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Beyond Silence: Exploring the Potential of Gene Therapy for Regenerating Inner Ear Hair Cells

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Abstract*—Millions of people worldwide suffer from hearing impairment caused by the loss of inner ear hair cells. While cochlear implants and hearing aids have achieved some success, they are limited in their ability to restore natural hearing. Regenerative approaches, such as the use of viral vectors to deliver genes for hair cell regeneration, have shown promise in animal models, but their translation to clinical applications has proven challenging. This article provides an in-depth review of the current state of the art in AAV-mediated gene transfer for inner auditory hair cell regeneration, highlighting the potential benefits and pitfalls of this approach. We examine the critical factors that determine AAV vector efficiency and specificity for targeting inner ear cells, including the choice of promoter, capsid, and delivery route. Additionally, we analyze the preclinical and clinical studies that have investigated AAV-based therapies for hearing restoration, and we address unresolved safety concerns that need to be addressed in future research. Our review indicates that AAV gene therapy offers great promise for treating hearing loss due to hair cell damage. However, further research is required to optimize the delivery and integration of therapeutic genes and ensure long-term safety and efficacy. This comprehensive analysis provides a roadmap for the development of innovative regenerative therapies for hearing restoration and highlights the challenges and opportunities in inner auditory hair cell regeneration.

Keywords: Inner ear hair cells, hearing impairment, cochlear implants, hearing aids, regenerative approaches, viral vectors

I. INTRODUCTION

Hearing loss is a common and debilitating condition affecting millions worldwide. One promising approach to treating hearing loss is inner hair cell regeneration (IHCR), which involves regrowing the sensory cells responsible for converting sound waves into neural signals in the inner ear. These hair cells are critical for maintaining balance and detecting sound; their loss is a major cause of hearing impairment.

The inner ear is a complex system with several sensory organs, including the three semicircular canals' cochlea, utricle, saccule, and crista ampullaris. Each structure plays a crucial role in our ability to hear and maintain balance.

Researchers have made significant strides in understanding the mechanisms underlying IHCR and developing new therapies to promote hair cell regeneration in recent years. This article will provide a comprehensive overview of the current state of research on IHCR, including recent advances in our understanding of the molecular and cellular processes involved in hair cell regeneration. We will also discuss the potential of these new therapies to improve outcomes for patients with hearing loss and the challenges and limitations that remain to be addressed. This article aims to provide a valuable resource for researchers, clinicians, and patients interested in the latest inner hair cell regeneration developments.

II. HISTORY & NECESSITY OF IHCR

Inner Hair Cell Regeneration (IHCR) is a concept that has been studied and debated for decades. The first observations of hair cell regeneration in the inner ear were made in the 1930s when researchers noticed that new hair cells were occasionally found in the cochlea of birds and reptiles exposed to loud noise or ototoxic drugs (Forge, 1985).

However, it was not until the 1980s that the idea of hair cell regeneration in mammals gained traction. A study

Journal of Current Research in Engineering and Science
Bi-Annual Online Journal (ISSN : 2581 - 611X)
Volume 7-Issue1, August 2023
Paper: 30

published in 1987 by Ryals and Rubel demonstrated that hair cells could be induced to regenerate in the cochlea of young chickens (Gallus gallus) using aminoglycoside antibiotics (Ryals and Rubel, 1988). This was followed by similar studies in guinea pigs (Cavia porcellus) and mice (Mus musculus) that showed that hair cells could be induced to regenerate under certain conditions (Forge and Schacht, 2000).

In the 1990s and 2000s, researchers focused on the role of supporting cells in hair cell regeneration. Supporting cells are non-sensory cells that surround hair cells in the cochlea. Studies showed that supporting cells could be induced to divide and differentiate into hair cells under certain conditions, such as exposure to growth factors or deleting specific genes (Bramhall et al., 2014; Cox et al., 2014).

Researchers have recently turned to gene therapy to induce hair cell regeneration. One approach has been to use an adeno-associated virus (AAV) vector to deliver genes that can promote hair cell regeneration to supporting cells in the cochlea (Askew et al., 2015; Akil et al., 2019). These studies have shown promising results in animal models and paved the way for human clinical trials.

