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Review

Roles of supporting cells in the maintenance and regeneration of the damaged inner ear: A literature review

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A B S T R A C T

The inner ear sensory epithelium consists of two major types of cells: hair cells (HCs) and supporting cells (SCs). Critical functions of HCs in the perception of mechanical stimulation and mechanosensory transduction have long been elucidated. SCs are indispensable components of the sensory epithelia, and they maintain the structural integrity and ionic environment of the inner ear. Once delicate inner ear epithelia sustain injuries (for example, due to ototoxic drugs or noise exposure), SCs respond immediately to serve as repairers of the epithelium and as adapters to become HC progenitors, aiming at morphological and functional recovery of the inner ear. This regenerative process is extensive in non-mammals, but is limited in the mammalian inner ear, especially in the mature cochlea. This review aimed to discuss the important roles of SCs in the repair of the mammalian inner ear.

1. Introduction

The mammalian inner ear is a highly delicate apparatus comprising two major cell types: hair cells (HCs) which are responsible for mechanosensory transduction and the supporting cells (SCs) around them. HCs can sense the mechanical vibrations of auditory and balance signals and convert them into neural signals that can be detected by the brain. HCs are susceptible to damage, in terms of gene mutations, noise exposure, ototoxic drugs, and aging, resulting in inner ear dysfunction that presents as hearing loss or balance disorders. Unlike non-mammals, the mammalian inner ear lacks or only possess limited regenerative capability (Cox et al., 2014; Devare et al., 2018), leading to unrecoverable dysfunction.

The specific roles of SCs remain to be elucidated; however, emerging evidence indicates that they have many important functions in development, structural maintenance, and ion homeostasis (Tritsch et al., 2007; Wilcox et al., 2001; Zhang et al., 2005). Deficits in SCs result in a clear loss of function in the inner ear (Wilcox et al., 2001; Zhang et al., 2005). Several studies have recently proposed that SCs may act as epithelial repairers to sustain the integrity of the sensory epithelium and as HC progenitors for the renewal or regeneration of HCs (Bucks et al., 2017; McLean et al., 2016). A subgroup of SCs (namely, Lgr5^{+}) has been assessed to share many common properties with stem cells (Cox et al., 2014) and exhibits critical activities in the self-renewal and regeneration processes in normal or pathological states of the inner ear (Bramhall et al., 2014; Cox et al., 2014; McLean et al., 2017).

The spontaneous repair and regenerative activities of SCs are important for the morphological and functional recovery of the inner ear. However, the process is limited in the mammalian inner ear, especially for adults (Collado et al., 2011), leading to permanent impairment of hearing and balance. Several studies have been conducted to further enhance the SC reprogramming process and, thus, improve the quantity and maturity of regenerated HCs. A comprehensive examination of these achievements is likely to facilitate future strategies aimed at the full regeneration of HCs and the realisation of inner ear function recovery.

This review aimed to focus on the important roles of SCs in the damaged inner ear and discuss the growing information concerning the process of initial repair and SC reprogramming, which allows for the adaptation of cell fates for HC regeneration. (Fig. 1)

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2. The initial wound-healing process following HC loss

The apical surface of the inner ear epithelium is tightly sealed by the reticular lamina, which is composed of tight and adherent junctions formed between the HC and SC membranes (Leonova and Raphael, 1997). The two kinds of fluids in the inner ear, namely, the endolymph and perilymph, are separated. The endolymphatic fluid contains a high concentration of K^+, a low concentration of Na^+, and is in contact with the apical surface of the inner ear; the perilymphatic fluid has a high concentration of Na^+ and low concentration of K^+ (similar to the intracellular fluid) and is in contact with the basolateral surfaces. Endocochlear potential, which is essential for HC depolarisation, is generated along the reticular lamina. The reticular lamina between the two distinct fluids also provides a critical physical barrier that protects the HCs from the toxic cellular endolymph.

