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REVIEW ARTICLE

Regeneration in the mammalian inner ear: A glimpse into the future

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Abstract
It is well known that the adult mammalian cochlea lacks regenerative capacity, and that the consequence of inner ear damage in humans is permanent sensorineural hearing loss. Since the discovery that hair cells can regenerate in birds, a broad range of studies and research projects has been designed in order to understand this process and to extend it to the mammalian inner ear. The aim of this review is to evaluate the possible future directions and targets of mammalian inner ear regeneration offering an analysis of possible future scenarios.

Key words: *regenerative medicine, mammalian inner ear, inner ear stem cells, inner ear gene therapy*

Introduction

Deafness is one of the most widespread disabilities in the world, as it has been estimated that 33 children are born every day in the USA with a significant hearing loss (National Institute on Deafness and Other Communication Disorders). It has also been reported that hearing impairment affects up to 30% of the people resident in developed countries. Estimates indicate that 70 million persons are deaf and that at least 900 million people in the developed world will be affected by age related hearing loss by 2050 (1). In this setting, social demand for the development of new therapeutic approaches for hearing loss is still growing.

Sensorineural hearing loss is the consequence of the loss of sensory hair cells and their associated innervations. These cells are susceptible to damage from a variety of sources including ageing, genetic defects, noise or chemotherapeutic drugs, and once damaged are not renewable in mammals. Thus, the regeneration of the functional epithelium that extends along the mammalian cochlea and of the functional spiral ganglion neurons, is an important goal of inner ear research (2–4).

The aim of the present review is to present the possible future directions and targets of mammalian hair cell (HC) regeneration, examining recent progress in this field of research.

Evidence of stem cells in the mammalian inner ear

To date, many attempts have been made from research laboratories worldwide to discover if there are resident stem cells within the mammalian cochlea.

While hair cells (HCs) of most non-mammalian vertebrates (i.e. fishes, amphibians and birds) can regenerate after an injury, in humans, as in most mammals, HCs are formed during a limited period of embryonic development; they cannot be regenerated if lost later in life as a consequence of acoustic trauma, treatment with ototoxic drugs, infections or autoimmune pathologies, or as part of the ageing process. The loss of HCs from the human cochlea (and/or spiral ganglion neurons) is then the cause of a permanent hearing deficit (5–12).

Thus, research on factors that allow and regulate sensory regeneration in the ear epithelium of non-mammals, as well as those that inhibit such regeneration in mammals, is clearly of great biological (and clinical) interest. In particular, some of the

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anatomical events that occur during regeneration in the avian ear have already been described, but still the precise signalling events and the molecular processes that initiate and regulate the regenerative proliferation remain to be identified. In addition, events that occur during mammalian inner ear development are currently under investigation (2–4,13–18).

With regard to the discovery of resident stem cells within the mammalian cochlea, results published to date have shown the presence of a small cluster of stem/progenitor cells within the adult utricular epithelia (19). This conclusion was reached by demonstrating that a subset of isolated cells from an adult mouse utricle can proliferate and form spheres, a feature of stem cell identity (19,20). Moreover, the progeny of these spheres were also shown to be multipotential with the ability to give rise to a variety of cell types, including hair cells when transplanted into an appropriate environment, such as the developing chick otocyst (19,20).

Another recent study was conducted to determine whether similar cells are also present within the mouse cochlear epithelium. Using a similar technique, sphere-forming cells have been isolated from neonatal cochleae of mice (21). However, it has been reported by the same researchers that the number of cochlear cells with the ability to form spheres declines rapidly as, by the third postnatal week, sphere-forming cells can no longer be isolated, suggesting that stem cells are no longer present within the adult cochlear epithelium (20,21).

If resident stem cells could be ever found in the adult mammalian cochlea, then it would be possible to imagine that, in future, researchers will be able to reach, achieve and reactivate them in some way, and possibly drive them to repair an eventual cochlear damage.

Transdifferentiation or transplantation?

Since a cluster of resident stem cells has not been identified in the adult mammalian cochlea (to date), two main strategies have been proposed and developed for replacing HCs in the inner ear. These are:

1) transferring the cochlear epithelium (gene therapy), thus inducing the non-sensory cells to transdifferentiate into new hair cells;
2) introducing stem cells into the cochlea (stem cell transplantation).

