The Hilltop Review

Volume 12
Issue 1 *Fall*

Article 3

December 2019

Strategies to Restore Hearing

Sydney N. Sheltz-Kempf
Western Michigan University

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

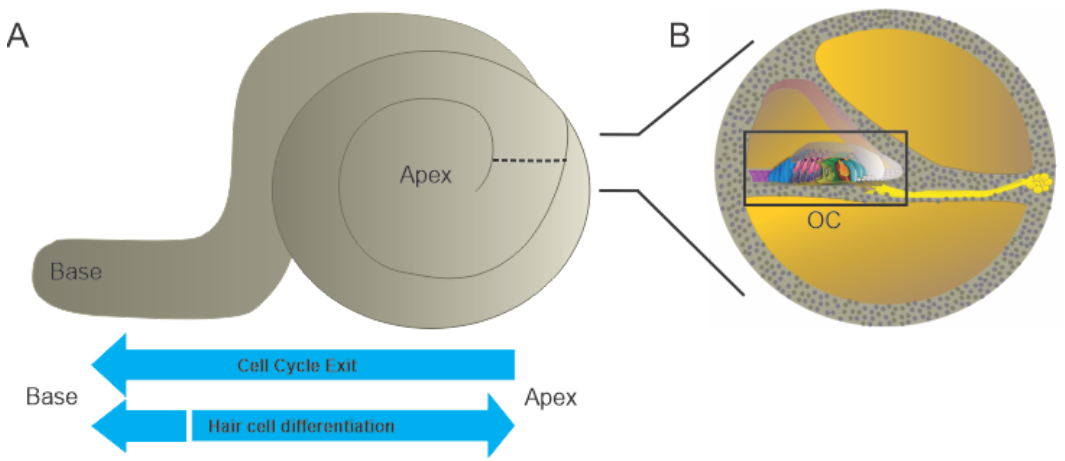


Figure 1 Cochlear Cross-Section: (A) A cartoon representation of the basal-to-apical organization of the cochlea. Cell cycle exit occurs from apex to base, while hair cell differentiation begins into the mid-basal region and progresses outwardly in both directions. (B) Cross-section of cochlea depicting OC within the cochlear duct, in reference to scala vestibule and scala tympani.

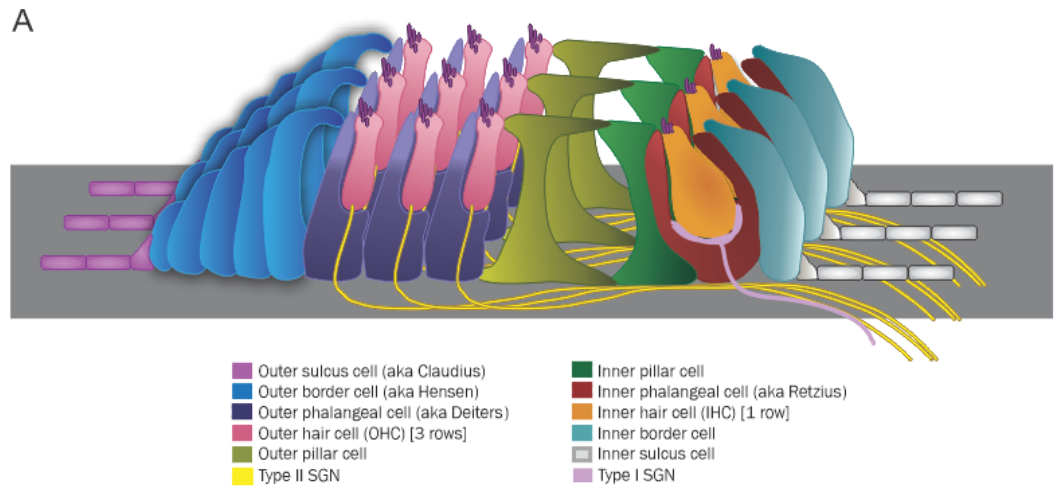
The inner ear is split into two regions: the vestibular region and the cochlea. While the vestibular system provides a sense of balance, the cochlea is responsible for the sense of hearing as a result of a highly organized arrangement of specific cell types. These cell types can be split into three different categories:

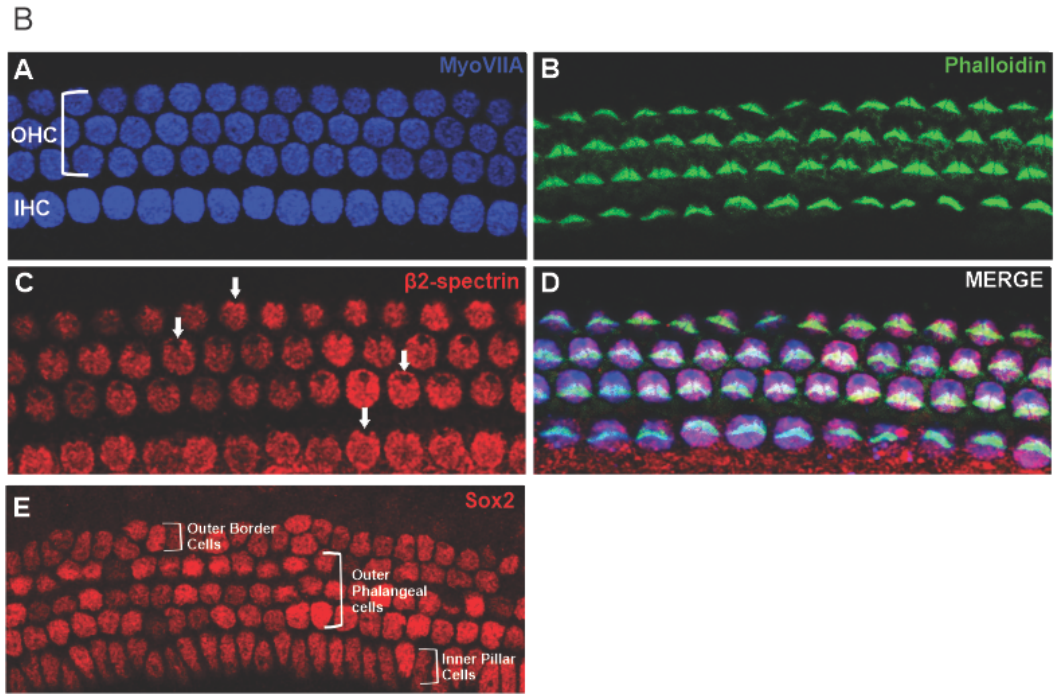
the mechanosensory cells responsible for hearing, the supporting cells that help maintain these mechanosensory cells, and the spiral ganglion neurons (SGNs) that transmit the electrical signal produced by the mechanosensory cells to the hindbrain [11, 12, 13, 22, 56]. These mechanosensory cells are called 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).





(continued) Figure 2 Cell Types in OC: (A) Drawing depicting cell types in the OC. The GER contains inner sulcus cells (grey), and the LER contains the outer sulcus cells (magenta). The GER is separate from the OC by inner border cells (pale blue) while the LER is separate the OC by outer border cells (turquoise). A single row of IHC (orange) lies closest to the GER, while three rows of OHC (pink) lay closest to the LER. Type I SGN (lilac) synapse onto IHC supported by inner phalangeal cells (red) and Type II SGN (yellow) synapse onto OHC supported by outer phalangeal cells (purple). The tunnel of Corti is formed by inner pillar cells (dark green) and outer pillar cells (yellow-green). (B) IHC of OC cell types. MyosinVIIA (blue) stains hair cells, and Phalloidin (green) stains stereocilia. Beta2-spectrin (red, C) marks HC polarity, while *Sox2* (red, E) stains supporting cells. The merged panel in D visualizes normal hair cell polarity.