When sensitive HCs are exposed to specific trauma, including acoustic overstimulation, ototoxic drugs, and presbycusis-related changes, they undergo either necrosis or apoptosis, leading to a deficit in the reticular lamina. SCs respond immediately to maintain the fluid barrier of the sensory epithelium to prevent toxic high-K^+ endolymph from contacting the basolateral surfaces of the SCs and surviving HCs (Leonova and Raphael, 1997). The process includes reorganisation of both adherent junctions, localised between two SCs, and tight junctions, connecting the apical surface of both HCs and SCs in the reticular lamina (Raphael and Altschuler, 1991). The cytoskeletal protein of HCs and SCs, actin filaments, have also been found to be rearranged, and the SCs extend among nearby HCs under the cell surface (Leonova and Raphael, 1997; Raphael and Altschuler, 1991). The upper part of the dying HCs, including the stereocilia bundles, then separate from the cell bodies. This event is also considered to depend on actomyosin contractile activity (Anttonen et al., 2014). Newly polymerised F-actin belts have been shown to dynamically reinforce the junctional extensions of the SCs (Anttonen et al., 2014). Consequently, the surface is resealed following the expulsion of the upper part of the HCs (Bird et al., 2010; Burns and Corwin, 2014). The initial scar formation process maintains the integrity of the sensory epithelium and prevents cellular damage, which allows for subsequent HC regeneration.

The F-actin of mammals gets thicker after birth, whereas that in non-mammals remains thin throughout adulthood. The strengthening of F-actin is supposed to restrict the ability of vestibular SCs to repair the sensory epithelium following impairments in adult mammals (Burns and Corwin, 2014). In addition, the discrepancy in F-actin thickness between adult mammals and non-mammals coincides with the disparate capacities of SC reprogramming for HC regeneration; thus, post-natally strengthened F-actin in the mammalian utricle has been proposed to be responsible for the limiting HC replacement (Burns et al., 2008; Burns and Corwin, 2014).

After expelling the apical portion of dead HCs, SCs engulf the residual HC debris. This phenomenon has been observed in both the cochlea and vestibular system of various non-mammals and mammals (Anttonen et al., 2014; Bird et al., 2010; Monzack et al., 2015). The ultrastructural change of this process was recorded using series of scanning electron microscopy (Anttonen et al., 2014). The apoptotic HC bodies progressively shrink, coupled with the swelling of the phalangeal processes of SCs, which fill the interstitial spaces. The swelling SC phalangeal process phagocytosed the apoptotic bodies, the degrading nuclei, and organelles. Phalangeal swelling then decreases concomitantly with successful clearance of the apoptotic body-like structures. Besides the phagocytosis process, apoptotic HCs that have inadequate contact with SCs, such as outer hair cells (OHCs) in the first row, undergo secondary necrosis because the apoptotic cells are not promptly cleared by phagocytes (Anttonen et al., 2014). Thus, SC phalangeal

