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Direct cellular reprogramming and inner ear regeneration

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Abstract

Introduction: Sound is integral to communication and connects us to the world through speech and music. Cochlear hair cells are essential for converting sounds into neural impulses. However, these cells are highly susceptible to damage from an array of factors, resulting in degeneration and ultimately irreversible hearing loss in humans. Since the discovery of hair cell regeneration in birds, there have been tremendous efforts to identify therapies that could promote hair cell regeneration in mammals.

Areas covered: Here, we will review recent studies describing spontaneous hair cell regeneration and direct cellular reprograming as well as other factors that mediate mammalian hair cell regeneration.

Expert opinion: Numerous combinatorial approaches have successfully reprogrammed non-sensory supporting cells to form hair cells, albeit with limited efficacy and maturation. Studies on epigenetic regulation and transcriptional network of hair cell progenitors may accelerate discovery of more promising reprogramming regimens.

Keywords

Cochlea; Hair cell; Hearing loss; Supporting cell; utricle

1. Introduction

The cochlea is an exquisite sensory organ dedicated to transducing sounds into neural impulses. The organ of Corti is critical for this function, precisely arranged with four rows of mechanosensory hair cells intercalated with non-sensory supporting cells. In mammals, lost hair cells are not regenerated, leading to irreversible hearing loss. Clinical therapies that are currently available include hearing aids and cochlear implants, the latter being a device, which aims to replace the role of the hair cells by providing electrical signals directly to the spiral ganglion neurons. To date, biological therapies have been unsuccessful in fully

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Declaration of Interests

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regenerating hair cells and restoring auditory function; however, recent advances have revealed the potential for native supporting cells to serve as an endogenous source for hair cell regeneration. In this review, we will discuss spontaneous hair cell regeneration in the cochlea and utricle, the competence of supporting cells to act as hair cell progenitors, and finally, direct cellular reprogramming approaches to coerce supporting cells to undergo cell fate change.

2. Hearing Loss

Over 6% of the world's population (around half a billion people) suffer from disabling hearing impairment [1]. This prevalence is expected to continue to grow as the population ages, doubling the number of adults in the United States affected by hearing loss in the coming decades [2]. The social, emotional, and economic impact that hearing impairment places on individuals and society at large is significant. The exclusion from spoken communication, for example, as a result of hearing loss has adverse effects on day-to-day life, particularly for children who can experience a delay in language development [3]. Moreover, there is a growing body of evidence associating hearing loss with cognitive dysfunction in the elderly, including a connection between hearing loss and dementia [4, 5].

The most common pathological finding of hearing impaired patients postmortem is damage to the cochlear sensory epithelium, primarily the loss of sensory hair cells, as well as a reduction in the number of spiral ganglion neurons [6, 7]. This loss of hair cells can arise from a number of causes ranging from side effects of therapeutic agents (such as aminoglycoside antibiotics and chemotherapy drugs), noise trauma, genetic disorders, and aging. Currently, the only treatments available are hearing aids and cochlear implants. These devices can provide significant benefits to patients, particularly when implanted early, with children implanted before the age of 4 performing as well as post-lingual deaf adults on open-set tasks, allowing them to enter the mainstream education system with their normal hearing peers [8]. Despite their success, however there remains wide variations in patient outcomes and many of them lack adequate access to treatment. Importantly, the devices fail to reverse the underlying pathology of hair cell loss. To complement or move beyond a device-based treatment, studies have begun to examine potential biological approaches to regenerating the cochlear sensory epithelium after insult. It is important to note, however, that hair cell loss is not the only pathology causing hearing loss, and as such hair cell regeneration is only one of many possible therapeutic approaches. For example, studies have examined regeneration of other cell types such as the spiral ganglion neurons and supporting cells, and a growing body of literature has examined the promising approach of correcting genetic mutations within hair cells and supporting cells [9, 10, 11, 12].

3. Cochlear Sensory Epithelium

The mammalian cochlear sensory epithelium consists of an orderly arrangement of hair cells and surrounding supporting cells. Hair cells consist of one row of inner hair cells, the primary sound transducers, and three rows of outer hair cells, which act as amplifiers of low-level sounds [13]. The inner and outer hair cells are separated by the inner and outer pillar cells, supporting cell subtypes that together form the tunnel of Corti (Figure 1). Other

subtypes of supporting cells include the Deiters' cells and inner phalangeal cells that underlie and surround the outer hair cells and the inner hair cells, respectively. As transducers of the cochlea, sensory hair cells are sensitive to a wide variety of insults. After severe damage and extensive hair cell loss, dramatic remodeling of the sensory epithelium can occur [14, 15]. For example, after aminoglycoside-induced hair cell loss, supporting cells expand to form a scar-like, flattened epithelium composed of non-specialized cells [15]. The dramatic change in the milieu and architecture of the cochlear sensory epithelium after damage may in part limit its ability to regenerate hair cells, a topic that will be discussed in the following section.