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,

38]. 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

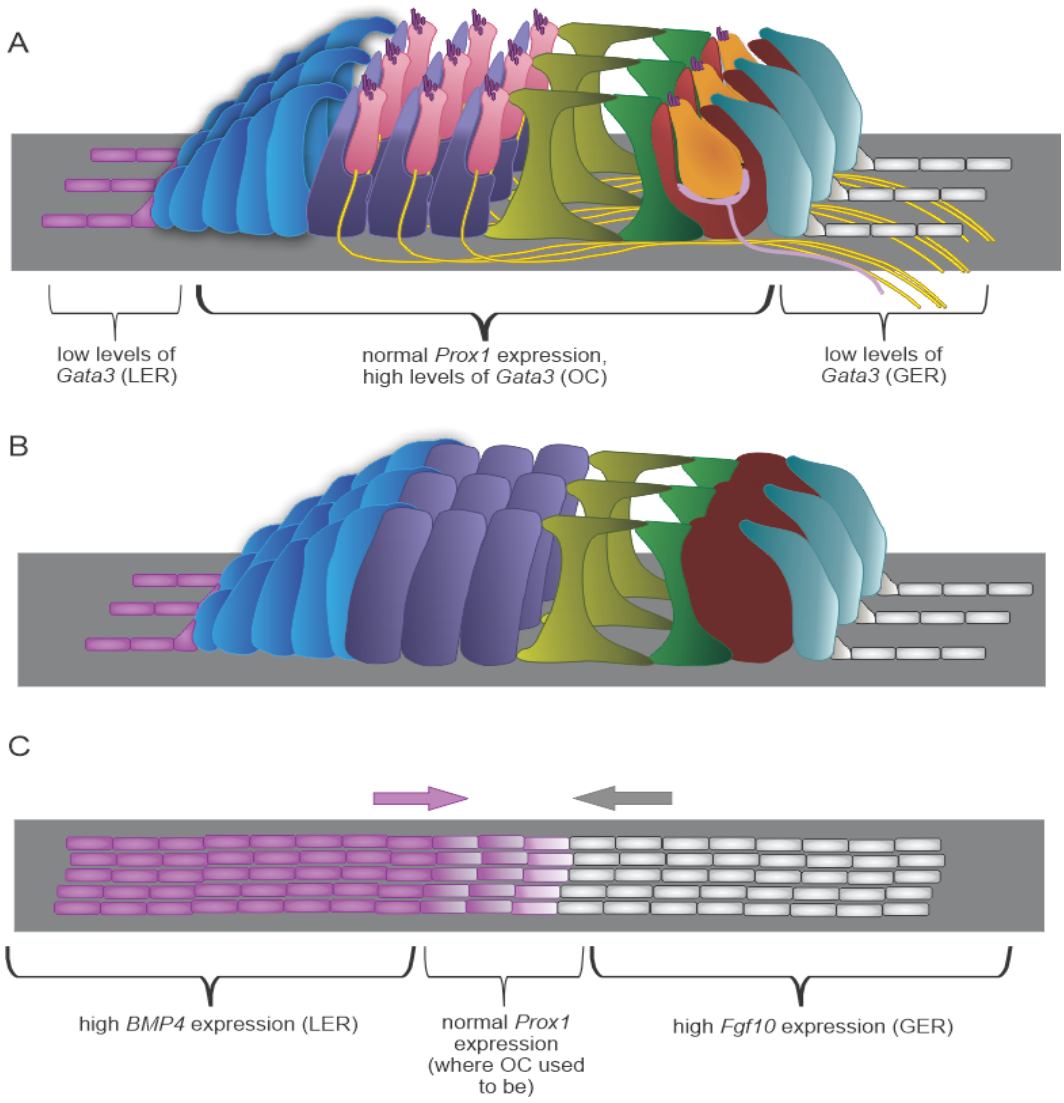


Figure 3: Flat Epithelia via Phalangeal Scarring (A) Cartoon depiction of normal cell types in OC. (B) Phalangeal scarring occurs when the hair cells die and the inner and outer phalangeal cells expand to fill the gap. (C) Between a few days to a few months, the rest of the sensory cells die after phalangeal scarring. They are replaced by the inner and outer sulcus cells in the GER and LER joining together to fill the gap.

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

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.