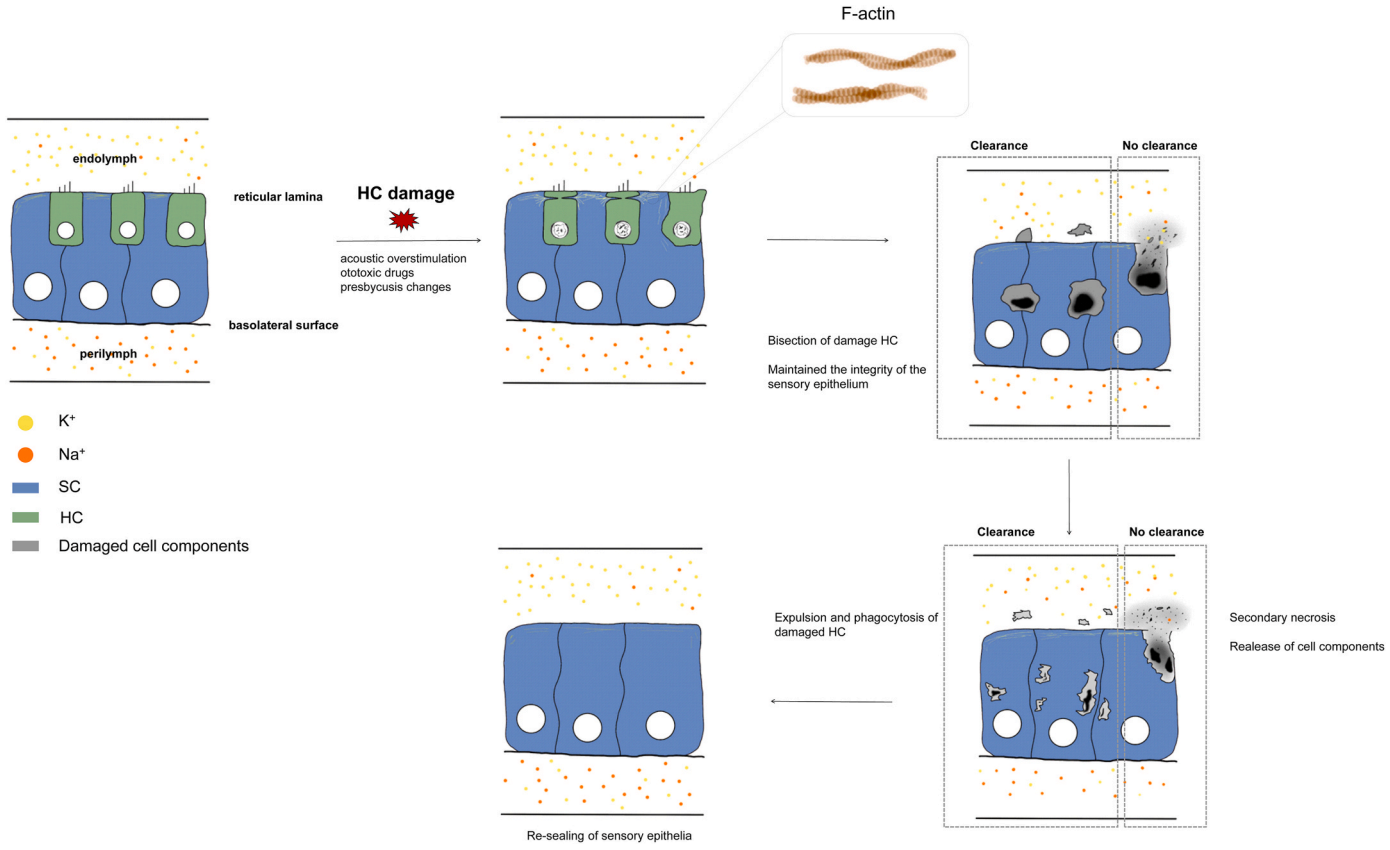


Fig. 1. The initial wound-healing process after HC loss. Upon HC loss, SCs extend under the apical part of HCs with the participation of the actin filament and the dying HC body is bisected. The HC debris is then phagocytosed and digested by adjacent SCs. HCs that lack sufficient contact with SCs will not be engulfed immediately and may undergo secondary necrosis. The epithelial surface is finally resealed by the SCs. HC, hair cell; SC: supporting cell.

swelling has been shown to be an immediate and transient response to ototoxic trauma, providing a homeostatic environment for the residual sensory epithelium and preventing secondary HC necrosis. Altered ion transport and connexin 30-mediated intercellular communication have been shown to be possible triggers for this event in the avian cochlea (Jagger et al., 2014).

Like the remnant clearance process of the cochlea, vestibular SCs also respond immediately to eliminate apoptotic HC bodies and constrict the apex of HCs following the loss of membrane integrity, which has been observed in the adult mouse utricle following HC damage (Bucks et al., 2017; Monzack et al., 2015). These studies emphasise that functional SCs of both cochlea and vestibular end organs are indispensable for the maintenance of permissive homeostasis which is prerequisite of the subsequent HC regeneration.

The repair process in the flat epithelium, in which SCs are almost completely lost after extreme damage to the inner ear (He et al., 2020; Wang et al., 2017), remains unclear. Understanding HC death and wound-healing mechanisms, including the coordination of intercellular signalling, hair cell apoptosis, apical surface closure, and debris engulfment, is essential for prevention of HC loss.