Inner Hair Cell Regeneration (IHCR) is a critical field of research that holds immense promise for addressing the problem of hearing loss. The World Health Organization estimates that approximately 466 million people worldwide suffer from disabling hearing loss, which is expected to increase to over 900 million by 2050 (1). This represents a significant public health burden, with profound implications for affected individuals' quality of life, mental health, and social and economic well-being (2).

Renewing inner hair cells in the cochlea is a promising approach to restoring hearing [1]. Compared to current treatments like cochlear implants, hair cell regeneration has the potential to restore more natural hearing and avoid the limitations associated with current treatments [2]. Multiple studies have demonstrated successful hair cell regeneration in animal models [3][4], with some studies even achieving partial hearing restoration [5]. Additionally, adeno-associated virus (AAV) vectors have shown the potential in delivering gene therapy to support hair cell regeneration [6]. Furthermore, recent advancements in stem cell research have opened up new possibilities for hair cell regeneration in humans [7]. Overall, regenerating inner hair cells presents a promising avenue for restoring hearing and should be further explored as a potential treatment option.

Despite these advancements, IHCR is still a complex and largely unsolved problem. The factors that regulate hair cell regeneration in the inner ear are poorly understood, and the ability to induce hair cell regeneration in mature mammals is still limited (Cox et al., 2014). However, the ongoing research in this area can potentially lead to new therapies for hearing loss in humans.

III. Feasibility of IHCR

The feasibility of IHCR has been debated for many years, and there have been various approaches to achieve it. One approach is gene therapy to regenerate hair cells in the inner ear. Several studies have shown that gene therapy using viral vectors, such as an adeno-associated virus (AAV), can successfully deliver genes to the cochlea and induce hair cell regeneration [8-9]. Other studies have shown that gene therapy can also target supporting cells in the cochlea, which can differentiate into new hair cells [10-11].

However, there are still challenges to overcome before IHCR becomes a reality. One challenge is the limited capacity of the inner ear to regenerate hair cells. The regenerative ability of hair cells declines with age, making it difficult to achieve IHCR in older patients [12]. Additionally, the specific mechanisms that regulate hair cell regeneration are not fully understood, and more research is needed to identify and manipulate these mechanisms [13].

Despite these challenges, recent advancements in gene therapy and stem cell research have brought us closer to achieving IHCR. For example, new techniques, such as nanoparticles, have been developed to enhance gene delivery efficiency to the inner ear [14]. In addition, researchers have discovered new types of stem cells in the inner ear that have the potential to differentiate into hair cells [15].

In conclusion, the feasibility of IHCR is still a work in progress, but recent advancements in gene therapy and stem cell research have brought us closer to achieving this goal. While challenges remain, continued research and development in this field will bring us closer to a future where IHCR can be a viable treatment option for hearing loss.

IV. State of the Art Experiments in IHCR

Several experimental approaches have been developed to regenerate inner hair cells in the cochlea, ranging from gene therapy to stem cell transplantation. This section will discuss the state-of-the-art experiments in IHCR and their potential for clinical translation.

A. Gene Therapy

The use of adeno-associated viral (AAV) vectors for gene therapy has shown promising results in regenerating inner hair cells in animal models [16]. A study conducted by Akil et al. demonstrated the successful regeneration of inner hair cells in guinea pigs using AAV vectors [17]. The vectors were engineered to deliver the Atoh1 gene, critical in inner

Journal of Current Research in Engineering and Science
Bi-Annual Online Journal (ISSN : 2581 - 611X)
Volume 7-Issue1, August 2023
Paper: 30

ear development. Another study by Suzuki et al. showed that AAV vectors could deliver the Math1 gene, which is also involved in inner ear development, to support cells in the cochlea, leading to the regeneration of inner hair cells in mice [18].

B. Stem Cell Transplantation

Stem cell transplantation is another approach investigated for IHCR. One study conducted by Chen et al. demonstrated the regeneration of inner hair cells in the cochlea of mice using embryonic stem cells. Another study by Izumikawa et al. used induced pluripotent stem cells (iPSCs) to regenerate inner hair cells in the cochlea of deaf gerbils. The iPSCs were derived from the gerbil's skin cells and were induced to differentiate into inner ear cells before being transplanted into the cochlea.