Gene Expression

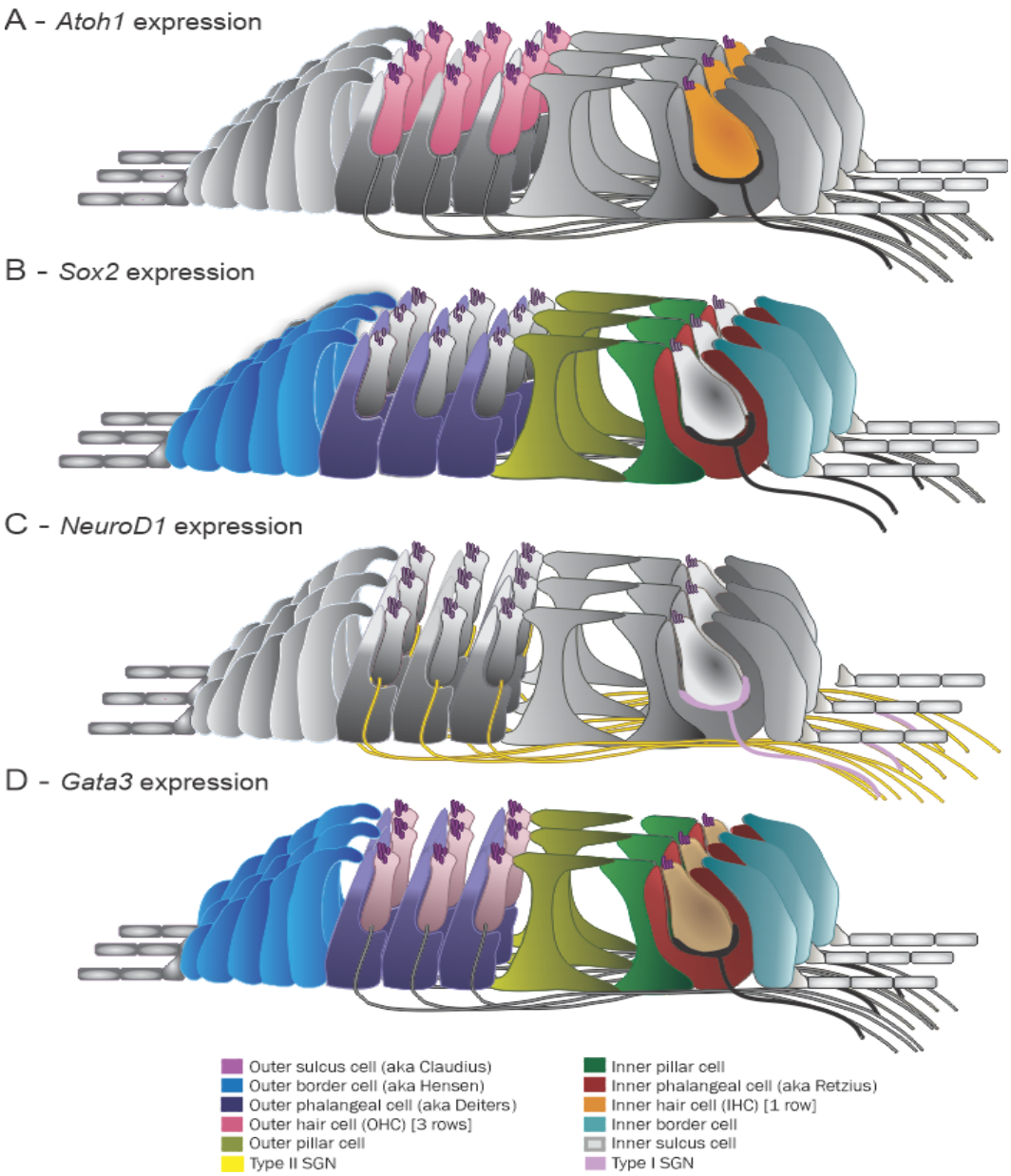


Figure 4 Gene Expression within Specific Cell Types in OC: continued on next page

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,

(continued) Figure 4 Gene Expression within Specific Cell Types in OC at P0 (A) *Atoh1* is expressed in both inner and outer hair cells (B) *Sox2* is expressed in all supporting cell types (C) *NeuroD1* is only expressed in SGN and (D) *Gata3* is highly expressed in supporting cells but expressed in hair cells in lower levels. *Neurog1* is not shown because it is expressed in the proneurosensory domain and not in these cell types at P0.

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 proneurosensory 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

population[14, 44]. Therefore, the regeneration of sensory hair cells would be preferable to the currently available treatment options.

Flat Epithelial Models of Hearing Loss

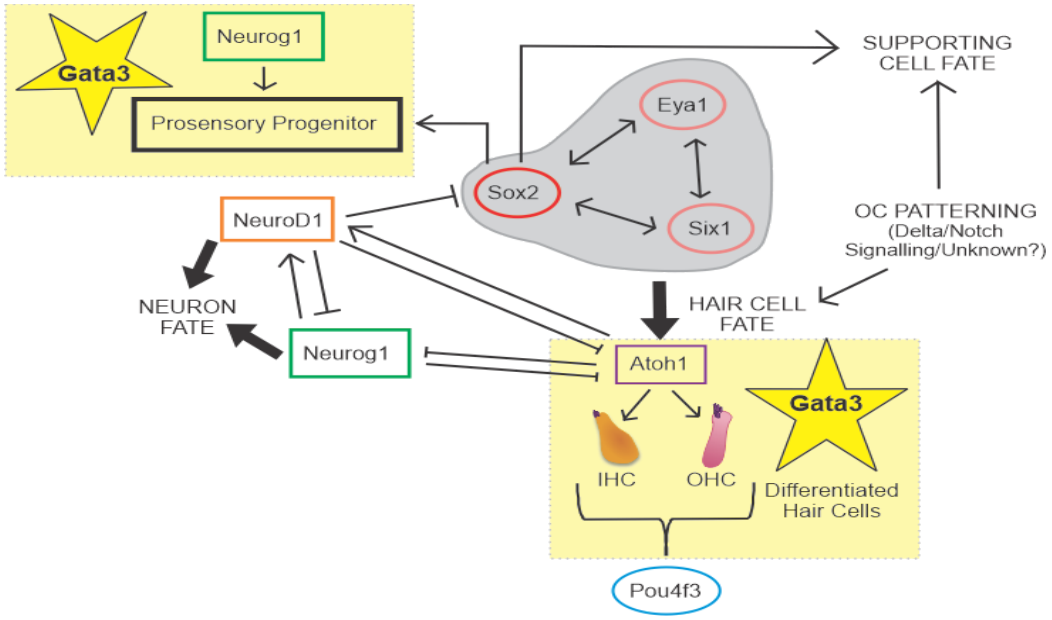


Figure 5 Transcription Factor Network in Inner Ear Development and Differentiation Prosensory progenitors can be pushed towards a neuronal or hair cell fate based on the expression of different transcription factors. Expression of *NeuroD1* and *Neurog1* will trigger neuronal fate, while *Atoh1* expression is driven by the SOX2/EYA1/SIX1 complex for a hair cell fate. Patterning between hair cell and supporting cells is determined by Delta/Notch signaling pathways, and possibly other factors. The yellow boxes and stars indicate possible roles for *Gata3* in either early development in combination with *Neurog1*, or with long-term maintenance and survival that cooperates with the expression of *Pou4f3* in mature hair cells.

