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Literature Review on Outer Hair Cell Regeneration and Potential Hearing Restoration

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Abstract

The cochlea is the portion of the inner ear responsible for hearing, containing both the organ of Corti and auditory hair cells. Sound wave vibrations mechanically bend the stereocilia on the three rows of outer hair cells and one row of inner hair cells. These hair cells transduce this mechanical energy into electrical impulses that are relayed from the auditory nerve to the brain, where sounds are processed. In this review I provide an overview of inner ear anatomy, ear physiology, and auditory transduction. I then move on to specifically focus on the importance of outer hair cells, their regeneration, and their importance for hearing restoration.

Background

Hearing Loss

The widespread prevalence of hearing loss in society significantly affects the lives of many; in the U.S., there are approximately 30 million individuals over the age of 12 who suffer from hearing loss in both ears, and 48 million who have hearing loss in at least one ear (Agrawal et al., 2008; Lin et al., 2011, as cited in National Academies of Sciences, Engineering, and Medicine et al., 2016). Worldwide, the World Health Organization estimates around 430 million people (5% of population) have disabling hearing loss, and this number is estimated to increase to over 700 million by 2050 (*Deafness and Hearing Loss*, 2021). Undetected and undertreated, hearing loss presents as a large barrier to communication, and can negatively impact relationships, job opportunities, and other social interactions. This may result in social isolation, depression, and amongst the elderly, decreased quality of life. Hearing loss can also negatively affect overall health by being associated with increases in cognitive decline (Reed et al., 2019). In addition to affecting the personal lives of millions, hearing loss has a large impact on the economy as well, with estimated total global economic costs to be over $981 billion due to direct medical costs, disability costs, and loss of productivity costs (McDaid et al., 2021)

Causes of Hearing Loss

The most common type of hearing loss is sensorineural hearing loss, which is due to damage or maldevelopment of the cells in the inner ear. This includes damage to auditory mechanosensory hair cells, genetic disorders leading to aberrations in inner ear structure, or damage to the auditory nerve (Tanna et al., 2021). Conductive hearing loss can also occur in individuals, which is when sound waves are unable to reach the inner ear due to some obstruction or malformation in the outer or middle ear (Tanna et al., 2021). However, this review will discuss sensorineural hearing loss that results from damaged or lost hair cells, specifically focusing on outer hair cells (OHCs), which are found to be the most susceptible to damage (Kemp, 2009). OHCs function to amplify and fine tune sounds that enter the cochlea. If they are damaged, this decreases sensitivity to sounds, resulting in hearing loss (Han et al., 2020). As depicted in figure 1, various factors can play a role in damaging hair cells, such as aging, excessively loud noise exposure, ototoxic medications, genetic mutations, or infection (Cheng, 2019). Hair cells tend to degrade with age due to years of continuous exposure to noise, and eventually they die. Excessively loud/long noise exposure damages hair cells by overworking

them, which causes swelling of the hair cells and damage to the stereocilia, which then causes apoptosis and hair cell loss (Harrison, 2012). There are also certain medications, such as aminoglycosides (antibiotics) and cisplatin (chemotherapy drug), that induce apoptotic cell death in hair cells, especially outer hair cells (Wu et al., 2021). Viral infections can cause hearing loss by directly damaging inner ear structures or causing inflammation. Viruses such as measles, mumps, and rubella can cause sensorineural hearing loss, which is why it is strongly recommended babies
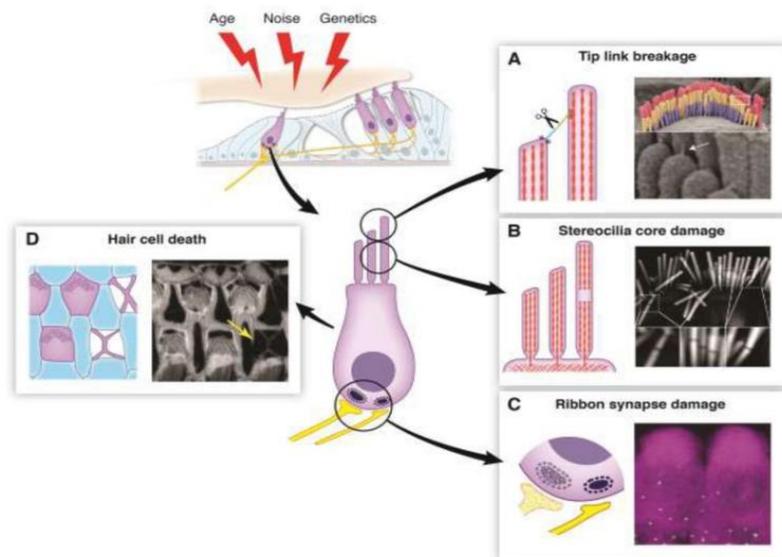