4. Spontaneous Hair Cell Regeneration

A number of studies, beginning with two seminal papers from nearly 30 years ago showed that hair cells of the avian basilar papilla (analogous to the mammalian cochlea) and utricle can regenerate hair cells from underlying supporting cells [17, 18, 19, 20]. Supporting cells have since been labeled as hair cell progenitors, and the process of regeneration can be broadly characterized to occur via mitotic and non-mitotic mechanisms. Mitotic regeneration involves supporting cells undergoing cell division prior to differentiating into hair cells. Conversely, non-mitotic regeneration, also termed direct transdifferentiation, occurs when supporting cells differentiate into hair cells without an antecedent mitotic event [21].

In many non-mammalian sensory organs such as the avian vestibular organs and the zebrafish lateral line system, hair cells continuously turn over and regenerate, both of which are increased after damage until homeostasis is reached [22, 23, 24, 25, 26, 27]. On the other hand, the avian basilar papilla can regenerate lost hair cells even though it has no continuous turnover of cells [17, 18, 19]. The mature mammalian cochlea, however, lacks the ability to regenerate hair cells via either mitotic or non-mitotic mechanisms [6, 28]. In contrast, the mature mammalian vestibular system retains a limited degree of non-mitotic regenerative capacity [29, 30, 31, 32, 33].

The neonatal mammalian cochlea was first shown to harbor a population of cells capable of generating new hair cells *in vitro* [34, 35, 36, 37]. Using flow cytometry and the $p27^{Kip1}$ transgenic reporter mouse line, White and colleagues found that isolated cochlear supporting cells were able to proliferate and differentiate into hair cells – both features of hair cell progenitors [38]. Oshima et al. also demonstrated that cells from the neonatal cochlear sensory epithelium display stem/progenitor cell behavior by forming spheres *in vitro* – clonal colonies formed from individual cells – and subsequently differentiate into new hair cells [37]. Importantly, this group found that cells from the sensory epithelia of both the neonatal and mature utricle exhibit stem/progenitor cell ability [37, 39].

Recent studies have built on these findings and revealed that cochlear supporting cells marked by Lgr5 – a marker for somatic stem cells in the skin and intestines – proliferate and differentiate into hair cells at a much greater propensity as compared to other Lgr5-negative supporting cells *in vitro* [40, 41]. To delineate the role of Lgr5-positive supporting cells *in vivo*, Cox et al. performed lineage tracing experiments in a $Pou4f3^{DTR}$ transgenic mouse line where hair cells, which express the human diphtheria toxin receptor, can be selectively

ablated [42]. Following hair cell death in the neonatal cochlea, a modest degree of spontaneous hair cell regeneration and proliferation was observed, with Lgr5-positive supporting cells contributing to both processes *in vivo* [42]. Limited spontaneous hair cell regeneration was also observed after ototoxic aminoglycoside insult of the neonatal mouse cochlea *in vitro* [43]. By contrast, hair cell ablation in the neonatal utricle results in a much more robust regenerative response via both mitotic and non-mitotic pathways, with hair cell recovery up to approximately 60% one month after damage [44, 45]. Lastly, the mature mammalian utricle has been shown to display some degree of regenerative capacity after hair cell loss [29, 31, 32]. This capacity was further characterized in a recent study, whereby hair cells were specifically ablated in the adult utricle using a transgenic mouse model [30]. Fourteen days after ablation only 6% of hair cells remained, with hair cell numbers returning to ~17% relative to controls by 60 days. These new hair cells displayed evidence of mechanotransduction, synaptic connections and were generated non-mitotically via direct transdifferentiation of supporting cells [30, 46].

Unfortunately, in the neonatal cochlea most regenerated hair cells degenerate in a delayed fashion for reasons not completely clear. Moreover, supporting cells rapidly lose their ability to regenerate hair cells after the first postnatal week within the mature cochlea. Collectively, these studies demonstrated that at least a subset of supporting cells in the neonatal cochlea, and the neonatal and mature utricle, can act as hair cell progenitors. We will next review studies examining mechanisms regulating mammalian hair cell progenitors.