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

1. Ahmed, M., Wong, E. Y., Sun, J., Xu, J., Wang, F. and Xu, X. (2012). Eya1-Six1 interaction is sufficient to induce hair cell fate in the cochlea by activating *Atoh1* expression in cooperation with *Sox2*. *Dev Cell*. **2**, 377-390.
2. Ahmed, R. M., Hannigan, I. P., MacDougall, H. G., Chan, R. C. and Halmagyi, G. M. (2012). Gentamicin ototoxicity: a 23-year selected case series of 103 patients. *Med. J Aust*. **701**, 701-704.
3. Barclay, M., Ryan, A. F. and Housley, G. D. (2011). Type I vs. type II spiral ganglion neurons exhibit differential survival and neuritogenesis during cochlear development. *Neural Dev.* **6**, 33.
4. Benito-Gonzalez, A. and Doetzlhofer, A. (2014). Hey1 and hey2 control the spatial and temporal pattern of mammalian auditory hair cell differentiation downstream of hedgehog signaling. *J Neurosci*. **34**,12865–76.
5. Bermingham, N. A., Hassan, B. A., Price, S. D., Vollrath, M. A., Ben-Arie, N., Eatock, R. A., Bellen, H. J., Lysakowski, A., and Zoghbi, H. Y. (1991). Math1: An essential gene for the generation of inner ear hair cells. *Science.* 1999. **284**, 1837-1841.
6. Cai, T., Seymour, M., Zhang, H., Pereira, F., and Groves, A. (2013). Conditional deletion of *Atoh1* reveals distinct critical periods for survival and function of hair cells in the organ of Corti. *J Neurosci*. **33**, 10110-10122.
7. Chumak, T., Bohuslavova, R., Macova, I., Dodd, N., Bucklova, D., Fritzsch, B., Syka, J. and Pavlinkova, G. (2016). Deterioration of the medial olivocochlear efferent system accelerates age-related hearing loss in Pax2-Isl1 transgenic mice. *Molec. Neurobio*. **53**, 2368-2383.
8. Doetzlhofer, A., Basch, M. L., Ohyama, T., Gessler, M., Groves, A. K. and Segil, N. (2009). Hey2 regulation by FGF provides a Notch-independent mechanism for maintaining pillar cell fate in the organ of Corti. *Dev Cell*. **16**, 58–69.
9. Duncan, J. S., and Fritzsch, B. (2013). Continued expression of *Gata3* is necessary for cochlear neurosensory development. *PLoS One.* **8**, 62046.
10. Duncan, J. S., Lim, K. C., Engel, J. D. and Fritzsch, B. (2011). Limited inner ear morphogenesis and neurosensory development are possible in the absence of *Gata3. Int J Dev Biol.* **55,** 297-303.
11. Durruthy-Durruthy, R., Gottlieb, A., Hartman, B. H., Waldhaus, J., Laske, R. D., Altman, R. and Heller, S. (2014). Reconstruction of the mouse otocyst and early neuroblast lineage at single-cell resolution. *Cell*. **157**, 964–78.
12. Dvorakova, M., Jahan, I., Macova, I., Chumak, T., Bohuslavova, R., Syka, J., Fritzsch, B. and Pavlinkova, G. (2016). Incomplete and delayed *Sox2* deletion defines residual ear neurosensory development and maintenance. *Sci Rep*. **6**, 38253.