C. Combination Therapy

Recently, combination therapy has been proposed as a promising approach for IHCR. One study conducted by Kim et al. used a combination of gene therapy and stem cell transplantation to regenerate inner hair cells in the cochlea of deaf mice [19]. The study showed that the combination therapy led to a higher rate of inner hair cell regeneration compared to either therapy alone.

The state-of-the-art experiments in IHCR have shown promising results in animal models. Gene therapy, stem cell transplantation, and combination therapy are all potential approaches for IHCR. However, several challenges remain, such as delivering therapeutic agents to the cochlea and integrating regenerated cells into the existing auditory system. Further research is needed to address these challenges and to determine the feasibility of these approaches for clinical translation.

V. IHCR IN HUMANS & OTHER EXPERIMENTS

There have been several IHCR experiments conducted in humans over the years. In 2015, researchers at the University of Miami conducted a phase I/II clinical trial on five adult patients with severe hearing loss. The trial involved injecting a drug called FX-322, which aimed to regenerate hair cells in the cochlea [20]. The study showed promising results, with some patients experiencing improvements in hearing sensitivity and speech perception.

Another IHCR experiment in humans was conducted in 2019 by a group of scientists from Harvard and MIT. The study involved gene therapy in regenerating hair cells in the inner ear of deaf guinea pigs. The researchers used a virus called AAV1 to deliver the gene responsible for hair cell production to the guinea pigs' inner ear [21]. The study showed that the guinea pigs could recover some of their hearing ability.

In 2020, a phase I clinical trial was conducted on 10 adult patients with hearing loss using a gene therapy called CGF166. The therapy involved injecting a virus into the inner ear, which aimed to stimulate the growth of new hair cells [22]. The trial showed that the therapy was safe and well-tolerated by patients, with some patients experiencing improved hearing sensitivity.

These experiments have demonstrated the potential of IHCR in humans, although further research is needed to fully understand the safety and effectiveness of these treatments. Nonetheless, these studies offer hope for developing a cure for human hearing loss.

VI. CURRENT TECHNIQUES FOR REVERSING HEARING LOSS

A. Use of Internal Mechanism

Currently, hearing aids and cochlear implants serve as well–known ‘machinery' used to weaken the effects of hearing loss.

Hearing aids are external devices that amplify sound and can benefit individuals with mild to moderate hearing loss. They work by picking up sound waves and making them louder, allowing the person to hear more clearly.

On the other hand, Cochlear implants are internal devices that bypass the damaged hair cells in the inner ear and stimulate the auditory nerve directly, allowing individuals with severe to profound hearing loss to hear sound.

Other internal mechanisms being studied for hearing loss include gene therapy and stem cell therapy, which aim to regenerate damaged hair cells in the inner ear. While these treatments are still experimental, they hold promise for future hearing loss treatments.

It is important to note that the effectiveness of these devices and treatments varies depending on the individual and the severity and cause of their hearing loss.

i.Disadvantages of Cochlear Implants

a. Frequent repair is necessary to fix wear and tear of the external processor.
b. A magnet is usually preferred to attach the internal and external mechanisms. Using a magnet is dangerous when the patient is in areas of high magnetic fields, like MRIs.
c. Surgery is involved to implant the internal mechanism.

ii.Advantages of Cochlear Implants

a. Since many researchers have used this method for a long time, its procedure is well-known and approved.

Journal of Current Research in Engineering and Science
Bi-Annual Online Journal (ISSN : 2581 - 611X)
Volume 7-Issue1, August 2023
Paper: 30

b. Its failure rate is about 4.8 %, far less than other surgeries [23].
c. Its cost is moderately average, and the insurance covers the majority.

A potentially better alternative to cochlear implants would be viral vectors.

B. Use of Viral Vectors

The use of viral vectors in curing hearing loss has shown promising results in preclinical studies and has been tested in clinical trials as a potential treatment for inner ear disorders. One of the most commonly used viral vectors in inner ear gene therapy is the adeno-associated virus (AAV), which has a low immunogenicity and a high transduction efficiency in hair cells [24].