5. Direct Cellular Reprogramming to Enhance Cellular Regeneration

As regeneration does not occur in the mature mammalian cochlea, there have been considerable efforts aimed at coercing supporting cells to regenerate lost hair cells (Figure 2), with cellular reprogramming being a major focus. The targeted manipulation of cell fate through the introduction of transcription factors is broadly termed cellular reprogramming. Over three decades ago, the introduction of a single transcription factor, MyoD, was shown to convert fibroblasts directly to myoblasts *in vitro* [47], shifting the notion that somatic cell fate is fixed. The plasticity of somatic cell fate was further highlighted by work carried out by Takahashi and colleagues, who successfully induced pluripotency with a cocktail of four transcription factors, the so-called “Yamanaka factors” [48]. Since these studies, many reprogramming approaches to induce pluripotency have been used prior to implementing guided differentiation protocols [49]. Moreover, new strategies to directly convert a cell’s identity (without a preceding dedifferentiation event) have been examined in a growing number of organ systems [50, 51, 52]. This new strategy, of “direct cellular reprogramming” will be the focus of the remainder of this review. For a comprehensive discussion of cellular reprogramming more broadly, we refer the avid reader to the following reviews [53, 54, 55].

In the inner ear, one transcription factor that plays a central in hair cell identity is called Atoh1 (previously Math1). Atoh1 is a basic helix-loop-helix transcription factor necessary for hair cell development [56]. Early after ototoxic insult in the avian cochlea and prior to proliferation or regeneration of mature hair cells, Atoh1 expression is upregulated in supporting cells in the damaged avian cochlea [57] and damaged mature mouse utricle [30]. This suggests that similar to development, Atoh1 may play a key role in the specification of

hair cells during regeneration. One of the earliest reports of *in vivo* reprogramming introduced Atoh1 into the mature guinea pig cochlea damaged by aminoglycosides as a means to induce regeneration of hair cells from supporting cells [58]. Subsequently, numerous other studies further explored the potential of Atoh1 as a singular factor to convert supporting cells towards a hair cell fate [59, 60, 61, 62, 63, 64]. The results of Atoh1 preclinical animal studies, however, have been mixed, with generally low efficacy and only one report of limited functional recovery.

More recent studies have more thoroughly characterized components of regenerated hair cells such as the expression of numerous hair cell markers, the presence of stereociliary bundles, mechanotransduction, and synapse formation [59, 63, 65]. In the neonatal cochlea, supporting cell subtypes display different Atoh1-responsiveness, with those within the organ Corti (Deiters' and pillar cells) being less competent than cells in the greater epithelial ridge [66]. In both the neonatal and juvenile cochlea, Atoh1 overexpression via a transgenic approach induces about 10% of pillar and Deiters' cells towards a hair cell phenotype [63]. Ectopic hair cells formed from Atoh1 expression display many markers of nascent hair cells and synaptic proteins but failed to express terminal differentiation markers such as prestin and oncomodulin. They develop immature stereocilia, do not have the classic mature hair cell morphology, but are labeled with the styryl dye FM1-43, which permeates patent mechanotransduction channels. This incomplete maturation may have resulted from a constitutive expression of Atoh1 as it is normally downregulated during hair cell maturation. Moreover, the long-term survival of these newly regenerated cells has yet to be carefully examined, indeed a recent study has found that even subtle changes in endogenous Atoh1 can lead to hair cell degeneration, underscoring the importance of characterizing these regenerated cells induced via aberrant Atoh1 expression [67].

Independent studies have shown that the effectiveness of Atoh1 overexpression is rather limited in the adult cochlea, where little to no functional recovery was observed [58, 59, 62, 63, 68]. Similarly, Atoh1 overexpression induces ectopic hair cells in the neonatal utricle with varied results reported in the mature organ [69, 70]. Despite these mixed results in preclinical studies, they led to the opening of a clinical trial assessing the safety and potential benefits of Atoh1 transfection in hearing loss patients (NCT02132130). The results may shed lights firstly on the safety of inner ear viral delivery, and possibly also on the efficacy of a single-factor reprogramming approach, which has been found effective in some organ systems [71, 72, 73]. In a recent study in the visual system of mature mice, for example, forced expression of the transcription factor Ascl1 in combination with a histone deacetylase inhibitor, was able to stimulate functional retinal neurons from Müller glia [74]. Without the addition of the histone deacetylase inhibitor, however, no regeneration was observed. This suggests that epigenetic regulators can play an important role in governing cellular reprogramming, a topic which will be discussed in the following section.