3. SCs as resources for HC regeneration

Mature SCs of non-mammals retain a potential ability to dedifferentiate, which appears to be reactivated through HC loss, so that SCs proliferate, and daughter cells then differentiate into new HCs and SCs (Cruz et al., 2015). However, mature mammalian cochlear SCs lack the ability to regenerate HCs either mitotically or non-mitotically (McGill and Schuknecht, 1976; Soucek et al., 1986). In contrast, mature vestibular mammalian SCs retain a limited ability for HCs regeneration via direct trans-differentiation (Golub et al., 2012; Kawamoto et al., 2009). Recent investigations have identified specific subgroups of SCs as progenitors for HC regeneration that allow the active turnover of injured HCs in the neonatal cochlea (Bramhall et al., 2014; Cox et al., 2014; Wang et al., 2015) and utricle (Lin et al., 2015; Wang et al., 2015), with or without exogenous transcriptional factors. However, this capability declines sharply during the post-natal stage (Cox et al., 2014; Burns and Corwin, 2014). The mechanisms underlying this diversity remain unclear, with suggestions that they are partly related to changes in epigenetic status (Ahmed and Streit, 2018; Jorstad et al., 2017) and to the morphological complexity of SCs (Burns and Corwin, 2014).

A fate-mapping study conducted by Bucks et al. showed that type II utricular HCs in adult mice undergo turnover in their normal states (Bucks et al., 2017). SCs play important roles in this self-renewal process, including phagocytosis of both type I and type II HCs and restoration of type II HCs. Furthermore, they reported that after diphtheria toxin (DT)-mediated ablation of most HCs in a Pou4f3^{DTR} transgenic mouse line, SC-transdifferentiated HCs increased six times compared with normal conditions. Morphological identification revealed that only type II HCs were generated during spontaneous regeneration. In a study by Golub et al., using the same mouse model (Golub et al., 2012), DT treatment reduced utricular HC numbers to 6% of normal subjects by 14 days post-DT. The HC numbers increased over time and reached 17% of untreated controls by day 60. They further examined the mitotic activity of SCs, confirming that no proliferation had occurred and the number of SCs had decreased.

In contrast to vestibular SCs, auditory SCs in adult mammals lack the capability for HC renewal, either mitotically or non-mitotically (McGill and Schuknecht, 1976; Soucek et al., 1986). SC-derived HC regeneration has only been observed in the cochlea of neonatal mammals. Cox et al. first showed that neonatal mammalian cochlear SCs retain the ability of HC mitotic regeneration after injury in vivo (Cox et al., 2014). In that study, the genetic strategies Pou4f3$^{DTR/+}$, Atoh1-CreER™, and ROSA26$^{DTA/+}$ were used to selectively kill neonatal mouse cochlear HCs in vivo. Fate-mapping studies have shown that HCs regenerate from SCs either mitotically or non-mitotically. The regenerated cells exhibited immature stereocilium bundles and expressed five HC markers, including the terminal differentiation HC marker, namely, prestin. However, most new HCs failed to survive beyond post-natal day 15. They further investigated the time period during which SC-based HC regeneration could occur and showed that it was limited to the first post-natal week. An almost simultaneous investigation conducted by Bramhall et al. verified SC-based HC regeneration in post-natal mammalian cochlea in vitro (Bramhall et al., 2014). This research proposed that the ability of HC generation was limited to Lgr5-positive SCs.

Further studies have shown the existence of progenitor cells in the mammalian inner ear (Oshima et al., 2007; Sinkkonen et al., 2011). Cells from the neonatal cochlear sensory epithelium give rise to a self-renewing neurosphere which subsequently differentiates into new HCs (Oshima et al., 2007). Using the technologies of flow cytometry and lineage tracing with a p27^{Kip1} transgenic mouse, White et al. first reported that mammalian cochlear SCs could act as progenitor cells, dividing and trans-differentiating into HCs upon isolation (White et al., 2006). The hypothesis that mammalian SCs serve as inner ear progenitor cells was further confirmed, based on findings that SCs in lower vertebrates act as precursors during HC regeneration (Ma et al., 2008) and that SCs express a number of stem cell markers, including Sox2, glutamate aspartate transporter, and Musashi (Oesterle et al., 2008; Sakaguchi et al., 2004; Shi et al., 2012). Several studies have been conducted to distinguish SC subgroups which can proliferate and possess stem cell properties. Lgr5 is a target of the Wnt pathway and taken as the marker of adult stem cells (Barker et al., 2007). Shi et al. (2012) used this marker to isolate cells from the cochlea and found that Lgr5-positive mammalian SCs, which are present at P60 in the inner pillar cell and third row of Deiters' cells, exhibited a capacity for dividing and for vigorous neurosphere formation, followed by trans-differentiation to HCs. In contrast, Lgr5-negtive SCs presented much weaker neurosphere formation and failed to produce HCs. The precursor role of Lgr5-positive cochlear SCs has been further verified both in vitro and in vivo through other subsequent studies (Bramhall et al., 2014; Chai et al., 2012; Cox et al., 2014; McLean et al., 2017), which showed that active Wnt signalling stimulated the mitosis of SCs which were Lgr5-positive (Chai et al., 2012) In addition, the progenitor features of Lgr5-positive SCs in vestibular end organs have also been elucidated (Lin et al., 2015; Wang et al., 2015).