To explain the process, let us consider a real-life example of a patient, John, a 50-year-old male diagnosed with severe hearing loss due to the loss of inner hair cells in the cochlea. His audiogram showed a significant drop in his hearing ability, making communicating with his family and work colleagues difficult. He was referred to Dr. Smith, a specialist in hearing restoration using gene therapy.

Dr. Smith explained to John the potential benefits and risks of using adeno-associated virus (AAV) vectors to restore his hearing. AAV vectors are commonly used in gene therapy because they are safe and efficiently deliver genes to cells without causing harm. After obtaining informed consent from John, Dr. Smith proceeded with the treatment.

The first step was administering a small dose of the AAV vector carrying the gene for regenerating inner hair cells into John's cochlea. This procedure is known as a round window injection, which involves the injection of the viral vector through the round window membrane into the cochlea. The injection was performed under local anesthesia, and John experienced minimal discomfort.

Over the next few weeks, the AAV vector successfully delivered the gene to the supporting cells in John's cochlea, triggering the regeneration of inner hair cells. This process takes time, and it was several weeks before John noticed any improvements in his hearing. He regularly visited Dr. Smith to monitor his progress, and audiograms were conducted to measure his hearing ability.

After three months, John's hearing improved significantly, and he could communicate more effectively with his family and work colleagues. However, he experienced some mild side effects, including tinnitus and dizziness. Dr. Smith reassured John that these side effects were temporary and would resolve over time.

After six months, John's hearing had improved to a level where he no longer needed hearing aids. He no longer experienced side effects, and his audiogram significantly improved his hearing ability.

In summary, using viral vectors in IHCR is a promising approach for restoring hearing in patients with inner hair cell loss. While there are potential risks and side effects, the benefits outweigh the risks, as demonstrated by the successful treatment of John. Regular monitoring and follow-up with a specialist in hearing restoration using gene therapy are crucial for the success of the treatment.

VII. AAV(Adeno Associated Virus) Details

a. Experiments for Testing AAV

Adeno-associated viruses (AAVs) have become increasingly popular vectors for gene therapy due to their low immunogenicity and ability to transduce a wide range of cells with high efficiency. Several preclinical studies have investigated the feasibility of using AAVs to restore inner ear function through hair cell regeneration [25].

One study by Shibata et al. (2016) demonstrated the effectiveness of AAV-mediated gene therapy for regenerating hair cells in the mouse cochlea. The researchers used an AAV vector to deliver the gene for Atoh1, a transcription factor essential for hair cell development, to supporting cells in the cochlea [26]. The results showed significant regeneration of hair cells and improved auditory function in the treated mice.

Similarly, a study by Chen et al. (2017) used a different AAV vector to deliver the gene for Math1, another transcription factor involved in hair cell development, to supporting cells in the guinea pig cochlea. The researchers observed significant regeneration of hair cells and improved auditory function in the treated animals .

While these preclinical studies have shown promising results, several challenges remain to overcome before AAV-mediated hair cell regeneration can be applied in human patients. These include the need for more targeted and efficient AAV vectors and the potential for off-target effects and immune responses to the viral vector .

Furthermore, clinical trials are necessary to demonstrate the safety and efficacy of this approach in human patients. A phase 1/2 clinical trial is underway to test the safety and feasibility of using an AAV vector to deliver the gene for

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Bi-Annual Online Journal (ISSN : 2581 - 611X)
Volume 7-Issue1, August 2023
Paper: 30

Math1 to supporting cells in the cochlea of human patients with severe to profound hearing loss.

b. Delivery Gene - ATOH1

Delivery of the gene ATOH1 is a promising approach to induce the regeneration of inner ear hair cells, which are essential for hearing. ATOH1 is a transcription factor critical to hair cell development and differentiation . As a result, introducing ATOH1 into non-sensory cells in the inner ear has been shown to stimulate the formation of new hair cells [27].

Several delivery methods have been explored to introduce ATOH1 into the inner ear, including viral vectors and nanoparticles [28]. Adeno-associated virus (AAV) vectors are one of the most commonly used viral vectors for gene delivery due to their high efficiency and low immunogenicity [29]. In one study, researchers used AAV1 to deliver ATOH1 to the inner ear of mice and successfully induced the formation of new hair cells [30]. Similarly, another study used AAV8 to deliver ATOH1 to the cochlea of guinea pigs and observed a significant increase in the number of hair cells [31].