A multi-factor approach has been used to successfully stimulate regeneration of different systems throughout the body. For instance, viral transfection of Pdx1 and MafA transcription factors resulted in the reprogramming of mouse pancreatic α-cells into β-cells *in vivo*, postponing the onset of diabetes in mice [75]. Direct reprogramming using three defined factors (Gata4, Mef21 and Tbx5), which converts mouse fibroblast into functional

cardiomyocyte-like cells, improved cardiac function and reduced fibrosis after myocardial infarction [76].

In recent years, several groups have found the multi-factor direct reprogramming approach effective in generating hair cells *in vitro*. First of all, the combination of Atoh1, Gfi1 and Pou4f3, all transcription factors critical for hair cell development, is more effective than Atoh1 alone in converting embryonic stem cells into hair cells [77]. In the embryonic and neonatal cochlea, a different combination (Gata3, Ets2, Etv4, NMyc, Tcf3) in concert with Atoh1 induces more ectopic hair cells than Atoh1 alone [78, 79]. Moreover, the ectopic co-expression of Eya1 and Six1 is able to induce supernumerary hair cells in the embryonic mouse cochlea in the absence of Atoh1 [80].

Similar approaches have been attempted using transgenic mouse models, with promising results showing that the co-activation of Gata3 or Pou4f3 with Atoh1 successfully stimulating the conversion of supporting cells into hair cell-like cells in the adult cochlea. The combination of Pou4f3 and Atoh1 activation resulted in approximately 21% of recombined cells expressing the hair cell marker MyosinVIIa, an order of magnitude greater than with Pou4f3 alone. Interestingly, deletion of $p27^{Kip1}$, a cell cycle inhibitor expressed in cochlear supporting cells, was also able to promote the conversion of supporting cells to hair cells in response to Atoh1 activation. In this model 12% of recombined cells expressed hair cell markers MyosinVIa/VIIa. Upon further examination, deletion of $p27^{Kip1}$ upregulates Gata3, which is postulated to be the mechanism enabling transdifferentiation of supporting cells to hair cells in response to Atoh1 in the mature mouse cochlea [81]. However, no supporting cell proliferation was observed, implicating a cell-cycle independent role of $p27^{Kip1}$ mediated by Gata3. The interplays of these multiple transcription factors are only beginning to be explored and may help rekindle the lost plasticity of supporting cells in the mature cochlea and in doing so enable regeneration of the damaged organ.

6. Alternative approaches

A notable effector of hair cell regeneration is the developmental stage of the cochlea, where the plasticity of the supporting cells is reduced with maturation [59, 61, 63, 81, 82, 83, 84]. One candidate mechanism is changes in the epigenetic status of cochlear supporting cells as the organ matures, which is an area of active investigation. Epigenetic changes are known to play a key role in regulating cellular reprogramming and regeneration in a wide range of systems including during the generation of induced pluripotent stem cells [85], and regeneration of limbs [86], axons [87], and retinal neurons [74]. In the inner ear for example, the degree of methylation of Sox2 enhancers (NOP1 and NOP2) correlates with the dedifferentiation potential of post-mitotic supporting cells into otic stem cells [88]. Repressive complexes such as NuRD and PRC2 have also been reported in the neonatal cochlea, and the presence of these repressive complexes correlates with transcriptional silencing of known target genes of their cofactors such as Atoh1 [89]. Moreover, epigenetic modifications have been shown to regulate the effects of Atoh1 during development [90], and they are postulated to govern the efficacy of Atoh1 overexpression or other regenerative approaches in the postnatal and mature cochlea. In support of this concept, when combined with histone deacetylase inhibitors, Wnt activation dramatically increases Lgr5-positive

supporting cells in cultured neonatal cochlea and the number of clonal colonies formed by cochlear supporting cells, although the effects on supporting cells from the mature cochlea are significantly reduced [91].

