

Chapter 2

Therapeutic Targets and Possible Strategies for Regenerative Medicine for the Inner Ear

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Abstract Regenerative medicine aiming for the functional recovery of the inner ear has several targets for therapeutics in tissue, cells, or cell organelle. Among various therapeutic targets, sensory hair cells have been paid considerable attention because of their importance in inner ear functions. At present practical methods for hair cell regeneration have not been developed. However, experimental studies have revealed possible strategies for regeneration according to development of new technologies. This chapter reviews therapeutic targets for regenerative medicine in the inner ear and possible strategies to realize regeneration of the inner ear.

Keywords Cell transplantation • Dedifferentiation • Self-repair • Technological regeneration • Transdifferentiation

2.1 Therapeutic Targets

The inner ear consists of two components from the point of view of function. The cochlea is an organ corresponding to hearing and the vestibules and semicircular canals are organs for vestibular function. In the cochlea, the sensory hair cell is included in main targets for the treatment of sensorineural hearing loss (SNHL). Hair cell regeneration has long been a central issue in research for inner ear regeneration, because hair cells are crucial for conversion of sound stimuli to neural signals, and studies of human temporal bones have demonstrated that the degeneration of hair cells is a main etiology for SNHL. The primary step for conversion of

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sound stimuli to neural signals is the tilt of stereociliary bundles located at the top of hair cells. Therefore, the stereocilia is a crucial cellular component of functional hair cells. In addition, for the tilt of stereocilia, the presence of the tectorial membrane is critical. The second step for conversion of sound stimuli to neural signals is the depolarization of hair cells, which requires a high concentration of potassium ions in the endolymph and several ion channels in hair cells. In the maintenance of high potassium in the endolymph, the function of the cochlear lateral wall including the stria vascularis and spiral ligament is inevitable. The third step is the release of neurotransmitters from hair cells to afferent dendrites of spiral ganglion neurons through synaptic contacts. At synaptic contacts between inner hair cells and afferent dendrites of spiral ganglion neurons, inner hair cells have specific organelle, ribbon synapses at their bottom. Considering regeneration of hair cells with normal function, regeneration of several cellular components of hair cells is necessary, and several cochlear components should be regenerated. All of these cellular components and cochlear components are included in therapeutic targets. An ultimate goal is complete regeneration of hair cells with necessary cellular components. However, even regeneration of one important component in hair cells would be efficacious for hearing recovery if other important components are maintained.

Beside hair cells, there are a number of cochlear components that are included in therapeutic targets. As mentioned above, the stria vascularis and spiral ligament in the cochlear lateral wall should be included in therapeutic targets. These components have a network of the gap junction, which is crucial for the maintenance of high potassium in the endolymph. Mutation of genes associated with the gap junction is the most frequent cause of congenital SNHL. Insufficient formation of the tectorial membrane also causes congenital SNHL. The spiral ganglion neuron is also an important therapeutic target. Spiral ganglion neurons play a crucial role in the transmission of auditory signals from hair cells to the central systems. Additionally, their loss diminishes clinical benefits of cochlear implantation, which is a medical device for restoration of hearing in patients with profound SNHL.

In the vestibular end organs, the hair cell is also a central player in their functionality similarly to the cochlea. Although adaptive frequencies of vestibular hair cells are quite different from cochlear hair cells, vestibular hair cells also convert mechanical stimuli to neural signals. The tilt of stereocilia in vestibular hair cells is induced by the movement of the cupula, a gelatinous component located above the stereocilia of vestibular hair cells. The otolith organs additionally have the otoconia, small particles composed of a combination of a gelatinous matrix and calcium carbonate on the cupula. The tilt of stereocilia in vestibular hair cells induces the depolarization of vestibular hair cells leading to the release of neurotransmitters into the synaptic contacts between hair cells and afferent dendrites of vestibular ganglion neurons. In the vestibular end organs, hair cells, cupula, and otoconia, vestibular ganglion neurons can be therapeutic targets. Synaptic contacts between hair cells and vestibular ganglion neurons are also included in therapeutic targets.

2.2 Possible Strategies

Recent studies have demonstrated that the mammalian inner ear has the capacity for regeneration, although it is limited. The presence of stem cell-like cells in the mammalian inner ear has been reported [1–4]. Some reports have demonstrated functional restoration of mammalian inner ear [5, 6]. However, the quality for the functionality of regenerated inner ears is not satisfactory. Further investigations are required before clinical application. Investigations for regeneration in the mammalian inner ear have been done referring to findings in other vertebrates including birds, in which hair cell regeneration occurs, or findings in developmental processes of the mammalian inner ear. Here we introduce possible strategies for induction of regeneration according to the stage or level of degeneration in the inner ear (Fig. 2.1). The sensory hair cells and spiral ganglion neurons have been primary targets for studies of inner ear regeneration. Therefore, regeneration of hair cells and spiral ganglion neurons is used as a model for discussion on possible strategies.

