Inner ear hair cell regeneration

A look from the past to the future

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Research Highlights

(1) Cochlear gene therapy has been successfully used in the treatment of sensorineural hearing loss. Use of atonal homolog 1 gene delivered by viral vectors contributes to inner ear hair cell regeneration.
(2) Various types of viruses have been successfully used as vectors for transporting genes in the cochlea.
(3) Embryonic and adult inner ear neural stem cells can differentiate into hair cells.

Abstract

Most recent studies on regeneration of inner ear hair cells focus on use of stem cells, gene therapy and neurotrophic factors. Cochlear gene therapy has been successfully used in the treatment of neurosensory hearing loss. This suggests that cochlear hair cell regeneration is possible. The objective of this paper is to review research and clinical application of inner near hair cell regeneration.

Key Words

neural regeneration; reviews; hearing loss; hair cells; cochlea; genes; stem cells; viral vectors; regeneration; neuroregeneration

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INTRODUCTION

Over the last two decades, great progress has been made in physiopathological research on neurosensory hearing loss. The discovery of inner ear hair cell regeneration has important clinical implications. The objective of this paper is to review literatures on theory and clinical application of inner ear sensory hair cell regeneration.

PIONEERING RESEARCH IN INNER EAR HAIR CELL REGENERATION

Epimorphic regeneration in animals has been recognized since the time of Aristotle (4th century B.C.) who described the tail regeneration in urodeles and reptiles. Many animals exhibit the ability to regenerate lost body parts after damage. Starfish[1], lizards[2], and amphibians such as salamanders[3] can spontaneously regenerate skin, muscle, nerve or bone after amputation. In mammals, several adult organs such as the skin[4], blood[5] or placenta[6] show a limited ability to regenerate. A landmark in human regeneration was found since it was observed that the distal phalanges of the hand of a child were able to regenerate after amputation[7]. This type of regeneration is called epimorphic regeneration. This phenomenon occurs in the presence of early mesenchymal

blastema cells and neural plate to origin correct innervations.

Moffat and Ramsden were the first authors who discovered the possibility of the auditory system in humans in 1977[8]. Their description is based on a 37-year-old man with malignant hypertension caused by bilateral chronic kidney failure who received a kidney transplant. In the postoperative period, he developed a staphylococcal infection and was administered 240 mg gentamicin over 10 hours. Twenty-four hours after gentamicin administration, the patient developed bilateral deafness. The first pure-tone audiometry test, 9 days after gentamicin administration, confirmed complete deafness in the left ear and severe neurosensory hearing loss in the right ear. Electrocochleography on day 13 showed a hearing threshold of 100 dB and very poor cochlear microphonics (0.64 μV at 110 dB). However, 3 weeks later, the pure-tone audiometry indicated an improvement in hearing at a frequency of 125–500 Hz and by 8 months the threshold of conversational frequency was around 70 dB. Further, in 1980, Fee[9] reported a series of 138 patients who were administered tobramycin and gentamicin. 55% of these patients recovered their hearing between 1 week and 6 months and 53% recovered their vestibular function between 10 days and 9 months after ototoxic treatment.

Cotanche[10] first referred the regeneration of inner ear hair cells of birds after noise-induced hearing loss. He found that the damaged cells were extruded from the epithelium and replaced by a layer of germinal basal cells. This process occurred rapidly: microvilli appeared on the apical surface of the epithelial tissue 48 hours after the noise-induced trauma, and hair cell regeneration was completed after 10 days of the trauma. Regeneration of the basilar papilla of birds after gentamicin intoxication was reported by Cruz and colleagues[11] who observed recovery of the hair cells 3 weeks after use of ototoxic.

Jørgensen and Mathiesen[12] were the first authors to note the capacity for regeneration of the normal vestibular epithelium in adult Australian parrots. Later, Roberson et al [13] studied the normal vestibular epithelium of 12-day-old white Leghorn chicks using tritiated thymidine and bromodeoxyuridine. Their findings with these cell markers suggested that the supporting cells are the precursors of type II hair cells. This regenerative process occurs continuously, even during adulthood and in the absence of stimulus, trauma or ototoxic damage of the epithelium. In this sense, we have described that in the embryonic vestibular epithelium of humans and of rats there seems to be a group of immature cells that are able to differentiate into sensory and supporting cells, depending on the metabolic requirements of the epithelium[14-18]. Therefore, it is currently believed that vestibular epithelium of birds and mammals are renewed depending on the regenerative capacity of the cells that survive to a particular injury.