Figure 1. Diagram showing vulnerable locations in a hair cell and different types of damage that may occur due to various factors. (Wagner & Shin, 2019)

ages 12-15 months get the MMR vaccine. Cytomegalovirus, in the herpes virus family, has hearing loss as a prevalent consequence (Cohen et al., 2014). Hair cell damage manifests in multiple ways exhibited in figure 1, including tip link breakage (which interferes with mechanotransduction), damage to the stereocilia F-actin core, and ribbon synapse damage. Although these damages can be repaired to some extent, an accumulation of continuous damages can result in hair cell death. Another large contributor to hair cell death are reactive oxygen species, which are created due to increased calcium levels and metabolic demands caused by intense sound stimulation (Wagner & Shin, 2019).

Importance of Regenerating Hair Cells

Mammals are unable to spontaneously regenerate hair cells after they are lost, which is why hearing loss is permanent (Cheng, 2019). Significant research has been and is currently being conducted to search for ways to regenerate these hair cells. When it comes to hair cell damage, OHCs are more likely to be affected than their IHC counterparts, affected earlier, and are in general more vulnerable to damage, especially noise induced damage (Waqas et al., 2018). Additionally, losing even a single row of OHCs significantly impacts the basilar membrane, affecting overall cochlear amplification and suggesting that all three rows are required in order to hear properly (Murakoshi et al., 2015). Discovering a way to regenerate or replace damaged and dead OHCs would be groundbreaking in the scientific and medical fields, and life changing for millions of people throughout the world.

Ear Development

Each section of the ear develops from various tissues. The outer ear is derived from the ectoderm, with the external auditory meatus developing from the first pharyngeal cleft and the

pinna developing from hillocks, which come from both pharyngeal arches. The middle ear is derived from the endoderm, with the ossicle bones developing from neural crest mesenchyme, and the ossicle muscles developing from mesoderm of the two pharyngeal arches (Helwany & Tadi, 2021). The inner ear is derived from the ectoderm as well. Around gestational week 4 in humans, the ectoderm adjacent to rhombomeres 5-6 of the hindbrain becomes the otic placode, which turns into the otic primordium, eventually forming the otic vesicle. From here, the otic labyrinth (three-dimensional inner ear) develops, along with the cochlea (Chacko et al., 2019). Within the otic labyrinth is the cochlear duct, containing prosensory cells from which hair cells develop (Atkinson et al., 2015). Various genes and transcription factors are required for proper ear development and fully functioning auditory and vestibular components, including V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB), Pax 2, Pax 6, Pax 8, peripherin, and class III β-Tubulin (Chacko et al., 2019).

Anatomy and Hearing Transduction

The process of hearing consists of sound waves entering the ear, moving through the sections of the ear, and getting transduced into action potentials that go through the auditory nerve to the brain, and are then interpreted by the brain as sound. Sound waves are air molecules that are compressed and travel in all directions. Every part of the ear plays a specific role in moving the sound waves along, and these parts of the ear are displayed in figure 2.

The outer ear is made up of the pinna/auricle and the external auditory meatus (EAM), often referred to as the ear canal. Brownell (1997) explains how the function of the pinna is to collect sound waves that are coming from the environment and funnel them into the EAM. In the EAM, these sound waves are amplified and passed down to the tympanic membrane. The EAM contains earwax that traps dust and particles to prevent them from going deeper into the ear. The tympanic membrane, the ossicles, the oval window, and the
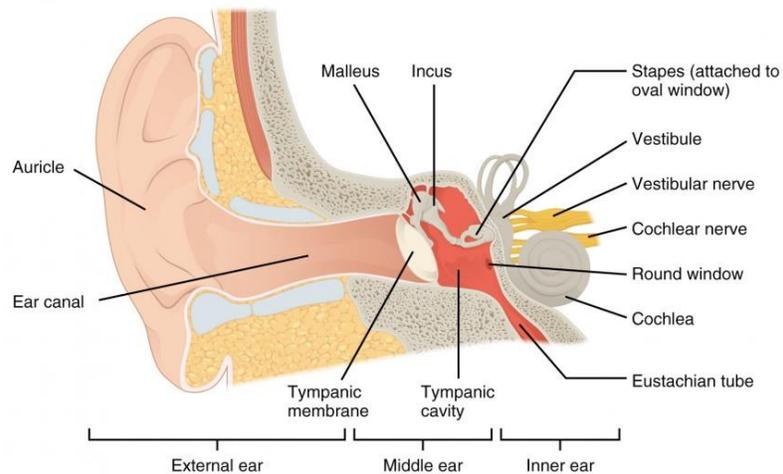