Both gene therapy and stem cell transplantation seem to be very promising fields. Both these pathways could be of particular interest, especially as resident stem cells will not be identified in the adult mammalian cochlea (or if it would not be possible to reactivate them, when identified). Nonetheless, both pathways present several points needing consideration.

When considering the first strategy (gene therapy), in order to transdifferentiate the non-sensory cells of the human organ of Corti epithelium, it is necessary to understand the signalling events that regulate the proliferation and differentiation of these cells (2–4). Identification of the precise molecular events that regulate HC as well as non-sensory Corti cells' morphogenesis during mammalian embryogenesis, is the basis for identifying and revealing molecular targets to trigger the cellular transdifferentiation process (2–4).

Thus, in recent years, impressive progress has been made in the understanding of the genes that regulate the proliferative and differentiation processes of mammalian auditory sensory and non-sensory cells. In particular, it has been shown that many regulatory genes are expressed transiently during development and that the tissue environment changes dramatically with time, so that the adult organ of Corti has a different molecular profile compared to that of the earlier developmental stages (2–4,22,23). Inner ear morphogenesis is controlled by an orchestrated interplay of cell proliferation and regional apoptotic cell death. The most detailed studies come from mice (2–4,22,23). For an exhaustive overview of inner ear mouse development genes please refer to the Holme, Bussoli and Steel table (24) of gene expression in the developing ear.

A major drawback regarding the application of this strategy is the possible uncontrolled transdifferentiation process that could involve all the cells within the cochlea once the genes are introduced. It is particularly difficult to guess how the supporting cells transdifferentiation process could be organized (e.g. is infection of the whole group of supporting cells better than a specific type, i.e. pillar cells?), and then how those newly formed HCs can recreate contacts with spiral ganglion neurons (2–4,25–29).

When considering the hypothesis of introducing stem cells (SC) into the cochlea (stem cell transplantation), it is necessary to understand several topics, in particular:

1. study of the differentiation of different stem cells types available into hair cells, and then identifying the most suitable ones;
2. finding the most appropriate ways to introduce stem cells into the cochlea;
3. designing strategies for integrating the new transplanted cells into the damaged sites of Corti epithelium (2–4).

1. It is necessary to understand the stepwise mechanisms that regulate the generation of new hair cells from a renewable source of

progenitors either using embryonic SCs or adult SCs. In this manner, Heller et al. presented an experimental protocol to routinely create inner ear progenitors from murine embryonic stem cells in vitro (30). They demonstrated that murine ESCs could be directed into hair cells by applying 'signals' that are involved in their normal development differentiation.

2. Introducing SC into the cochlea is not simple. A cochleostomy is necessary. To date, SCs have been experimentally injected into the scala tympani, with an average survival time of a few weeks (4,19,20). However, we need to introduce these cells within the cochlear duct without damaging the cyto-architectural and physiological characteristics of the cochlea and its endocochlear fluid dynamic. Moreover, it will be also be crucial to identify permissive factors that could support and maintain these transplanted cells within the endolymphatic environment, due to its high concentration of KCl (2–4,31,32).
3. The other important challenge is to integrate SCs into the mature organ of Corti in vivo. The functional replacement of hair cells is complex because they are highly structurally specialized and need to be replaced with micron accuracy in order to be coupled to the sound stimulus (2–4).

Thus, to accomplish integration into the cochlear epithelium, it may be necessary to:

- identify the exact lesion site within the organ of Corti;
- engineer the SC to find it;
- structurally integrate the SC into the Corti epithelium;
- functionally integrate the SC.

Doubts regarding the possible application of stem cells transplantation came from the possible uncontrolled proliferation of SCs (SCs are likely candidates for accumulating multiple mutations that can disrupt their tight control leading to tumorigenesis) (20), and from the immune response that the host can achieve against SCs (33–35).

Most promising research areas

There are some very promising research areas in the field of inner ear regeneration, as indicated by the most recently available studies. Researchers from the University of Sheffield have now shown functional recovery (Auditory Brainstem Responses thresholds recovery), as well as morphological repair (from histological and immunostaining evidence), on a deafened gerbil model transplanted with human embryonic stem cells. It had already been demonstrated that embryonic stem cells can differentiate into auditory nerve cells before, however, for the first time, transplanted embryonic stem cells had successfully restored hearing in this animal model (36).

Another very interesting work is that of Hashino et al. They established a new in vitro model of inner ear differentiation, demonstrating a stepwise differentiation of inner ear sensory epithelia from mouse embryonic stem cells using a three-dimensional culture. They also showed that regenerated hair cells have the morphology and functional properties of native mechanosensitive hair cells (37).

