC or have different effects that change along the length of the cochlea.

Once the field has a more complete understanding of the flat epithelia, gene manipulation in this environment will be the next step in order to regenerate a functional OC. One gene that may be important in altering the flat epithelia of hearing loss patients is Gata3 (Figure 5). As previously described, Gata3 is widely expressed throughout the early development of the ear, but it becomes restricted at P0 and later ages [9, 10]. It is known that differential levels of transcription factors determine the level of their function, which raises another interesting question: why does Gata3 have varying levels of expression throughout the ear? The implication is that Gata3 either has several different roles throughout development or that Gata3 plays a specific role that is restricted to certain prosensory regions as the cells within the ear differentiate. One way to tease these questions apart is to determine the effects of Gata3 within the flat epithelia. It has been previously shown that there is a constant low level of Gata3 expression in cells that border the OC within the GER and LER, which would be present in a flat epithelia disease model created from either the DTR approach or the antibiotic method [57]. Furthermore, Gata3 has been shown to be necessary for the proper function of ATOH1, both initially and long-term [10, 75]. Regarding the second method, it has been previously suggested that overexpression of Gata3 may have a protective function against antibiotic ototoxicity because of its role in long-term maintenance of ectopic and regenerated hair cells. Regardless of the method, the field will benefit from the complete characterization of the flat epithelia model, with and without phalangeal scarring. Furthermore, a more complete understanding of the role of Gata3 in the development and long-term maintenance of sensory cell types in the OC can direct studies for other novel treatments.

**Conclusion**

This paper discussed strategies within the field to restore hearing in the context of a flat epithelia model that could alleviate the limitations of current treatment options and avoid the obstacles associated with cellular restoration attempts. A review of important genes required for the development, differentiation, and long-term maintenance of the OC demonstrates that any future direction to regenerate hair cells necessitates a better understanding of the gene
expression as a whole. Specifically, regeneration of hair cells requires a more complete understanding of the genes and cells present during the phalangeal scarring process and within the resulting flat epithelia environment. As previously suggested, this understanding could be achieved through the development of a characterized flat epithelia, followed by complete regeneration of various sensory cell types in the correct location that respond appropriately to noise stimuli [21]. Future directions will likely focus on the manipulation of genes that influence hair cell development, for example, *Gata3*, within this flat environment. This knowledge will assist in the design of treatments for hearing loss that does not require certain cell types, like cochlear implants or hearing aids. Furthermore, these novel treatment options can target the flat epithelia remaining in all types of hearing loss.
Bibliography