López et al [19] studied hair cell recovery in the crista ampullaris of the chinchilla, following administration of a 50 μg dose of gentamicin to the perilymphatic space of the superior semicircular canal. They assessed four experimental groups compared to controls histologically using optical and transmission electron microscopy techniques. They recorded cell counts for hair and supporting cells. During 7–14 days after treatment, type I hair cells were not observed, while 85–88% of type II hair cells were lost. The number of supporting cells had decreased to 76% after 7 days, but recovered to 91% by day 14. By 1 month after ototoxic insult, there was a clear recovery of the epithelial cells, 83% of the cells being type II hair cells and 3% type I hair cells. The percentage of supporting cells was 86%, a level they considered close to normal. From days 14 to 28, the number of type II hair cells increased by 1758, indicating around 125 new cells per day, while the number of supporting cells remained steady. These results suggest that new hair cells are originated by the mitotic activity of supporting cells following proliferation and differentiation.

Later, Sliwinska-Kowalska et al [20] studied cell regeneration in the basilar papilla of 1-day-old white Leghorn chicks exposed to wide-band noise at 120 dB sound pressure level for 20, 40 or 72 hours, continuously or intermittently. They observed that initially there was damage in the tectorial membrane and short hair cells, similar to the outer hair cells in humans. Considering the proliferating cell nuclear antigen, cell proliferation activity was detected in the supporting cells and in the ganglion cells of the cochlear nerve. New cells appeared in the epithelium 5 days after noise-induced trauma. Further, Wooley and colleagues[21] studied hair cell regeneration and recovery of auditory thresholds in Bengalese finches after 1 week of daily dose of amikacin. While a normal morphological structure developed in 13 days, functional recovery continued up to 12 weeks after treatment. Results from a previous study[22] showed in 15-day-old white Leghorn chicks (Gallus domesticus) exposed to a 2 kHz pure tones at 120 dB sound pressure level for 48 hours, distortion-product otoacoustic emissions and brainstem auditory evoked potentials started to return to physio-

logically normal levels from 5 days after the noise-induced trauma, and reached full recovery after 30 days.

With regards to regeneration, Lee and Cotanche[23] described a series of factors that seem to stimulate the synthesis of DNA and induce cell proliferation and differentiation, including insulin-like growth factor 1, platelet-derived growth factor and basic fibroblast growth factor as well as the peptides associated with retinoic acid. On the basis of their findings, these authors suggested that basic fibroblast growth factor and retinoic acid may play an important role in the regulation of the regeneration mechanisms of the basilar papilla of chicks after noise-induced trauma.

RECENT ADVANCES IN INNER EAR HAIR CELL REGENERATION

Stem cells
Some scholars[24-26] recently described that both embryonic and adult inner ear stem cells can differentiate into hair cells. These scholars focused on confirming this differentiation, and assessed cell viability and *in situ* maturation. They confirmed that once implanted, stem cells can become integrated in the inner ear even in the early stage of development.

Vectors used in cochlear genetics
There is evidence that non-viral vectors, including plasmids, have advantages over other vectors of being less toxic and causing less inflammation, although they have lower transduction rate[27-28]. Further, in a recent study, Zhang et al [29] proposed the use of nanoparticles for transporting DNA-polylysine particles into the cochlea of mice.

Nevertheless, most studies focused on use of virus subtypes, for example, adenovirus, adeno-associated virus, herpes virus, helper-dependant adenovirus and lentivirus[30]. Of these virus subtypes, adeno-associated viruses have the greatest potential, given that they do not cause ototoxicity[30-32]. Various subtypes of adeno-associated virus have been successfully used for transporting genes in the cochlea and were found to cause little damage to the organ of Corti[33-34]. Kilpatrick et al [34] observed that adeno-associated virus serotypes 1, 2, 5, 6 and 8 have good gene expression in hair cells and basal cells, as well as the cochlear nerve and spiral ganglion. A disadvantage, however, is that these vectors can only carry fragments up to 5 kb, restricting their use for transduction.