Figure 2. Diagram showing the structures of the external, middle, and inner ear. (https://courses.lumenlearning.com/suny-ap1/chapter/audition-and-somatosensation/)

Eustachian tube make up the middle ear. As these sound waves come into contact with the tympanic membrane, it causes this membrane to vibrate. These vibrations then cause the ossicles to start moving. The ossicles consist of the malleus, incus, and stapes, and these tiny bones function to amplify and transfer sound waves. As the sound waves cause the ossicles to vibrate, the stapes bone pushes against the oval window, causing it to vibrate and causing a buildup of pressure (Brownell, 1997). The Eustachian tube, which is normally closed, helps relieve this pressure buildup by equalizing pressure on both sides of the tympanic membrane; this can be done by swallowing, because the Eustachian tube is connected to the palate (Szymanski & Agarwal, 2021). When the oval window vibrates, it transmits the vibrations to the cochlea, and

pressure waves occur in the scala vestibuli, which contains perilymph fluid. Perilymph fluid has a high concentration of sodium and a low concentration of potassium. These pressure waves put additional pressure on the vestibular membrane, which causes an increase in pressure in the endolymph (which has higher concentrations of potassium than sodium) and increases pressure in the basilar membrane.

Attached to the basilar membrane are hair cells and their stereocilia; oscillation of the basilar membrane causes the hair cells and stereocilia to move. Because the stereocilia are embedded in a stiff, stationary tectorial membrane, when the basilar membrane vibrates, the stereocilia are mechanically bent back and forth. As these hair cells become mechanically deformed, mechanically gated ion channels in the hair cells open and close, resulting in depolarizing and hyperpolarizing membrane potential changes (as shown in figure 3). The gating of these channels is regulated by tension of elastic structures in the hair bundle which act as gating springs that pull on the molecular gates of transduction channels. Transduction of sound waves into action potentials occurs in the hair cells, located in the organ of Corti. There are two types of hair cells, inner and outer. Inner hair cells (IHCs) function to transform mechanical forces of sound into electrical impulses of hearing. When these IHCs depolarize, as shown in figure 3, the basilar membrane is deflected upward and the rate of neurotransmitter release increases. When these cells hyperpolarize, the basilar membrane is deflected downward and firing rates of the primary afferents are decreased. The OHCs receive signals from the brain over efferent fibers and act to enhance the response of the IHCs. Cell depolarization and hyperpolarization affects the size of the OHCs as well. Depolarization causes contraction of OHCs, while hyperpolarization causes elongation of OHCs (Frolenkov, 2006). This ability of OHCs to change in size depending on membrane potential is called electromotility (Frolenkov, 2006), and it helps OHCs function properly by aiding in cochlear amplification, which increases frequency tuning and sound sensitivity (Yu & Zhao, 2009). It is important to note that hair cells do not use second messenger systems in transduction. Hair cells are also able to adapt to sustained stimuli (Eatock, 2000). When fluid movement increases the tension in the gating springs, ion channels open, and calcium entry into the hair cells interacts with calmodulin and causes myosin molecules, that hold each tip link in place, to move down from the tip of the stereocilia. Tip links are actin connections that physically connect stereocilia (Sakaguchi et al., 2009).

Building up a good foundation of knowledge is crucial in order to take the next steps in an experiment. Studying normal hair cell development, particularly OHC development, can help with the process of understanding how to successfully regenerate OHCs.
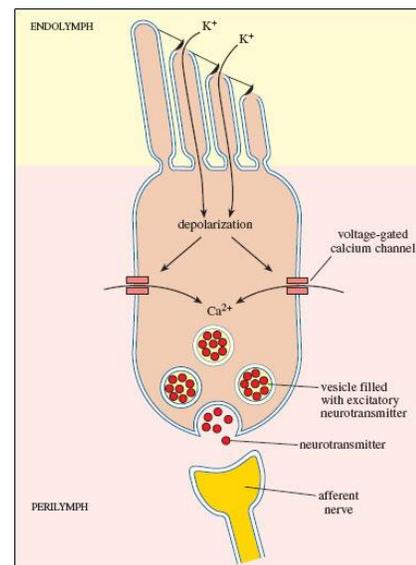