13. Fariñas, I., Jones, K. R., Tessarollo, L., Vigers, A. J., Huang, E., Kirstein, M., de Caprona, D. C., Coppola, V., Backus, C., Reichardt, L. F. and Fritzsch, B.(2001). Spatial shaping of cochlear innervation by temporally regulated neurotrophin expression. *J Neuroscience*. **21**, 6170–80.
14. Géléoc, G. S. and Holt, J. R. (2014). Sound strategies for hearing restoration. *Science*. **344**, 1241-062.
15. Gibson, W. S. (1967). Deafness due to orally administered neomycin. *Arch Otolaryngol*. **86**, 63-165.
16. Golub, J., Tong, L., Ngyuen, T., Hume, C., Palmiter, R., Rubel, E. and Stone, J. (2012). Hair cell replacement in adult mouse utricles after targeted ablation of hair cells with diphtheria toxin. *J Neurosci*. **32**, 15093-15105.
17. Govaerts, P. J., Claes, J., van de Heyning, P. H., Marquet, P. G. and De Broe, M. E. (1990). Aminoglycoside-induced ototoxicity. *Toxicol Lett*. **52**, 227-251.
18. Hoppe, U. and G. Hesse. (2017). Hearing aids: Indications, technology, adaptation, and quality control. *GMS Curr Top Otorhinolaryngol Head Neck Surg*. **16**.
19. Iurato, S. and Zellforsch, Z. (1962). Submicroscopic structure of the membranous labyrinth**.** *Zeitschrift für Zellforschung und Mikroskopische Anatomie.* **56**, 40-96.
20. Izumikawa, M., Batts, S. A., Miyazawa, T., Swiderski, D. L. and Raphael, Y. (2008). Response of the flat cochlear epithelium to forced expression of *Atoh1*. *Hear Res.* **240**, 52-56.
21. Jahan, I., Pan, N., Elliott, K. and Fritzsch, B. (2015). Gaps in comprehension of ear development impede successful human hearing organ restoration. *Bioessays*. **37**, 1016-1027.
22. Jahan, I., Pan, N., Kersigo, J. and Fritzsch, B. (2010). *NeuroD1* suppresses hair cell differentiation in ear ganglia and regulates hair cell subtype development in the cochlea. *PLoS One*. **5**, e11661.
23. Karis, A., Pata, I., van Doorninck, J. H., Grosveld, F., de Zeeuw, C. I., de Caprona, D. and Fritzsch, B. (2001). Transcription factor *GATA3* alters pathway selection of olivocochlear neurons and affects morphogenesis of the ear. *J Comp Neurol*. **429**, 615-620.
24. Kelly, M. C., Chang, Q., Pan, A., Lin, X. and Chen, P. (2012). *Atoh1* directs the formation of sensory mosaics and induces cell proliferation in the postnatal mammalian cochlea in vivo. *J Neurosci*. **32**, 6699-6710.
25. Kiernan, A. E., Pelling, A. L., Leung, K. K., Tang, A. S., Bell, D. M., Tease, C., Lovell-Badge, R., Steel, K. P., and Cheah, S. (2005). *Sox2* is required for sensory organ development in the mammalian inner ear. *Nature*, 2005. **434**, 1031-1035.
26. Kim, Y. H., and Raphael, Y. (2007). Cell division and maintenance of epithelial integrity in the deafened auditory epithelium. *Cell Cycle*. **6**, 612-619.

27. Korver, A. M., Smith, R. J., Van Camp, G., Schleiss, M. R., Bitner-Glindzicz, M. A., Lustig, L. R., Usami, S. I. and Boudewyns, A. N. (2017). Congenital hearing loss. *Nat Rev Dis Primers*. **12**, 16094.
28. Kujawa, S. and Liberman, M. (2006). Acceleration of age-related hearing loss by early noise exposure: Evidence of misspent youth. *J Neurosci*. **26**, 2115-2123.
29. Kujawa, S. G. and Liberman, M. C. (2009). Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J. Neurosci.* **29**, 14077–14085.
30. Lee Y. S., Liu F. and Segil N. (2006). A morphogenetic wave of p27Kip1 transcription directs cell cycle exit during organ of Corti development. *Development*. **133**, 2817–2826.
31. Lee, M. and Park, Y. (2018). Potential of gene and cell therapy for inner ear hair cells. *BioMed Research Int*. **2**, 11.
32. Lee, M. L., Hackelberg, S., Green, K. L, Lunghamer, K. G, Kurioka, T., Loomis, B. R. Swiderski, D. L, Duncan, R. K. and Raphael, Y. (2017). Survival of human embryonic stem cells implanted in the Guinea pig auditory epithelium. *Sci Rep*. 7.
33. Lesica, N. (2018). Hearing aids: Limitations and opportunities. *The Hear J*. **71**, 43-46.
34. Lillevali, K., Haugas, M., Matilainen, T., Pussinen, C., Karis, A. and Salminen, M. (2006). *Gata3* is required for early morphogenesis and Fgf10 expression during otic development. *Mech Dev.* **123,** 415-429.
35. Lin, F. R., Niparko, J. K. and Ferrucci, L. (2011). Hearing loss prevalence in the united states. *Arch Int Med*. **171**, 1851-1853.
36. Liu, Z., Dearman, J. A., Cox, B. C., Walters, B. J., Zhang, L., Ayrault, O., Zindy, F., Gan, L., Roussel, M. F., and Zuo, J. (2012). Age-dependent in vivo conversion of mouse cochlear pillar and deiters' cells to immature hair cells by *Atoh1* ectopic expression. *J Neurosci*. **32**, 6600-10.
37. Ma, Q., Chen, Z., del Barco Barrantes, I., de la Pompa, J. L. and Anderson. D. J. (1998). Neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. *Neuron*. **20**, 469–482.
38. Macova, I., Pysanenko, K., Chumak, T., Dvorakova, M., Bohuslavova, R., Syka, J., Fritzsch, B. and Pavlinkova, G. (2019). *NeuroD1* is essential for the primary tonotopic organization and related auditory information processing in the midbrain. *J Neurosci*. **39**, 984-1004.
39. Mathers, C., Smith, A. W. and Concha, M. (2000). Global burden of hearing loss in the year 2000. *World Health Rep*.
40. McCormack, A. and Fortnum, H. (2013). Why do people fitted with hearing aids not wear them? *Int J Audiol*. **52**, 360-368.
41. McDermott, H. (2004). Music perception with cochlea implants: A review. *Trends Amplif*. **8**, 49-82.