2.2.1 *Self-Repair*

Before hair cells or spiral ganglion neurons disappear, the induction of self-repair may be a pragmatic strategy. For this purpose, there are two possible strategies. One is the promotion of survival of hair cells and subsequent reconstruction of cellular components in hair cells by spontaneous activity. This can be expressed as the protection from cell death. Several agents for promotion of survival or protection from cell death have been reported. Some of such candidates have been examined for their efficacy and safety in clinical trials [7, 8]. On the other hand, there is no specific report describing the induction of reconstruction of cellular components in damaged hair cells. In spiral ganglion neurons, reconstruction of synaptic contacts with the inner hair cells or cochlear nucleus neurons is a key issue in regeneration of cellular components. For this purpose, further investigations should be required to reveal mechanisms for maturation of functional hair cells and spiral ganglion neurons.

2.2.2 *Transdifferentiation*

After hair cells have gone, three different possible strategies can be applied depending on the condition of the remaining supporting cells. If sufficient numbers of healthy supporting cells still remain in the sensory epithelium, the induction of transdifferentiation of supporting cells to hair cells can be a strategy for hair cell regeneration. Hair cells and supporting cells share a common progenitor in the development. In fate determination of progenitor cells in the sensory epithelium,

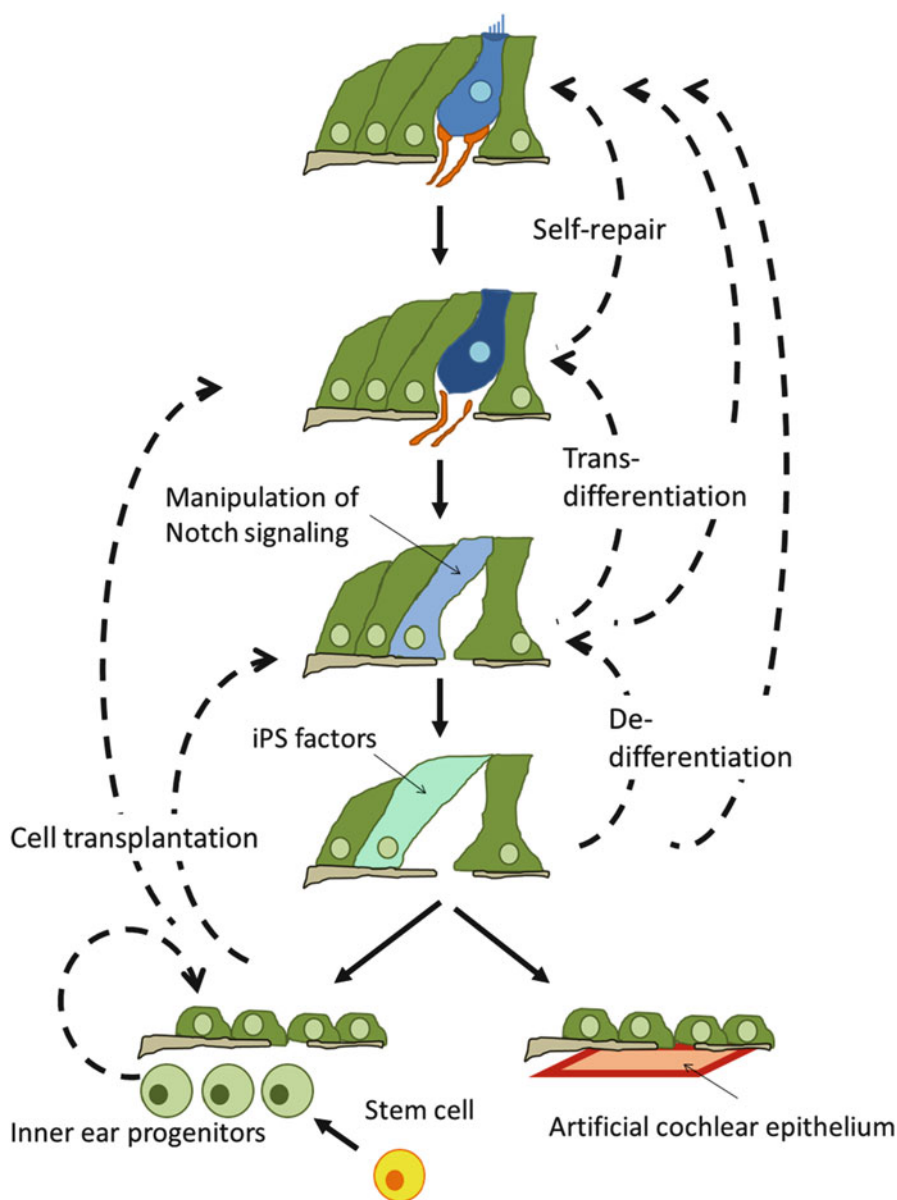


Fig. 2.1 Schematic drawing of the strategies for hair cell regeneration

the Notch signaling plays a key role [9, 10]. The manipulation of Notch signaling has been used for induction of transdifferentiation of supporting cells. Transdifferentiation of supporting cells to hair cells was firstly demonstrated by means of gene transfer. Introduction of *Atoh1* gene into supporting cells using