Figure 3. Diagram displaying what occurs when a hair cell depolarizes. (https://www.open.edu/openlearn/science-maths-technology/biology/hearing/content-section-3.6)

Hair Cell Development

Before looking into potential hair cell regeneration, it is important to first understand how hair cells develop. There are two types of hair cells: one row of IHCs and three rows of OHCs, as displayed in figure 4. These hair cells are located in the organ of Corti, which is located within the cochlea of the

Figure 4. Diagram displaying the arrangement of inner and outer hair cells. (Lee et al., 2017)

inner ear. This section will discuss the process hair cells undergo to fully mature and become functional sensory cells within the auditory system.
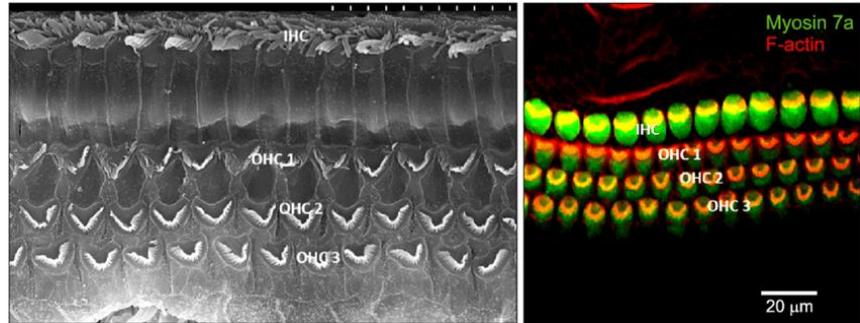
ATOH1

There are several basic helix-loop-helix (bHLH) transcription factors that play a role in hair cell development, such as ATOH1 (which is the first known to be expressed, according to Zhong et al., 2019), NEUROG1, NEUROD1, and ASCL1 (Bertrand et al., 2002; Fritzsch, 2003; Tiveron et al., 2003; Jahan et al., 2013, as cited in Cheng, 2019). SOX2 also plays a major role in overall inner ear development. ATOH1 in particular is a crucial component in hair cell formation, differentiation, survival, and maturation. Overall, *Atoh1* determines if a cell commits to becoming a hair cell or not; absence of *Atoh1* means absence of hair cells (Jahan et al., 2015). Called *HATH1* in humans (Mulvaney & Dabdoub, 2012), *Atoh1* expression begins in the prosensory epithelium of the cochlea, and as development continues, it is only expressed in cells that specifically develop into hair cells. As shown in Figure 5, after *Atoh1* determines the fate of the cell, *Gfi1*, *Pou4f3*, and *Barhl1* genes all function to help hair cells survive and mature into fully developed, fully functioning hair cells (Zhong et al., 2019). It is unknown what ATOH1 specifically targets within hair cells while being expressed, whether it affects genes that determine specific hair cell subtypes (OHC vs IHC) or if it just targets hair cells in general (Groves et al., 2013). However, it has been found that ATOH1 initiates a positive-feedback loop by binding to its own promoter region, which allows for *Atoh1* to be continually expressed (Helms et al., 2000, as cited in Wu & Kelley, 2012). Studies found that reduction of *Atoh1* in embryonic stages leads to hair cell loss and formation of abnormal hair cell bundles in transgenic mice, while other studies that knocked *Atoh1* out of mice displayed a lack of formation of cochlear or vestibular hair cells (Cheng, 2019). Therefore, *Atoh1* is required to be expressed continuously, through all developmental time points, in order to get a hair cell that is fully developed and fully functional (Atkinson et al., 2015).

SOX2

SOX2 is a transcription factor that is crucial for inner ear development. It is responsible for neural specification (Evsen et al., 2013; Puligilla et al., 2010; Steevens et al., 2017, as cited in Steevens et al., 2019), sensory specification (Kiernan et al., 2005, as cited in Steevens et al., 2019), and sensory differentiation (Dabdoub et al., 2008; Kempfle et al., 2016, as cited in Steevens et al., 2019), and is very time specific. In studies with mice, around embryonic days 8.5-9.5 (E8.5-9.5), SOX2 is expressed on the lateral wall of the otocyst, where it helps form