In addition to Lgr5-positive SCs, other SC subsets, such as CD326^{+}/CD146^{low}/CD271^{low} SCs (Sinkkonen et al., 2011) and p27^{Kip1}-positive SCs (White et al., 2006), have also been identified as having progenitor profiles.

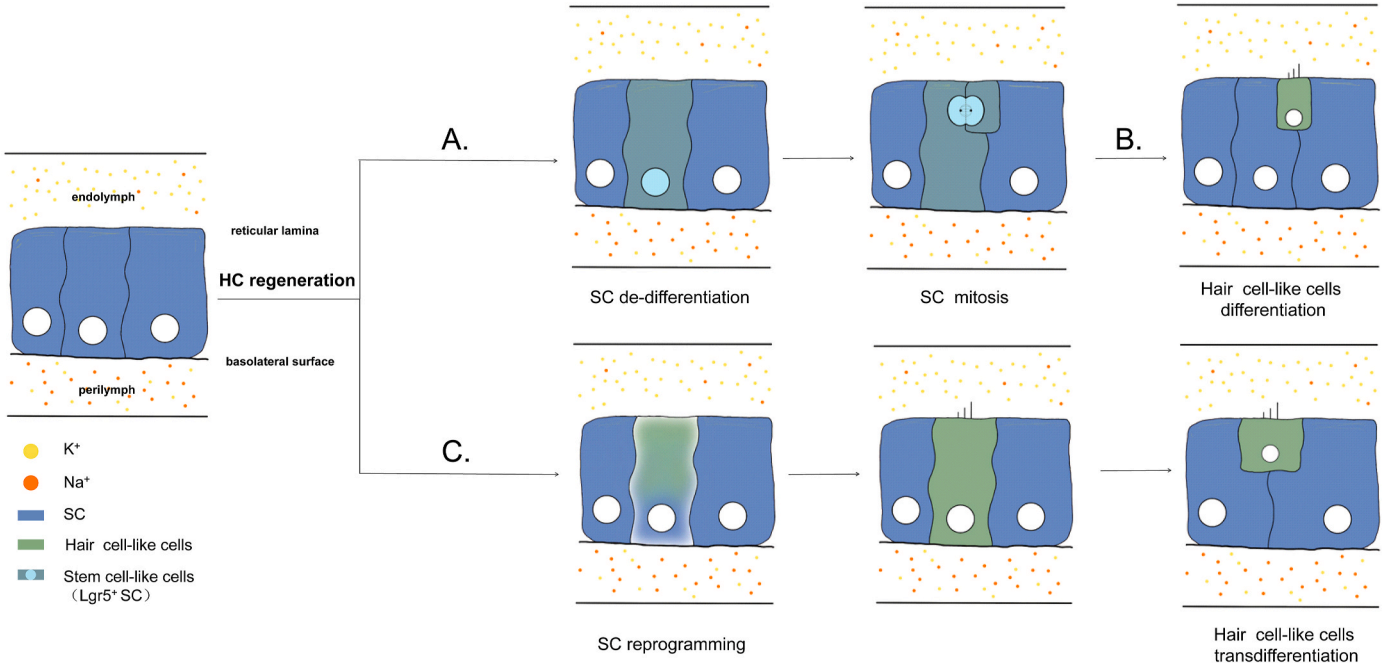
4. Strategies to enhance SC reprogramming and advanced epithelial renewal

Since SC-based HC regeneration is not present in adult mammalian cochlea and limited in neonatal cochlea and vestibular end organs of different stages, considerable efforts aiming at the cellular reprogramming of SCs to strengthen SC-based HC regeneration have been made. The term cellular reprogramming refers to the process through which cell fate is converted through the manipulation of transcription factors (Atkinson et al., 2019). This concept was first introduced approximately 30 years ago, when Davis et al. (1987) successfully redirected fibroblasts to myoblasts through treatment with a single transcription factor. Since then, several studies have attempted to induce the conversion of cell fates within various systems (Guo et al., 2014; Qian et al., 2012; Song et al., 2016).