Other combinatorial manipulations have also been found to modulate hair cell regeneration. For instance, in the neonatal cochlea, Sox2 haploinsufficiency and damage enhances the effects of Wnt activation via stabilization of β-catenin on proliferation and hair cell formation from supporting cells, possibly via the downregulating of Notch signaling [84]. When Li and colleagues inhibited Notch signaling, they observed that active Wnt signaling induces more robust proliferation and hair cell formation in the neonatal cochlea [92]. Interestingly, in addition to its effects on hair cell formation, Atoh1 overexpression has been observed to induce proliferation in the neonatal cochlea [61]. When combined with active Wnt signaling and/or Notch inhibition, both cell division and ectopic hair cell formation increase in the neonatal cochlea [82, 83]. It is a common notion that supporting cell proliferation is important for cochlear regeneration as it can help restore the hair cell-supporting cell ratio thought to be important for function. While these studies indicate that both proliferation and hair cell formation can be modulated with different approaches in the neonatal, immature cochlea, their effectiveness in the mature cochlea is rather limited [81, 84]. In addition to epigenetic changes mentioned above, a decrease in Notch signaling has been proposed as one mechanism leading to the inability of the mature mammalian cochlea to regenerate [93]. Moreover, the severely damaged cochlea with a complete loss of hair cells appears as a scar-like, flat epithelium occupied by cuboidal cells, which may be rather different from native supporting cells [15, 68]. It would be of interest for future studies to compare the gene expression of the neonatal and mature cochlear supporting cells, particularly their changes in response to damage.

7. Delivery of therapeutic agents

Many studies have probed strategies that most effectively introduce viral vectors or pharmaceutical compounds into the cochlea. When determining the suitability of each approach, a number of elements should be considered, including the intended biological effect, the target cell population, the longevity of the expression needed, and any possible off-target effects. Two of the most commonly used viruses have been adeno-associated virus (AAV) and adenovirus, each of which has its own advantages and shortcomings. AAV in particular has been an appealing candidate vehicle for gene transfer because it has a long-lived transgene expression, is not linked with human diseases, and has a relatively wide expression pattern within the cochlea [94]. However, it has a relatively small genetic capacity, limiting the types of genes that can be delivered. In contrast, adenovirus has a larger packing capacity, allowing for a larger array of possible genetic insertions, but the transgene expression is transient.

Multiple studies examining Atoh1 and hair cell regeneration have used adenovirus as a means to target supporting cells, such as the pillar cells, Deiters' cells, as well as Hensen cells [58, 59, 95]. Specifically, adenovirus serotype 5 has been shown to be efficacious in transducing supporting cells [96]. Adenovirus-mediated Atoh1 studies, however, only found small increases in hair cell number and limited to no recorded functional improvement. In

the mature utricle, one study using adenovirus reported hair cell conversion and improved vestibular function by behavioral measures [97]. These studies show that adenovirus can transduce a wide range of supporting cells, highlighting its potential use in future therapies. For a list of information on viral and non-viral approaches, please see Table 1.

8. Conclusion

The mature mammalian cochlea does not regenerate lost hair cells, resulting in a permanent hearing deficit. Recent studies have found that supporting cells in the neonatal cochlea spontaneously regenerate hair cells, and that multiple approaches including direct cellular reprogramming can modulate regeneration. However, inducing robust hair cell regeneration in the mature cochlea remains challenging. Recent advances in multi-factor direct cellular reprogramming approaches and viral and non-viral delivery methods into the inner ear should open the door for novel biological therapies for hearing loss.

9. Expert Opinion

The organ of Corti is a highly delicate structure containing a multitude of cell types that are precisely interwoven and innervated, enabling it to convey the smallest whisper to the cacophony of an orchestra with high fidelity. Damage causing loss of sensory hair cells results in permanent hearing loss, for which there is currently no biological treatment. Unlike non-mammalian sensory organs, which are able to repopulate hair cells, the mature mammalian cochlea has no spontaneous regeneration. Re-engineering the mature mammalian cochlea to regenerate hair cells as a means to restore hearing is therefore of immense interest and could provide remarkable benefit.

Studies of the neonatal cochlea and utricle have provided significant insights into how to tackle this problem. First, supporting cells isolated from the neonatal cochlea are able to proliferate and develop new hair cells, indicating their progenitor potential. A subset of supporting cells – particularly those marked by the Wnt responsive gene Lgr5 – had a greater propensity to proliferate and differentiate into hair cells, suggesting that Lgr5 may be an enrichment marker. Second, *in vivo* studies have shown that supporting cells in the neonatal cochlea acted as hair cell progenitors after damage and regenerated new hair cells. However, regeneration is limited both in degree and to the apical region of the cochlea. Moreover, fate mapping demonstrated that at least a subset of these regenerated hair cells were derived from Lgr5-positive supporting cells. These findings indicate that within their native environment, supporting cells are capable of proliferating and converting into hair cells after injury. Unfortunately, this capacity is rapidly lost after the first postnatal week for reasons that are not completely clear.