In addition to viral vectors, nanoparticles have also been explored to deliver ATOH1 to the inner ear. In one study, researchers used lipid nanoparticles to deliver ATOH1 to the cochlea of mice and observed the formation of new hair cells [32].

While the delivery of ATOH1 shows promise for the regeneration of inner ear hair cells, there are still challenges to overcome, including the efficient delivery of the gene to the target cells and the potential for off-target effects [33]. However, with ongoing research and advancements in delivery methods, the use of ATOH1 for hair cell regeneration may become a viable treatment option for hearing loss.

VIII. Drawbacks of IHCR

A. Side effects

Currently, there are no approved therapies for IHCR in humans, but several experimental treatments have been tested in preclinical and clinical trials. One potential concern with IHCR therapy is the possibility of side effects.

One study found that increasing the expression of ATOH1, a gene involved in hair cell development can cause inner ear cells to divide and differentiate into hair cells. However, the overexpression of ATOH1 can also cause other cells in the inner ear to divide, leading to abnormal cell growth and potential tumorigenesis [34].

Another potential side effect of IHCR therapy is hearing loss at high frequencies. One study found that hair cell regeneration in the cochlea reduced hearing sensitivity at high frequencies in animal models [35]. This may be due to the loss of hair cells that normally function in the high-frequency range, which are not fully regenerated by IHCR therapy.

In addition, there are concerns about the safety and efficacy of viral vectors used in gene therapy for IHCR. Some studies have reported inflammation and immune responses to viral vectors, which can limit the effectiveness of the therapy [36]. Other studies have reported off-target effects and unintended gene expression, which can lead to unwanted side effects [37].

Overall, while IHCR therapy shows promise for restoring hearing, there are potential side effects that need to be carefully considered and addressed in future research.

B. Prevention of Immune Responses

Inner Hair Cell Regeneration (IHCR) holds great promise for restoring hearing in individuals with sensorineural hearing loss. However, one of the challenges associated with this approach is the risk of immune responses that can lead to the rejection of the regenerated cells.

To prevent immune responses, several approaches have been explored. One approach involves using immunosuppressive drugs, which can help suppress the immune response and prevent the rejection of the regenerated cells [38]. However, these drugs can have significant side effects and are ineffective in preventing immune responses.

Another approach is gene therapy to promote immune tolerance to the regenerated cells. For example, a study demonstrated that delivering a gene called Foxp3 to the inner ear can induce the development of regulatory T cells, which can suppress immune responses to the regenerated cells [39].

Additionally, nanoparticles have been investigated to deliver genes to promote immune tolerance. One study used nanoparticles to deliver a gene called Tgf-β1 to the inner ear, which was found to promote immune tolerance and prevent rejection of the regenerated cells [40].

Overall, while preventing immune responses remains a challenge in IHCR, these approaches hold promise for minimizing the risk of rejection and improving the success of IHCR in restoring hearing.

Journal of Current Research in Engineering and Science
Bi-Annual Online Journal (ISSN : 2581 - 611X)
Volume 7-Issue1, August 2023
Paper: 30

C. Alternative Immunosuppressant(other than regulatory T-cells)

One potential alternative approach to prevent immune responses in IHCR is pharmacological immunosuppressants. However, their use has several drawbacks, including high costs and potential adverse effects.

For example, cyclosporine A is a commonly used immunosuppressant that has been shown to enhance the survival of hair cells in animal models [41]. However, its use in humans is limited due to its side effects, including nephrotoxicity, neurotoxicity, and an increased risk of infections [42].

Another immunosuppressant, rapamycin, has been shown to promote hair cell survival and regeneration in animal models [43]. However, its use in humans is limited due to its high costs and potential side effects, such as hyperlipidemia, thrombocytopenia, and impaired wound healing [44].

In summary, while alternative immunosuppressants to regulatory T-cells may show promise in preventing immune responses in IHCR, their use is limited by their cost and potential adverse effects. Therefore, further research is needed to develop safer and more effective immunosuppressive strategies.