mainly nonsensory regions in the cochlea (Abelló et al., 2010; Kiernan et al., 1997; Raft et al., 2004, as cited in Steevens et al., 2019). But between E10.5 and E11.5, SOX2 expression shifts to the medial part of the otocyst, which is where sensory regions, therefore hair cells, are formed. By E12.5, SOX2 is only expressed in sensory regions (Steevens et al., 2019). Within these sensory regions, SOX2 expression helps determine the fate of different cells (Steevens et al., 2019). Essentially, SOX2 helps restrict which region forms as the organ of Corti and which region does not, and then once it is in the organ of Corti, it helps cells differentiate into either hair cells or supporting cells. Studies done with mice found that mutations in *Sox2* decreased or completely killed hair cells and supporting cells, leading to hearing loss and deafness (Yang et al., 2019). SOX2 is also needed for *Atoh1* expression (Wu & Kelley, 2012). When *Sox2* expression occurs for an extended period of time, it inhibits *Atoh1,* therefore decreasing hair cell formation. When *Sox2* expression is decreased, hair cell formation increases (Dabdoub et al., 2008, as cited in Wu & Kelley, 2012). Notch signaling has also been found to be needed in order for *Sox2* to be expressed (Dabdoub et al., 2008).

Notch, Wnt/β-catenin, and FGF Signaling in Inner Ear Development
　　There are multiple signaling pathways that play a significant role in regulating expression of *Atoh1* and overall hair cell development. One of these pathways is the Notch signaling pathway (Jarriault et al., 1995; Lanford et al., 1999; Brooker et al., 2006, as cited in Cheng, 2019). Notch signaling utilizes lateral inhibition to control if prosensory cells differentiate into hair cells or supporting cells, and helps determine how these two types of cells are arranged in the cochlea (Kelley, 2003, as cited in Cheng, 2019). The way this process works is that specific Notch ligands *Jag2* and *Delta1* (Cheng, 2019) bind to the Notch receptor in cells. This initiates γ-secretase mediated cleavage, which causes Notch intracellular domain to be released. This causes inhibitory bHLH transcription factors to be released and expressed. Their expression downregulates *Atoh1* expression, therefore inhibiting cells from differentiating into hair cells. These

Figure 5. Image showing regulators in development of auditory sensory epithelium (Zhong et al., 2019)

cells, by default, develop into supporting cells (Wang et al., 2010). If γ-secretase is inhibited or the receptor *Notch1* is not present, this will upregulate *Atoh1* and cause more hair cells to form (Zhong et al., 2019).
　　A second crucial pathway that plays a role in determining cell fate is the Wnt/β-catenin signaling pathway. This pathway is important in separating regions within the inner ear that have hair cells, and regions that do not (Stevens et al., 2003, as cited in Cheng, 2019). Hair cell
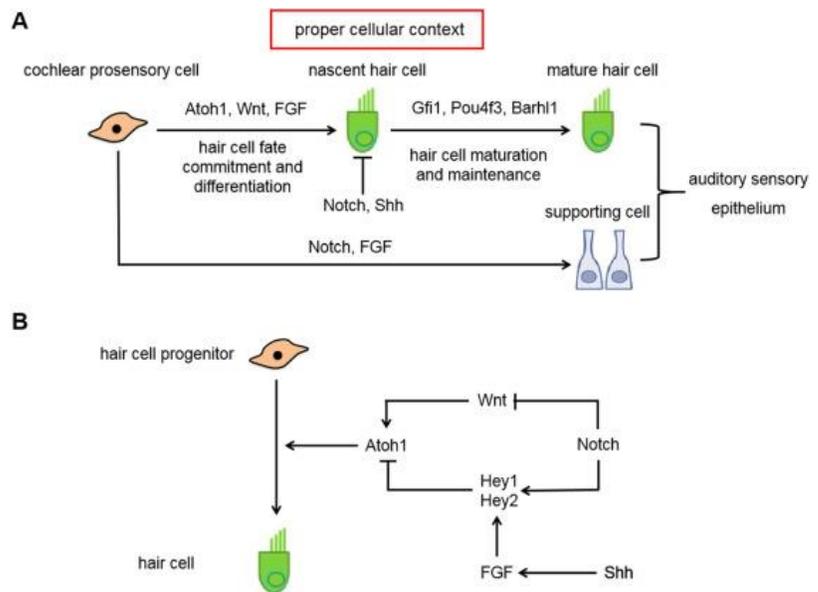
formation is inhibited when β-catenin is conditionally knocked out. Hair cell formation increases when β-catenin is upregulated (Shi et al., 2012; 2014; McLean et al., 2017, as cited in Cheng, 2019). It was also found that the two pathways interact; when Notch signaling is inhibited, β-catenin is expressed more (Shi et al., 2010, as cited in Cheng, 2019). This finding displays how β-catenin expression is needed for hair cells formation after Notch inhibition, and also for increased *Atoh1* expression (Li et al., 2015, as cited in Cheng, 2019). *Atoh1* has also been found to be post-translationally regulated by the CK1-Huwe1-ubiquitin-proteasome pathway. Increased levels of CK1 degrades Atoh1 while inhibition of CK1 maintains Atoh1 (Cheng, 2019).