In the inner ear, a lot of attention for SC reprogramming has focused on one transcription factor, namely, Atoh1, which is a basic helix-hoop-helix transcription factor involved in HC development (Bermingham et al., 1999). Embryonic ablation of Atoh1 causes failure in cochlear and vestibular HC generation, and the SCs undergo demise subsequently (Bermingham et al., 1999; Cai et al., 2013). In the limited spontaneous regeneration process of the adult mammalian utricle, Atoh1 is transiently upregulated in SCs (Golub et al., 2012; Wang et al., 2010),

implying the importance of Atoh1 for SC-based regeneration after damage. Izumikawa et al. induced HC regeneration and hearing restoration through Atoh1 overexpression using an adenovirus vector in adult guinea pig cochleae (Izumikawa et al., 2005). However, the origin of the regenerated HCs remains unclear. Using a transgenic mouse line of Atoh1-loxP and SC-specific CreER, Liu et al. specifically overexpressed Atoh1 in pillar and Deiters' cells from neonatal and juvenile mice cochleae and found that they were capable of converting into HCs, confirming the reprogramming potency of mammalian cochlear SCs to divert into an HC fate (Liu et al., 2012). Stereocilia and mechanoelectrical transduction channels were formed in the new HCs, which survived for >2 months; however, markers of mature OHCs, such as prestin and oncomodulin, were absent. Subsequently, several attempts have been made to further explore the capacity of Atoh1 to convert SCs to adopt an HC fate in both the cochlea (Atkinson et al., 2014; Kelly et al., 2012; Kraft et al., 2013) and vestibular systems (Guo et al., 2021; Sayyid et al., 2019; Schlecker et al., 2011) of mammals. While functional recovery has been observed in some studies undertaken within damaged mature cochleae (Izumikawa et al., 2005) and vestibular systems (Sayyid et al., 2019), most have reported to be lacking in the mature HC morphology and electrophysiology (Kelly et al., 2012; Kuo et al., 2015; Liu et al., 2012, 2014). Despite these different results, a clinical trial has been approved and launched within patients with deafness to further assess the safety and efficacy of the Atoh1-induced supporting cell reprogramming strategy (NCT02132130).

In addition to Atoh1, other transcription factors such as Pou4f3 and $p27^{Kip1}$ have also been shown to be capable of inducing cochlear SC reprogramming (Walters et al., 2017). LIN28 is an RNA-binding protein involved in self-renewal, lineage commitment, cell differentiation (Rehfeld et al., 2015) and metabolic reprogramming (Shinoda et al., 2013; Zhu et al., 2011). Golden et al. found that LIN28 served as a vital regulator for the timing of cochlea development. Additionally, it strengthened the plasticity of post-natal SCs through enhancing their capacity for cell conversion to an HC fate in response to Notch inhibition in the cochlea (Golden et al., 2015). LIN28 is also necessary and enough to restore Sox2+ SCs and initiate HC regeneration in a traumatised



A.SC de-differentiation

Lin28 Li XJ et al ,2020

Myc + Notch1 Shu Y et al ,2019

B.Differentiation of hair cell-like cells

Other regenerative signals of factors like Notch1 inhibition，Wnt activation and overexpression of Atoh1.

C.SC reprogramming and transdifferentiation of hair cell-like cells

Atoh1 + Ikzf2 Sun S et al ,2021

Atoh1 + Pou4f3 + Gfi1 Chen et al,2021

Atoh1 + Pou4f3 + Gfi1 + Six1 Menendez L et al,2019

Fig. 2. SC-resourced HC regeneration could be strengthened as follows: (i) Strategies such as overexpression of LIN28 or the combined application of Myc and Notch1 were designed to induce dedifferentiation of SCs to regain the properties of progenitor cells. They would then proliferate, and the daughter cells would differentiate into HC-like cells. (ii) Other strategies such us combined overexpression of Atoh1 and Ikzf2, were implemented to reprogramme SCs into HC-like cells directly. SC: supporting cell; HC, hair cell.