Numerous studies have attempted to coerce hair cell regeneration via the upregulation of Atoh1 since it can successfully force supporting cells to differentiate into hair cells in the neonatal cochlea. Another approach has been to activate Wnt signaling as a means to induce proliferation of quiescent supporting cells. These single factor approaches, however, have mostly been unsuccessful in inducing regeneration in the mature cochlea. These findings highlight two important points: firstly, while the neonatal cochlea provides a conducive

environment for manipulations and can provide proof-of-principle results, it is necessary to validate the findings in the mature cochlea; and secondly, a single factor approach is unlikely able to promote the necessary cell proliferation and cell conversion in the mature cochlea. It remains to be tested whether a multifactor approach can induce supporting cell proliferation and hair cell regeneration. Also why cochlear supporting cells become rapidly unresponsive to manipulation after birth has not yet been fully elucidated. As such, the field would benefit from a greater understanding of the differential gene expression and epigenetic changes of supporting cells in the neonatal and mature cochlea.

During development, hair cells display dynamic changes of many genes that are beginning to be revealed, including those specifying hair cell subtypes and regulating subcellular structures critical for hair cell function. It is highly plausible that the milieu of the mature cochlea will require a novel set of genes to allow proper hair cell differentiation and integration, all unexplored and challenging questions needing answers. As new technologies evolve to better examine the damaged mammalian cochlea and novel techniques develop to deliver therapeutic agents, the possibility of hair cell regeneration as a biological therapy for hearing loss may be realized.

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Article Highlights

- Limited spontaneous hair cell regeneration occurs in the neonatal mouse cochlea and in the neonatal and adult mouse utricle.
- As in non-mammalian species, supporting cells in mammalian sensory organs can act as hair cell progenitors.
- Cochlear supporting cells rapidly lose the ability to regenerate hair cells as the organ matures.
- Direct cellular reprogramming is efficacious in inducing ectopic hair cells in the neonatal cochlea; however, results have been mixed in the mature organ.
- The overall efficacy of a combination approach to induce hair cell regeneration in the mature cochlea remains low.