Fibroblast Growth Factor (FGF) signaling is another important pathway for inner ear and hair cell development. FGF has over 20 different ligands and different membrane bound receptors that help cells survive, proliferate, and differentiate (Jacques et al., 2012). There are many different *Fgf* genes that all play important roles in inner ear development, and when mutations arise, this phenotypically affects development in various ways. For example, if *Fgf3* becomes mutated, this prevents the otic vesicle from forming properly and being able to mature (Ebeid & Huh, 2017). FGF signaling has been found to regulate formation of the otocyst (Schimmang, 2007, as cited in Jacques et al., 2012). More importantly, various knockout experiments found that outer hair cells and supporting hair cells are not able to differentiate without FGF1 signaling (Ebeid & Huh, 2017).

Outer Hair Cell Development

While it is known that hair cells originate from the prosensory region of the otocyst (Wiwatpanit et al., 2018), and a significant amount is known about hair cell function and their role in hearing, the mechanisms that cause these hair cells to differentiate into outer and inner hair cells are still unclear (Li et al., 2018). However, there are certain proteins present in hair cells whose expression or lack of expression has been found to potentially play a role in hair cell specialization, most notably insulinoma-associated protein 1 (INSM1). Additionally, the motor protein prestin is needed in order for OHCs to function properly.

Insm1

INSM1 is a zinc finger protein that is expressed for a short period of time at the beginning of OHC development. It has been found that expression of INSM1 prevents early OHCs from differentiating into IHCs. INSM1 does not specifically cause hair cells to commit to becoming OHCs. Rather, it exhibits its effects after the OHC is developed, and prevents it from switching to an IHC. This ensures that the OHC remains an OHC through the full course of development. A study done by Wiwatpanit et al. (2018) compared transcriptomes of immature OHCs and IHCs, and those of OHCs that contained INSM1 and OHCs that did not. It was found that cells born embryonically as OHCs differentiate into mature IHCs if INSM1 is not present. This occurs because embryonic OHCs contain a set of IHC-enriched genes that, if exposed to an IHC-inducing gradient, cause them to differentiate into IHCs. INSM1 works to repress these genes, preventing them from responding to the IHC-inducing gradient and turning into IHCs. These cells can then fully develop as OHCs. To summarize, rather than directly affecting OHCs by activating OHC genes, INSM1 works by inhibiting IHC genes to prevent hair cells from differentiating into IHCs (Wiwatpanit et al., 2018).

Prestin

Prestin is an important component needed for proper OHC function and overall hearing. It is expressed in OHCs, but not expressed in IHCs. While prestin is important for the function of OHCs, it cannot make OHCs. Prestin is a voltage dependent motor protein located in the lateral walls of OHCs, and functions to generate electromotility forces in OHCs (Choi, 2011). The way it does this is by forming complexes with other proteins located in the lateral walls of OHCs. This coupling generates forces that change the length of the cell, which then affects the vibrations of the cochlear and tectorial membranes (Choi, 2011). Electromotility is crucial because it powers cochlear amplification and helps with frequency tuning and sound sensitivity. Absence of prestin or non-functional prestin arising from damage results in severe hearing loss because of loss of electromotility (Xia et al., 2013 and Choi, 2011). Calmodulin binding sites have been found in prestin, and certain studies have found evidence that when calmodulin binds, it makes the voltage operating point of prestin more negative, and affects OHC axial stiffness (He & Dallos, 1999; He et al., 2003, as cited in Keller et al., 2014). Calcium also plays a large role in OHC electromotility. In fact, a specific study done by Szönyi et al. found results suggesting that calcium and calmodulin play roles in increasing electromotility magnitude in OHCs (Szönyi et al., 2001).