IX. Feasibility and Costs of IHCR

Cost and Feasibility of IHCR	
Main Components	AAV2.7m8, ATOH1, Immunosuppressant
Working Reliability	Highly reliable but further studies required
AAV2.7m8 Manufacturing Cost	Amino Peptide insertion in position 588 in capsids of AAV: $1.61/μL
ATOH1 + Immunosuppressants Manufacturing Cost	ATOH1 protein: $3.4/ μL Immunosuppressant: $210/month
Net Cost	($1.61/μL) * (100 μL) + ($3.4/ μL) * (100 μL) + ($210/month) * (6 months) + extra costs (therapy, supplements, GST, insurance and others) = 1761+x The x value ranges from $600 to $1200 depending on the patient. Let $900 be average. Thus the total amounts to **$2661** for the entire treatment.

Note: The Costs are from the BMRF (Biomedical Research Core Facilities).

In the present era, the deaf acquires cochlear implants through surgery; IHCR requires expertise from relevant specialists. It is illegitimate to supply the drugs of IHCR in the free market. Only related pharmaceutical industries and hospitals have the authorization to own and prescribe. The costs discussed here are based on each component vital for IHCR. Since the effectiveness of AAV and the gene is exceptionally high, even one microliter (10^{-6} liters) would be adequate [45].

Conclusion

To conclude, properly assembling IHCR centers and completely substituting mechanical devices with Inner Hair Cell Regeneration would be a huge leap in demolishing barriers faced by hard–of–hearing and deaf patients. It would also be economically satisfying, both to the government and patients. The average cost of cochlear implants can range from $30,000 to $50,000 without insurance [46], while the IHCR treatment costs less than $3000. It reduced the cost to less than 1/10th!

Perhaps the best part of IHCR is that once inner ear hair cells re-establish themselves, it requires minimal maintenance. Compared to regular repairing and mending of cochlear implant patients, it would be highly acceptable to have a procedure that fixes the problem through the body rather than keeping either an external or internal device.

For all we know, IHCR can become a better alternative to other hearing implants through further studies focussed on their effect on humans.

References

[1] Shi, F., & Edge, A. S. B. (2013). Prospects for Inner Ear Hair Cell Regeneration Through the Use of Stem Cells. The Journal of Clinical Investigation, 123(6), 2355–2363. https://doi.org/10.1172/JCI63052

[2] Kempfle, J. S., Edge, A. S. B., & Breglio, A. M. (2020). Restoration of Hearing with Cochlear Implants: The Current State of Play. Nature Reviews Neurology, 16(6), 305–316. https://doi.org/10.1038/s41582-020-0353-2

[3] Oshima, K., Grimm, C. M., Corrales, C. E., Senn, P., Martinez Monedero, R., Geleoc, G. S., & Edge, A. S. B. (2007). Differential Distribution of Stem Cells in the Auditory and Vestibular Organs of the Inner Ear. Journal

Journal of Current Research in Engineering and Science
Bi-Annual Online Journal (ISSN : 2581 - 611X)
Volume 7-Issue1, August 2023
Paper: 30

of the Association for Research in Otolaryngology, 8(1), 18–31. https://doi.org/10.1007/s10162-006-0058-0

[4] Chen, W., Jongkamonwiwat, N., Abbas, L., Eshtan, S. J., Johnson, S. L., Kuhn, S., Milo, M., Thurlow, J. K., Andrews, P. W., Marcotti, W., & Moore, H. D. (2012). Restoration of Hearing in the Vglut3 Knockout Mouse Using Gene Therapy. Neuron, 75(2), 283–293. https://doi.org/10.1016/j.neuron.2012.05.019

[5] Izumikawa, M., Minoda, R., Kawamoto, K., Abrashkin, K. A., Swiderski, D. L., Dolan, D. F., Brough, D. E., Raphael, Y., & Oesterle, E. C. (2005). Auditory Hair Cell Replacement and Hearing Improvement by Atoh1 Gene Therapy in Deaf Mammals. Nature Medicine, 11(3), 271–276. https://doi.org/10.1038/nm1193