42. McGovern, M., Zhou, L., Randle, M., and Cox, B. (2012). Spontaneous Hair Cell Regeneration Is Prevented by Increased Notch Signaling in Supporting Cells. *Front. Cell. Neurosci*. **12,** 1662.
43. Morris, J. K., Maklad, A., Hansen, L. A., Feng, F., Sorensen, C., Lee, K. F., Macklin, W. B, and Fritzsch, B. (2006). A disorganized innervation of the inner ear persists in the absence of ErbB2. *Brain res*. **1091**,186–99.
44. Müller, U. and Barr-Gillespie, P. G. (2015). New treatment options for hearing loss. *Nat Rev Drug Discovery*. **14**, 346-365.
45. National Institute on Deafness and other Communication Disorders (NIDCD), "Age-related hearing loss" (Publication 97-4235, NIH, 2016; https://www.nidcd.nih.gov/health/age-related-hearing-loss).
46. Oshima, K., Shin, K., Diensthuber, M., Peng, A. W., Ricci, A. J.,and Heller, S. (2010). Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells. *Cell*. **141**, 704– 716.
47. Pan, N., Jahan, I., Kersigo, J., Duncan, J. S., Kopecky, B. and Fritzsch, B. (2012). A novel *Atoh1* "self-terminating" mouse model reveals the necessity of proper *Atoh1* level and duration for hair cell differentiation and viability. *PLoS One*. **7**, 30358.
48. Pan, N., Jahan, I., Kersigo, J., Kopecky, B.,Santi, P., Johnson, S., Schmitz, H. and Fritzsch, B. (2011). Conditional deletion of *Atoh1* using Pax2-Cre results in viable mice without differentiated cochlear hair cells that have lost most of the organ of Corti. *Hear Res*. **275**, 66-80.
49. Pan, N., Jahan, I., Lee, J. E. and Fritzsch, B. (2009). Defects in the cerebella of conditional *NeuroD1* null mice correlate with effective Tg(*Atoh1*-cre) recombination and granule cell requirements for *NeuroD1* for differentiation. *Cell Tissue Res*. **337**, 407–28.
50. Park, Y. H., Wilson, K. F., Ueda, Y., Tung Wong, H., Beyer, L. A., Swiderski, D. L., Dolan, D. F., and Raphael, Y. (2014). Conditioning the cochlea to facilitate survival and integration of exogenous cells into the auditory epithelium. *Mol Ther*. **22**, 873–880.
51. Parker, M., Corliss, D., Gray, B., Anderson, J., Bobbin, R., Snyder, E. and Cotanche, D. (2007). Neural stem cell injected into the sound-damaged cochlea migrate throughout the cochlea and express markers of hair cells, supporting cells, and spiral ganglion cells. *Hear Res*. **2**, 29-43.
52. Pauley, S., Wright, T. J., Pirvola, U., Ornitz, D., Beisel, K. and Fritzsch, B. (2003). Expression and function of FGF10 in mammalian inner ear development. *Dev Dynamics*. **227**, 203-15.
53. Petrovic, J., Formosa-Jordan, P., Luna-Escalante, J. C., Abelló, G., Ibañes, M., Neves, J. and Giraldez, F. (2014). Ligand-dependent Notch signaling strength orchestrates lateral induction and lateral inhibition in the developing inner ear. *Development*. **141**, 2313–24.
54. Powers, R. J., Kulason, S., Atilgan, E., Brownell, W. E., Sun, S. X., Barr-Gillespie, P. G. and Spector, A. A. (2014). The Local Forces Acting on the Mechanotransduction Channel in Hair Cell Stereocilia. *Biophysical J*. **106**, 2519–2528.