Hair Cell Regeneration

Assistive devices such as hearing aids and cochlear implants are currently available to the public to help somewhat enhance hearing abilities. However, high costs for these devices can deter people, along with the fact that they cannot fully restore hearing to how it was before hair cell damage/loss occurred (Michels et al., 2019 and Tanna et al., 2021). Because of these factors, the most favorable way to completely treat hearing loss is to figure out how to regenerate hair cells that have been damaged or lost (Cheng, 2019). In mammals, auditory hair cells are not able to spontaneously regenerate after being damaged or lost, resulting in permanent hearing loss (Xu & Yang, 2021). A significant amount of research has gone into determining potential ways to regenerate hair cells, such as cell therapy, gene therapy, pharmacological therapy, and cell transplantation. The main issue with these methods is that the cells are not able to survive very long, and they lack the ability to mature into fully functional hair cells (Cheng, 2019). Additionally, human inner ear sensory cells are inaccessible, which complicates the research process and limits what can be studied about them (Menendez et al., 2020). ATOH1 and the Notch and Wnt signaling pathways once again come into play when discussing hair cell regeneration (Cheng, 2019). Many studies have been done with ATOH1, manipulating it in various ways to determine its effects on signaling pathways and on hair cells. Studies show that ATOH1 has been found to be a factor in potential hair cell regeneration. Gene and pharmacological therapy experiments using ATOH1 found that when it was overexpressed for a short period of time, it turned supporting cells into hair cells, therefore resulting in the formation of new hair cells. However, continuous overexpression has the opposite effect, and is actually damaging to hair cells (Izumikawa et al., 2005; Richardson and Atkinson, 2015, as cited in Cheng, 2019). Expression of β-catenin, important in the Wnt pathway, also was found to help create hair cells (Kuo et al., 2015, as cited in Mittal et al., 2017). Additionally, a study found that inhibition of the Notch pathway was found to help turn stem cells into hair cells (Li et al., 2015, as cited in Mittal et al., 2017).

Regeneration Approaches

There are three main approaches that are used by studies attempting to figure out how to regenerate hair cells. The first is using stem cells to form hair cells. Cells have been produced using this method that resemble hair cells, but it is unclear whether they are able to function as hair cells do. Additionally, only a few cells are produced, and they do not live very long after being formed (Shi et al., 2012; Bramhall et al., 2014; Cox et al., 2014, as cited in Mittal et al., 2017). Gene editing of stem cells has taken place as well, utilizing adeno-associated virus (AAV) vectors to deliver genes targeted at cochlear hair cells (Xu & Yang, 2021). A specific

Figure 6. Diagram showing potential of gene therapy in the inner ear to regenerate hair cells (Mittal et al., 2017)

study used this method to inject mice with the *VGLUT3* gene, and it actually improved hearing (Akil et al., 2012, as cited in Xu & Yang, 2021). Other studies are not as successful with this approach. The second approach attempts to use supporting cells to form hair cells, and has gotten many successful results. In addition to using Atoh1 to change supporting cells into hair cells (as mentioned earlier in this section), this approach utilizes a protease complex called γ-secretase, which also functions to turn supporting cells into hair cells (Mizutari et al., 2013, as cited in Mittal et al., 2017). An *in vivo* study found that hearing was restored in mice injected with γ-secretase, implying that this could be a step in the right direction for studies trying to recover hearing loss in humans (Mittal et al., 2017). The third approach consists of trying to get hair cells and supporting cells to begin dividing again (Mittal et al., 2017). This approach uses cell cycle activator *Myc* and inner ear progenitor gene *Notch1*. *Myc* is important because it causes cells to re-enter the cell cycle, and then begin dividing again. *Notch1* helps form the prosensory region of the otocyst. When these two are activated at the same time, it causes inner ear cells to enter the cell cycle again and begin dividing. These cells then become responsive to Atoh1 induction signals, and are able to differentiate into hair cells (Shu et al., 2019).
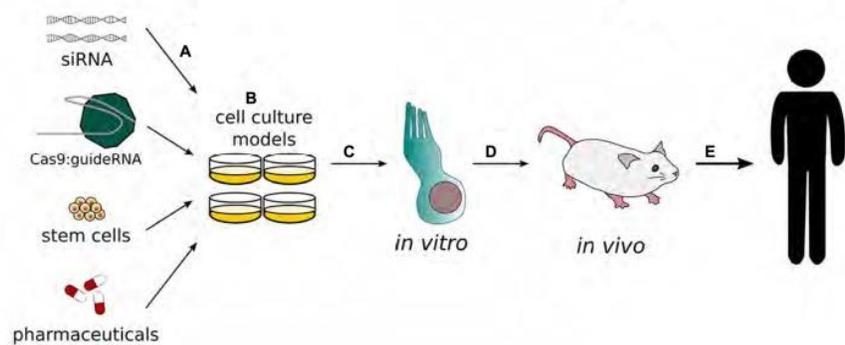
Outer Hair Cell Regeneration