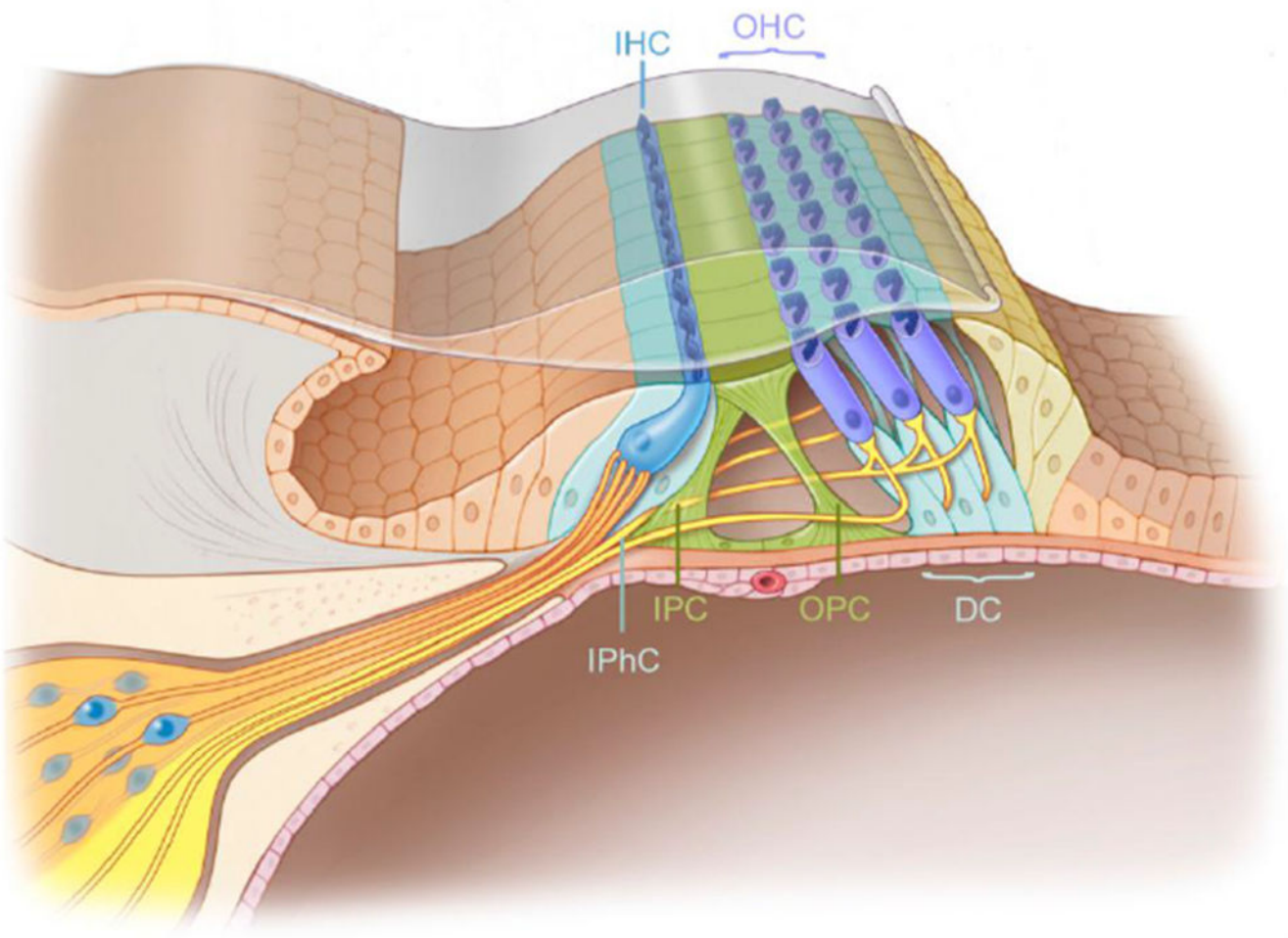

Figure 1.

The organ of Corti, the sensory domain of the cochlea, houses one row of inner hair cells and three rows of outer hair cells. Inner hair cells are supported by inner phalangeal cells and outer hair cells are supported by Deiters' cells, collectively referred to as supporting cells. The inner and outer hair cells are separated by the inner and outer pillar cells which form the tunnel of Corti. IHC: inner hair cell, OHC: outer hair cell, IPhC: Inner phalangeal cell, IP: Inner pillar cell, OP: Outer pillar cell, DC: Deiters' cell. Modified and reprinted from [16] under a CC BY license, with permission from Springer Nature, Understanding the cochlea (2017).

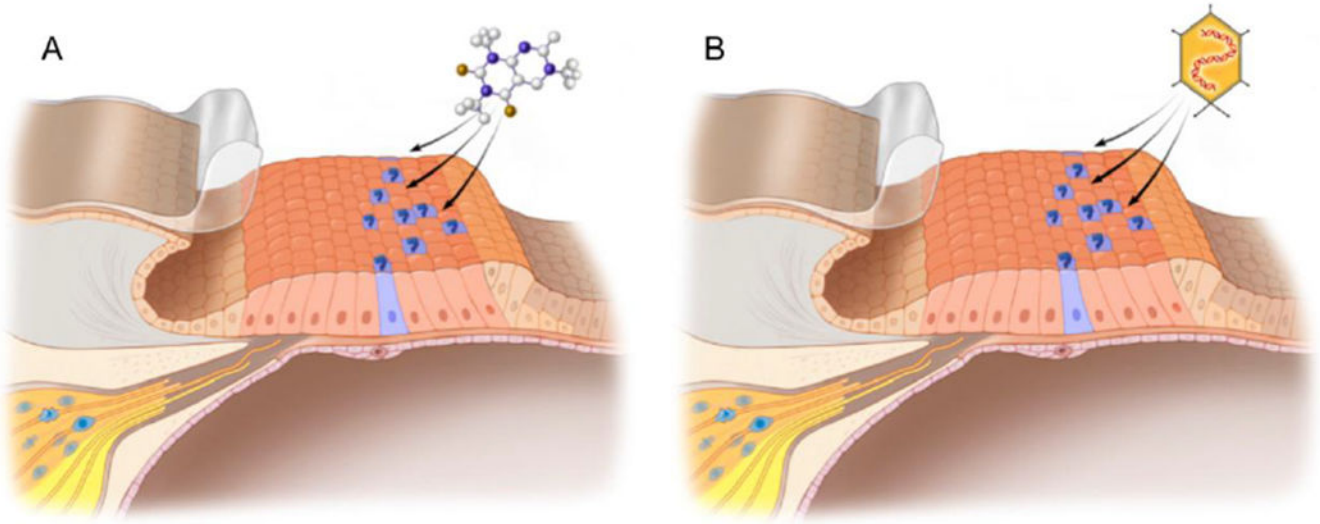

Figure 2.