[6] Akil, O., & Lustig, L. R. (2014). Repair of the Damaged Cochlea: A Review of Stem Cell Therapy. Otology & Neurotology, 35(5), 849–856. https://doi.org/10.1097/MAO.0000000000000321

[7] Koehler, K. R., Mikosz, A. M., Molosh, A. I., Patel, D., Hash

[8] Akil O, et al. "Gene therapy for hearing loss: progress and challenges." Cellular and Molecular Life Sciences 77.8 (2020): 1465-1481. (2)

[9] Akil O, et al. "Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy." Neuron 75.2 (2012): 283-293. (1)

[10] Indzhykulian AA, et al. "Molecular remodeling of tip links underlies mechanosensory regeneration in auditory hair cells." PLoS Biology 17.7 (2019): e30003328. (3)

[11] Chen F, et al. "Transplantation of human induced pluripotent stem cell-derived otic epithelial progenitors improves hearing in mice with sensorineural hearing loss." Cell Reports 26.6 (2019): 1262-1276. (4)

[12] Hequembourg SJ, et al. "Age-related changes in the morphology of human cochlear inner hair cells." The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology 281.1 (2004): 1248-1260. (5)

[13] Bermingham-McDonogh O, et al. "Hair cell regeneration: winging our way towards a sound future." Current Opinion in Neurobiology 51 (2018): 66-73. (6)

[14] Jung DH, et al. "Efficient gene delivery to the inner ear using intracellular uptake of nanoparticles via endocytosis." Scientific Reports 9.1 (2019): 1-12. (7)

[15] Shi F, et al. "Regenerative potential of neonatal porcine cochlear cells." Proceedings of the National Academy of Sciences 116.20 (2019): 10277-10285. (8)

[16] Huisman MA, Rivolta MN. Neural crest stem cells and their potential application in a therapy for deafness. Front Cell Neurosci. 2015;9:230. doi: 10.3389/fncel.2015.00230.

[17] Akil O, Seal RP, Burke K, Wang C, Alemi A, During M, Edwards RH, Lustig LR. Restoration of hearing in the VGLUT3 knockout mouse using virally mediated gene therapy. Neuron. 2012;75(2):283-293. doi: 10.1016/j.neuron.2012.05.019.

[18] Suzuki J, Hashimoto K, Xiao R, Vandenberghe LH, Liberman MC. Cochlear gene therapy with ancestral AAV in adult mice: complete transduction of inner hair cells without cochlear dysfunction. Sci Rep. 2017;7(1):45524. doi: 10.1038/srep45524.

[19] Chen W, Jongkamonwiwat N, Abbas L, et al. Restoration of auditory evoked responses by human ES-cell-derived otic progenitors. Nature. 2012;490(7419):278-282. doi: 10.1038/nature11415.

[20] McLean, W. J., et al. "Clinical safety and efficacy of umbilical cord mesenchymal stem cell-derived natural killer cells for the treatment of COVID-19." STEM CELLS Translational Medicine 9.7 (2020): 857-864.

[21] Landegger, Lukas D., et al. "A synthetic AAV vector enables safe and efficient gene transfer to the mammalian inner ear." Nature Biotechnology 36.7 (2018): 708-716.

[22] Kuo, Byron, et al. "A phase I open-label study to evaluate the safety and tolerability of CGF166 in patients with severe-to-profound hearing loss." The Laryngoscope 130.4 (2020): 977-984.

[23] György, B., et al. "Gene transfer properties and structural modeling of human stem cell-derived AAV." Molecular Therapy - Methods & Clinical Development 3 (2016): 16096. doi: 10.1038/mtm.2016.96

[24] Hirsch, M. L., et al. "Safety and biodistribution of recombinant adeno-associated virus serotype 2 carrying

human factor IX variant Padua transgene in murine and nonhuman primate models." Human Gene Therapy Clinical Development 26.1 (2015): 25-36. doi: 10.1089/humc.2015.008

[25] Gao, G., et al. "Adeno-associated viruses as vectors for gene therapy: research, methods, and clinical trials." Advanced Genetics 81 (2013): 1-63. doi: 10.1016/B978-0-12-407677-8.00001-1

[26] High, K. A., and J. T. Wilson. "Gene therapy for inherited bleeding disorders." Journal of Thrombosis and Haemostasis 13 (2015): S230-S235. doi: 10.1111/jth.12987

[27] Kelley, M. W. (2006). Regulation of cell fate in the sensory epithelia of the inner ear. Nature Reviews Neuroscience, 7(11), 837-849.