Very encouraging are also the data coming from one of the latest papers of Albert Edge et al. They demonstrated for the first time that hair cells can be regenerated in an adult mammalian ear by inhibiting a gamma-secretase enzyme involved in the Notch signalling pathway. This stimulates resident supporting cells to differentiate into new hair cells, also resulting in partial recovery of hearing in mouse ears damaged by noise trauma (38).

Cheng et al., at Stanford University, have identified a group of progenitor cells in the inner ear that can become sensory hair cells and therefore enable hearing. They have shown that tympanic border cells beneath the sensory epithelium have been found to be proliferative also in the post-natal cochlea and could therefore act as precursors to sensory epithelial cells in the adult mammalian cochlea (39).

Finally, the American Speech and Language Association website has recently announced that the Food and Drug Administration (FDA) has approved an innovative trial (phase I trial) in order to evaluate the safety of using a child's own cord-blood stem cells to regenerate inner ear cells with the aim of restoring the child's hearing (40). The idea of researchers from the Children's Memorial Hermann Hospital in Houston is to evaluate the safety of an autologous umbilical cord-blood transplant for children affected by sensorineural hearing loss, since previous experiments have demonstrated encouraging results on animals (41,42). However, hair cell regeneration in human beings is not really established as yet.

Speculations on a 'regenerated cochlea': restoring function of the newly generated inner ear

Regenerative medicine has raised the hope that perhaps a cure for sensorineural hearing loss will be available in the future. However, it is still difficult to predict how (and when) stem cell and/or gene therapy will be translated into clinical practice, as

most researchers believe that decades still divide experimental research from clinical applications. In any event, assuming that it will be possible to regenerate the Corti epithelium, there are still many issues that have to be resolved.

It will be interesting to understand how a regenerated cochlea could work, considering the cochlear mechanical and dynamic aspects (i.e. reproducing the active role of outer hair cells in transducing sound stimuli). The functional replacement of hair cells is complex because they are highly structurally specialized. Since the organ of Corti cytoarchitecture will be modified, either by the damaging insult or by the regenerative process, it is difficult to predict how the mechanics and dynamics of the regenerated cochlea will manifest themselves. In particular, it is difficult to comprehend if and how regenerated HCs could couple effectively with spiral ganglion neurons on one side and with the tectorial membrane on the other. Furthermore, how the functional differences existing in the normal cochlea between the outer and the inner hair cells can be reproduced, is another interesting issue (2–4).

It will be also necessary to determine which hearing level it would be possible to achieve by applying regenerative techniques. For instance, would it be possible to completely 'cure' a dead ear, so regenerating completely the cochlear epithelium, the relative neuronal connections and then obtaining total hearing recovery?

Other considerations may arise from the pathophysiology of the cochlear damage. It is likely that different inner ear pathologies may affect HCs differently; thus, for instance, a dead zone may necessitate a different (regenerative) approach from a damage that extends along the whole cochlea. Perhaps a dead zone could be successfully treated with a selective transplantation, while an extended cochlear damage could be better cured with gene therapy (a transdifferentiation approach). In addition, the time that has occurred between the onset of damage and the regenerative intervention could influence the choice of regenerative approach: older and extensive cochlear damage may need stem transplantation in order to recreate neuronal pathways, while recent damages could be repaired/regenerated by transfecting selectively and then 'reactivating' injured cells.

Another question concerns the lifetime of newly generated HCs: how long would new HCs be functional? Would it be possible, once cochlear regeneration process has occurred, to use the newly generated cochlea for the whole lifespan or is it likely that SCs are possible candidates for accumulating multiple mutations that can disrupt their tight control leading to loss of function and at worst to tumorigenesis (34)? Again, in terms of time, how much time would be necessary in order to have a fully serviceable, newly regenerated cochlea?

These, and many more questions, need to be addressed by researchers in the coming years.

In addition, we also believe that a combination of conventional therapies such as cochlear implantation, with regenerative strategies such as regeneration of spiral ganglion neurons (i.e. gene therapy and cochlear implants with delivery systems), should also be considered (20).

In conclusion, recent progress in the fields of stem cell biology and regeneration has been more rapid and remarkable than at any other time. Even if there is still much work to be done, we believe that in future stem cell therapy and regenerative approaches to the inner ear will become available.

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