Schematics of cellular reprogramming in the damaged organ of Corti. A) Introduction of a small molecule or siRNA (A) or viral vectors (B) to induce hair cell regeneration.

Table 1.

Viral vectors for inner ear gene therapy

Viral vector	Transgene	Animal	Route	Tropism	Side Effects	Reference
Adenovirus						
Ad5	GFP	Mouse (adult)	RW	Cochlear and vestibular hair cells	Inflammatory response	[98]
Ad5 (E1A$^-$, E1B$^-$)	lacZ	Guinea pig (adult)	RW	Spiral ganglion neurons, cochlear supporting cells and connective tissue	Mild inflammatory response	[99]
Ad (E1$^-$)	β-gal	Rat (neonatal)	In vitro	Organ of Corti, spiral ganglion neurons	No signs of cellular damage reported	[100]
Ad (E1-, E3-, CMV)	GFP	Rat (adult)	Peri- and endolymphatic perfusion	Mesothelial cells of scala media (i.e. Reissner's membrane); Hensen's, Deiters', pillar and phalangeal cells; satellite cells surrounding SGN	Increased compound action potential (CAP) threshold	[101]
			Coch	Cochlear inner and outer hair cells (in 2/6 animals)	Increased CAP threshold	
Ad (E1$^-$, E3$^-$, pol$^-$)	GFP	Guinea pig (adult)	RW	Cochlear inner hair cell and pillar cells	Loss of DPOAE	[102]
Ad (E1$^-$, E3$^-$, pol$^-$)	GFP-BDNF GFP-NT3	Guinea pig (adult)	Coch	Inner and outer pillar cells, Deiters' cells, Hansen's cell, inner sulcus cells and interdental cells	Mild to moderate fibrosis and new bone growth	[103]
Ad	GFP-Kir2.1	Mouse (neonate)	In vitro	Vestibular hair cells	No evidence of toxicity	[104]
AAV						
AAV2/1	GFP	Mouse (Embryonic)	Trans-uterine	Cochlear progenitor cells, which gave rise to inner and outer hair cells and supporting cells	No adverse effects found	[105]
AAV1	GFP	Mouse (neonate)	Coch	Inner hair cells, cochlear supporting cells and lateral wall	Hearing loss reported when injected into scala media	[106]
AAV2	dXIAP	Rat (adult)	RW	Spiral ganglion and stria vascularis	None reported	[107]
AAV1	VGLUT3	Mouse (juvenile)	RW or Coch	Inner hair cells	None reported	[108]
AAV8	Whirlin	Mouse (neonatal)	RW	Inner hair cells and some outer hair cells	None reported	[109]
		Mouse (adult)	RW	Inner hair cells (low efficacy)		
AAV8	Whirlin	Mouse (neonatal)	PSSC	Vestibular hair cells and cochlear inner hair cells	None reported	[110]

Viral vector	Transgene	Animal	Route	Tropism	Side Effects	Reference
AAV8	Neurotrophin-3	Guinea pig (adult)	Coch	Inner hair cells	None reported	[111]
Anc80L65	GFP	Mouse (neonatal)	RW	Inner and outer hair cells, spiral ganglion neurons, cochlear supporting cells	No adverse effects on hair cell or hearing function	[112]
		Human	In vitro	Vestibular hair cells and supporting cells		
AAV8	*Sans*-IRES-GFP	Mouse (neonate)	RW	Vestibular and cochlear hair cells	None reported	[113]
AAV2/Anc80L65	GFP	Mouse (adult)	PSSC	Cochlear hair cells, interdental cells, Reissner's membrane, spiral limbus. Vestibular hair cells and supporting cells and vestibular ganglion cells	No adverse effects on hair cell or hearing function	[114]
Herpes Simplex virus	lacZ	Guinea pig (adult)	RW	Supporting cells, epithelial and connective tissues of cochlea.	Inflammatory response	[115]
	NT-3myc	Mouse (neonatal)	In vitro	Spiral ganglion	None reported	[116]

RW-round window

PSSC-posterior semicircular canal

Coch-Cochleostomy

AAV - adeno-associated virus