55. Puligilla, C. and Kelley, M. W. (2017). Dual role for *Sox2* in specification of sensory competence and regulation of *Atoh1* function. *Dev Neurobio*. **77**, 3-13.
56. Raphael, Y. and Altschuler, R. A. (2003). Structure and innervation of the cochlea. *Brain research bulletin*. **60**, 397–422.
57. Raphael, Y., Kim, Y., Osumi, Y. and Izumikawa, M. (2007). Non-sensory cells in the deafened organ of Corti: Approaches for repair, *Int J Dev Bio.* **51**, 649-654.
58. Richardson, G. P. and Russell, I. J. (1991). Cochlear cultures as a model system for studying aminoglycoside induced ototoxicity. *Hear Res.* **53**, 293-311.
59. Robles, L. and Ruggero, M. (2001). Mechanics of the mammalian cochlea. *Physiol Rev*. **81**, 1305-1352.
60. Ruben, R. (1967). Development of the inner ear of the mouse: a radioautographic study of terminal mitoses. *Acta Otolaryngol*. **220**, 221-224.
61. Rybak, L. P., Mukherjea, D., Jajoo, S. and Ramkumar, V. (2009). Ciplatin ototoxicity and protection: clinical and experimental studies. *Tohoku J Exp Med*. **219**, 177-186.
62. Saito, M., Iwawaki, T., Taya, C., Yonekawa, H., Noda, M., Inui, Y., Mekada, E., Kimata, Y., Tsuru, A. and Kohno, K. (2001). Diphtheria toxin receptor-mediated conditional and targeted cell ablation in transgenic mice. *Nat Biotechnol*. **19**, 746-50.
63. Schimmang, T., Tan, J., Müller, M., Zimmermann, U., Rohbock, K., Kôpschall, I., et al. (2003). Lack of Bdnf and TrkB signalling in the postnatal cochlea leads to a spatial reshaping of innervation along the tonotopic axis and hearing loss. *Development*. **130**, 4741–4750.
64. Selimoglu, E. (2007). Aminoglycoside-induced ototoxicity. *Curr Pharm Des*. **13**, 119-126.
65. Sheth, S., Mukherjea, D., Rybak, L. P. and V. Ramhumar (2017). Mechanisms of Ciplatin-induced ototoxicity and otoprotection. *Front Cell Neurosci*. **11**, 3389.
66. Skinner, M. K., Rawls, A., Wilson-Rawls, J., Roalson, E. H. (2010). Basic helix-loop-helix transcription factor gene family phylogenetics and nomenclature. Differentiation; research in biological diversity. **80**, 1-14.
67. Song, Z., Jadali, A., Fritzsch, B. and Kwan, K. Y. (2017). *Neurog1* regulates CDK2 to promote proliferation I otic progenitors. *Stem Cell Rep*. **9**, 1516–1529.
68. Spoendlin, H. (1984). Factors inducing retrograde degeneration of the cochlear nerve. *Ann. Otol. Rhinol. Laryngol*. **Suppl.112**, 76–82.
69. Srinivasan, R., Dillard, M., Lagutin, O., Lin, F., Tsai, S., Tsai, M., Samokhvalov, I. and Oliver, G. (2007). Lineage tracing demonstrates the venous origin of the mammalian lymphatic vasculature. *Genes Dev*. **21**, 2422-2432.

70. Sugawara, M., Corfas, G. and Liberman, M. C. (2005). Influence of supporting cells on neuronal degeneration after hair cell loss. *J. Assoc. Res. Otolaryngol.* **6**, 136–147.
71. Sun, W. and Salvi, R. J. (2009). Brain derived neurotrophic factor and neurotrophic factor 3 modulate neurotransmitter receptor expressions on developing spiral ganglion neurons. *Neuroscience*. **164**, 1854–1866.
72. Svirsky, M. (2017). Cochlear implants and electronic hearing. *Physics Today*. **70**, 53-58.
73. Ulfendahl, M., Scarfone, E., Flock, A., Le Calvez, S. and Conradi, P. (2000). Perilymphatic fluid compartments and intercellular spaces of the inner ear and the organ of Corti. *NeuroImage*. **12**, 307-313.
74. Wallhagan, M. (2010). The stigma of hearing loss. *Gerontologist*. **50**, 66-75.
75. Walters, B. J., Coak, E., Dearman, J., Bailey, G., Yamashita, T., Kuo, B., and Zuo, J. (2017). In Vivo Interplay between p27Kip1, *Gata3*, *Atoh1*, and Pou4F3 converts non-sensory cells to hair cells in adult mice. *Cell Rep*. **19**, 307-320.
76. Weinstein, M. J., Leudemann, G. M. and Oden, E. M. (1963). Gentamicin, a new antibiotic complex from micromonospora. *J. Med. Chem*. **6**, 463-464.
77. Winterstein, A. G., Liu, W., Xu, D. and Antonelli, P. J. (2013). Sensorineural hearing loss associated with neomycin eardrops and nonintact tympanic membranes. *Otolaryngol Head Neck Surg*. **148**, 277-283.
78. Zeng, F. G., Rebscher, S., Harrison, W. V., Sun, X. and Feng, H. (2008). Cochlear implants: System design, integration and evaluation. *IEEE Rev Biomed Eng*. **1**, 115-142.