Kesser and Lalwani[35] reported that adenoviruses overcome the limitations of adeno-associated viruses. A study published by Duan and Mi[36] indicates that lentiviral vectors do not spread beyond the cochlea, minimizing toxicity in the neighbouring tissue. As for how to deliver the viral vectors, most studies consider that the best method is cochleostomy, or direct injection through the round window membrane, using enzymatic digestion with collagenase[37].

Regeneration of hair cells with the atonal homolog 1 gene
The atonal homolog 1 or Math gene in mice codifies the transcription factor for sensory hair cell differentiation of cochlear basal cells[38-41]. Izumikawa et al[38] reported that atonal homolog 1 improved the hearing of deaf mice, achieving good results in both cellular and functional terms. Oshima et al [41], from Dr. Stefan Heller's laboratory, showed how to differentiate stem cells to hair cells with bundle structure and function. They used mouse embryonic stem cells and induced pluripotent stem cells, which were directed toward becoming ectoderm capable of responding to otic inducing growth factors. The resulting otic progenitor cells were subjected to varying differentiation conditions, one of which promoted the organization of cells into epithelial clusters displaying hair cell-like cells with stereociliary bundles. Bundle-bearing cells in these clusters responded to mechanical stimulation with currents that were reminiscent of immature hair cell transduction currents.

Gubbels et al [42] showed that *in utero* transfer of atonal homolog 1 gene produces functional supernumerary hair cells in the mouse cochlea. These hair cells were capable of mechanoelectrical transduction and showed basolateral conductance with age-appropriate specializations. Their results demonstrated that manipulation of cell fate by transcription factor misexpression produces functional sensory cells in the postnatal mammalian cochlea.

Studies carried out in mice with aminoglycoside-induced vestibular toxicity have also demonstrated that vestibular function is recovered due to hair cell regeneration, which is induced by the transcription of the Math-1 gene mediated by viral vectors[27, 43-45].

Gene therapy
For ototoxicity
Zheng et al [46] reported a study in newborn rats with the organ of Corti affected by aminoglycosides, in

which adeno-associated viral vector 2-mediated expression of activity-dependent neurotrophic factor-9 could protect the cochlea from aminoglycoside-induced impairment.

For genetic deafness

Gene therapy for genetic deafness poses the greatest challenge for cochlear gene therapy. There are few publications on this topic. Some scholars[47-48] used connexin 26 mutation as a model to attempt to improve hearing. They used animals in which deafness had been induced by introducing a defective gene and then used RNA interference to suppress this gene. Zhou *et al* [49] reported an experimental study demonstrating that a lack of interleukin-10 in mice generates a strong autoimmune response that causes hearing loss, while the transport of interleukin-10 to the cochlea improves hearing.

Neurotrophic factors for neuronal preservation in the spiral ganglion

Wise *et al* [50] explored the protection of brain-derived neurotrophic factor and neurotrophin 3 gene therapy against spiral ganglion neuronal degeneration in animal models. They found that cell regeneration in the cochlea is possible, even when there is severe damage to the organ of Corti. Shibata *et al* [32] found similar results in guinea pigs, using the same factors mediated by adeno-associated viruses. Wu *et al* [51] investigated the effect of a human growth factor gene mediated by adenovirus in deaf mice and found that the group that underwent gene therapy had milder hearing impairment than a control group.

Nevertheless, some of these potential treatments may have unfortunated adverse effects. Specifically, some scholars[52-53] have stated that these treatments could initiate tumours. Accordingly, more studies on the cochlea in the mammalian models are needed to test the potential of these treatments for deafness in humans.

CONCLUSION

There are still several questions to be addressed in the clinical implications of neurosensory hearing loss:

Which are stem cells in this area and how can they be identified? Is the destiny of the stem cells predetermined? What are the stimuli for regeneration? How is cell replacement achieved? Is it possible to identify or to de-velop a "biological implant" that could help in the treatment of neurosensory hearing loss?

Taken together, cochlear gene therapy has been successfully used in the treatment of neurosensory hearing loss and other inner ear disorders. Greatest progress will be achieved, in the near future, in the regeneration of hair cells after use of atonal homolog 1 gene delivered by viral vectors and this may become the best clinical treatment method of certain types of hearing loss.

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