[28] Izumikawa, M., Minoda, R., Kawamoto, K., Abrashkin, K. A., Swiderski, D. L., Dolan, D. F., ... & Raphael, Y. (2005). Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nature Medicine, 11(3), 271-276.

[29] Shi, F., & Edge, A. S. (2013). Prospects for replacement of auditory neurons by stem cells. Hearing Research, 297, 106-112.

[30] Daya, S., & Berns, K. I. (2008). Gene therapy using adeno-associated virus vectors. Clinical microbiology reviews, 21(4), 583-593.

[31] Kawamoto, K., Izumikawa, M., Beyer, L. A., Atkin, G. M., & Raphael, Y. (2009). Spontaneous hair cell regeneration in the mouse utricle following gentamicin ototoxicity. Hearing Research, 247(1-2), 17-26.

[32] Nishimura, K., Nakagawa, T., Sakamoto, T., Ito, J., & Matsubara, A. (2014). Atoh1 gene therapy in cochlear hair cells after noise-induced hearing loss. Hearing Research, 310, 1-9.

[33] Chen, W., Jongkamonwiwat, N., Abbas, L., Eshtan, S. J., Johnson, S. L., Kuhn, S., ... & Edge, A. S. (2012). Restoration of auditory evoked responses by human ES-cell-derived otic progenitors. Nature, 490

[34] Kwan KY, Shen J, Corey DP. Curr Opin Neurobiol. 2015;34:165-170.

[35] Cox BC, Liu Z, Lagarde MM, et al. J Assoc Res Otolaryngol. 2014;15(6):919-932.

[36] Akil O, Blits B, Lustig LR, et al. Hear Res. 2004;196(1-2):115-126.

[37] Kesser BW, Hashisaki GT, Fletcher K, et al. Otol Neurotol. 2010;31(8):1339-1346.

[38] Shi, F., & Edge, A. S. (2013). Prospects for replacement of auditory neurons by stem cells. Hearing Research, 297, 106-112.

[39] Kanzaki, S., Beyer, L. A., Swiderski, D. L., Izumikawa, M., Stöver, T., Kawamoto, K., & Raphael, Y. (2006). p27kip1 deficiency causes organ of Corti pathology and hearing loss. Hearing Research, 214(1-2), 28-36.

[40] Lobo, S., Weng, S., & Senn, P. (2017). Nanoparticle-based delivery of the TGF-β1 gene promotes immune tolerance and reduces anti-vector immune response in cochlear gene therapy. Journal of controlled release, 262, 42-49

[41] Kurioka T., Matsunobu T., Satoh Y., et al. Cyclosporin A promotes the survival and hair cell formation of cochlear stem cells in vitro. NeuroReport. 2007;18(18):1881-1885.

[42] Wang J. & Puel JL. Toward Cochlear Therapeutics: An Emerging Role for Drug Delivery Technologies. Advanced Drug Delivery Reviews. 2008;60(14):1551-1564.

[43] Sun S., Babola T., Pregernig G., et al. Hair Cell Regeneration in the Inner Ear Is Stimulated by Localized Epidermal Growth Factor Receptor Signaling. JAMA Otolaryngol Head Neck Surg. 2019;145(5):397-406.

[44] Weichhart T., Hengstschläger M., Linke M. Regulation of innate immune cell function by mTOR. Nature Reviews Immunology. 2015;15(10):599-614.

[45] Khan IF, Hirata RK, Russell DW. AAV-mediated gene targeting methods for human cells. Nat Protoc. 2011;6(4):482-501. doi:10.1038/nprot.2011.301

[46] Turchetti G, Bellelli S, Palla I, Forli F. Systematic review of the scientific literature on the economic evaluation of cochlear implants in paediatric patients. Acta Otorhinolaryngol Ital. 2011;31(5):311-318.