While research is still being done on understanding generic hair cell regeneration, there is even less known about how to specifically regenerate OHCs. There are many factors that complicate the search for regeneration techniques. For one, it still is not very clear how cells specifically differentiate into OHCs or IHCs. We do know that ATOH1 controls both IHCs and OHCs, prestin controls OHCs, and *Fgf8* controls IHCs. However, we do not know the time or amount of expression required of these transcriptions factors/genes to get mature, fully functional OHCs or IHCs. Additionally, there are numerous signal pathways that all must coordinate to form and regenerate hair cells (Mittal et al., 2017). There are other important factors that need to be figured out as well; once it is determined how to regenerate hair cell subtypes, research efforts need to move towards focusing on how to regenerate those hair cell

subtypes in certain locations and in specific numbers. It is crucial that scientists are able to place certain numbers of specific hair cell subtypes where they want them, or else OHCs will regenerate in the IHC row (and vice versa), and this will impact hearing. There are more OHCs than IHCs, so there is a greater chance that an OHC will end up in the wrong location and cause improper arrangement. If OHC arrangement is not correct and if OHCs in general are lost, this affects the basilar membrane, which impacts cochlear amplification, which impacts hearing transduction (Murakoshi et al., 2015). Until more research is done to understand these factors completely, regeneration efforts will have to remain experimental and will not be able to be used on human subjects. One potential approach in the right direction consists of modifying genomes to help study hearing loss related genes. It is thought that in the future, CRISPR/Cas9 may be able to fix mutations in these genes so that they no longer negatively affect hearing. Cas9 complexes injected into mice OHCs have been found to successfully edit the genomes. These complexes are currently only effective in OHCs, not IHCs (Mittal et al., 2017).

There are several approaches currently being researched and tested on how to regenerate hair cells, but it is still unclear how to approach regenerating specific hair cell subtypes, particularly OHCs. This is because there are a lot of unknowns regarding the various mechanisms that go into hair cell formation and regeneration (Cheng, 2019). Additionally, different types of damage and their varying severities affect hair cells in different ways and impact different signaling cascades; it is incredibly difficult to come up with regeneration techniques when the cause of the problem is unknown and varies from case to case (Smith et al., 2016). Since OHCs are generally affected first and tend to be more vulnerable to damage, finding an effective and realistic method to regenerate them is crucial in the process to restore hearing in humans.

Conclusion

Hearing is one of the five senses through which the body processes sensory information from the environment. Hearing allows for communication and socialization with others, along with providing a sense of what is happening in the world. Being deprived of this ability can contribute to social isolation and amongst the elderly, decreased quality of life. Millions of people in the world currently suffer from some form of hearing loss, most commonly sensorineural hearing loss. While there are certain assistive devices available to aid with hearing, these devices are unable to completely restore hearing that has been lost. Once auditory hair cells are lost or damaged, they are unable to regenerate on their own because the cells are no longer able to divide. Outer hair cells are considered to be more susceptible to damage, therefore playing an overall larger role in hearing loss. Significant research efforts have been made to find ways to regenerate hair cells. For one, gene therapy has been used to make hair cells out of supporting cells by overexpressing ATOH1, which helped bring back hearing that had been lost (Izumikawa et al., 2005; Richardson and Atkinson, 2015, as cited in Cheng, 2019). AAV vectors have also been used, and a Zinn et al. study was able to create a specific vector that can target both IHCs and OHCs and promote regeneration, but is more efficient in OHCs (Zinn et al., 2015, as cited in Xu & Yang, 2021). While scientists currently do not know how to regenerate specific hair cell subtypes, such as outer hair cells, there have been several approaches used by numerous studies that have had hopeful results relating to the regeneration of hair cells in general. More research that leads to a deeper understanding of how hair cells develop, along with learning more

about genes that help them differentiate, will be very helpful in the process to understanding and completing hair cell regeneration (Jacques et al., 2012). With continued research, experimentation, and analysis, hopefully more can be learned about hair cell formation and differentiation. This knowledge can then be utilized to clarify different approaches to hair cell regeneration, and then new and different techniques can be applied with increasingly consistent results.

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