adenovirus vectors induced transdifferentiation of supporting cells into hair cells [11, 12]. In general, the inhibition of Notch signaling increases the expression of *Atoh1*, and its activation suppresses *Atoh1* expression. Next to gene transfer, pharmacological inhibition of Notch signaling was used for this purpose. Pharmacological inhibition of Notch signaling by gamma secretase inhibitors induced an increase of *Atoh1* expression in neonatal cochleae, leading to excessive generation of hair cells [13]. The activity of Notch signaling weakens in the supporting cells according to maturation. In adult cochleae, virtually no expression of Notch ligands and receptors was identified in supporting cells. However, during certain duration after damage, temporal activation of Notch signaling was found even in adult cochleae [14, 15]. Topical application of gamma secretase inhibitors into cochleae resulted in hair cell induction [6]. However, at present hearing restoration by this approach is not satisfactory, and the therapeutic time window is limited.

2.2.3 *Dedifferentiation*

In case that insufficient numbers of supporting cells remain, transdifferentiation is not an effective strategy. Transdifferentiation of one supporting cells is equal to the loss of one supporting cells. In such a case, induction of proliferation in supporting cells is necessary. In the mammalian cochlea, cell proliferation rarely occurs after birth, while in the avian cochlea (basilar papilla) supporting cells proliferate in response to hair cell loss [16]. In the avian cochlea, both transdifferentiation of supporting cells to hair cells and proliferation of supporting cells followed by differentiation into hair cells occur [16]. To induce proliferation of supporting cells in the mammalian cochlea, the downregulation of cell cycle inhibitors is necessary. One of the major cell cycle inhibitors in mammalian supporting cells is p27, an inhibitor of cyclin-dependent kinase. Genetic deletion of p27 resulted in excessive generation of hair cells [17, 18]. Knockdown of p27 in supporting cells induced reentry of cell cycle in supporting cells, but the majority of supporting cells that had reentered cell cycle fell into apoptosis [19]. Thus, cell cycle reentry of supporting cells is not sufficient for regeneration of hair cells, suggesting that alterations in characteristics of supporting cells may be critical. Recently, challenges for induction of dedifferentiation in supporting cells have been reported [20, 21]. One possible strategy for dedifferentiation is introduction of transcription factors for generation of the iPS cell. Detailed analysis of mechanisms for alterations in characteristics of supporting cells in the avian cochlea after hair cell loss could provide useful information to induce dedifferentiation of mammalian supporting cells.

2.2.4 Cell Transplantation

Another option in case that supporting cells are severely damaged is the introduction of exogenous stem cells into the inner ear. In the early 2000s, the approach of cell transplantation for hair cell regeneration has gained considerable attention, because stem cells have been believed to accumulate in damaged sites and have the potential to repair damaged organs. However, migration of transplanted stem cells into damaged sensory epithelia of the inner ear rarely occurred [22]. In addition, the circumstance of the endolymphatic space in the inner ear is hard for the survival of transplants, because of its high concentrations of potassium. On the other hand, recent progress in research for induction of pluripotent stem cells for differentiation into inner ear cells has demonstrated that generation of sensory hair cells from pluripotent stem cells is possible. In the near future, specific guidance cues for hair cell induction from pluripotent stem cells would be discovered. As for spiral ganglion neurons, a cell transplantation approach is realistic comparing with hair cells. Recently, functional restoration of spiral ganglion neurons by transplantation of human ES cell-derived neural progenitors has been reported [5]. The iPS cells have a similar potential for regeneration of spiral ganglion neurons to ES cells [23]. However, the use of iPS cells involves the risk of tumor formation [24]. To realize cell-based therapy for regeneration of spiral ganglion cells, autologous transplants that are fully differentiated and completely eliminated undifferentiated cells must be used.

2.2.5 Technological Regeneration

As an alteration of biological approach for hair cell regeneration, an artificial cochlear epithelium has been investigated as technological regeneration. A cochlear sensory epithelium converts mechanical vibration to electrical signals. In response to mechanical stimuli, a piezoelectric material generates electricity and so could be used in place of a cochlear epithelium as a bionic cochlear epithelium. A thin membrane of a piezoelectric material framed with silicon generated electricity in response to sound stimuli after implantation into a guinea pig cochlea [25]. Problems to be resolved included insufficient power of the device to stimulate spiral ganglion neurons and limited sensitivity for sound frequencies. A combination of technological and biological approaches may be required to resolve these problems. Neurite induction from spiral ganglion neurons to the device by gene therapy might contribute to reduction of required electrical power for stimulation of spiral ganglion neurons.

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