zebrafish lateral line (Ye et al., 2020). Its key role in controlling the reprogramming of mammalian SCs has been supported in a study by Li and Doetzlhofer (2020). They implemented loss- or gain-function of LIN28B in murine cochleae and reported that the developmental loss of supporting cell plasticity for HC regeneration was at least in part related to the decline of LIN28's mammalian target of rapamycin (mTOR) activity. The effect of LIN28 overexpression was further explored and found to be sufficient to allow mature SCs to dedifferentiate to obtain property of stem cells and adopt the HC fate in response to regenerative cues (Fig. 2 A). Notably, reactivation of LIN28 alone is not enough to allow SC proliferation and the generation of HCs in mammals. Additional regenerative signals like Wnt and Notch signalling must be present simultaneously (Fig. 2B). it is notable that this study was conducted in vitro using mice cochleae in the early post-natal stages. The effects of LIN28 in adult mammalian SCs require further investigation. Nevertheless, the finding that LIN28 facilitated SC reprogramming may shed light on future studies that combine LIN28-induced SC reprogramming with other regenerative factors, such as Atoh1 overexpression or Wnt activation, to realise HC regeneration in adult mammals.

Single-factor approaches inducing SC reprogramming have been demonstrated to be limited in terms of regeneration extent and functional restoration, especially in the mature cochlea. Therefore, multifactor strategies have been attempted in recent years to improve the quantity, morphology, and physiological maturity of regenerated HCs, with the aim of functional recovery of the sensory epithelium (Chen et al., 2021; Iyer et al., 2022; Li et al., 2022; Lu et al., 2022; Menendez et al., 2020; Shu et al., 2019; Sun et al., 2021). Coactivation of the cell cycle activator Myc and the inner ear progenitor gene Notch1 was achieved in a transgenic mouse model to reprogramme mature cochlear SCs to undergo mitosis and regain the ability to trans-differentiate into HCs in the presence of induction signals (Shu et al., 2019) (Fig. 2 A). Newly formed HC-like cells obtained neural innervation and possessed functional transduction channels. They further screened small chemical compounds to fulfil the manipulation of Myc and Notch1 genes with a drug-like approach in wild type adult mice. Mature cochlear SCs regained progenitor properties and transdifferentiated into HC-like cells under the cocktail manipulation, which represents a more clinically relevant approach (Quan et al., 2023). Another combination strategy involving four transcription factors, Six1, Atoh1, Pou4f3, and Gfi1, has been applied in vitro via viral transduction and shown to be capable of converting post-natal mouse cochlear SCs to induce HCs (Menendez et al., 2020), which not only appeared in HC-like morphology, but also exhibited transcriptomic and epigenetic profiles and electrophysiological properties (Fig. 2 C). However, the HC-like cells failed to express other key markers of mature HCs like prestin for OHCs and Gata3 for inner hair cells (IHCs) (Bardhan et al., 2019; Liberman et al., 2002). To obtain further maturation of regenerated HCs, Sun et al. explored the synergistic function of Atoh1 and Ikzf2, which are vital transcription regulators for specifying HC maturation (Chessum et al., 2018) (Fig. 2C). They used transgenic mouse lines to induce the co-overexpression of Atoh1 and Ikzf2 in mature cochlear SCs, specifically pillar and Deiters' cells. SCs were readily reprogrammed and adopted an OHC fate with remarkable prestin expression, especially with predamaged endogenous OHCs (Sun et al., 2021). This study was the first to identify SC-originated prestin-positive OHC-like cells in vivo in the adult mammalian cochlea. While the regenerated prestin-positive OHC-like cells were not sufficient to obtain hearing recovery, these results highlight SC-originated OHC regeneration via the synergistic function of Atoh1 and Ikzf2.

Another combination strategy involving Gfi1, Pou4f3, and Atoh1 (GPA) (Fig. 2C), was recently investigated in two studies involving neonatal and adult mammals, respectively (Chen et al., 2021; Iyer et al., 2022). Chen et al. (2021) co-expressed GPA in neonatal cochleae using two different CreER/loxP mouse lines and investigated the potential of different SC subtypes to reprogramme and differentiate into HCs. Newly formed IHCs arose exclusively from Lgr5 positive SCs. In contrast, the induced OHCs were originated from both Lgr5-and Fgfr3 positive SCs. Subtype-specific differentiation was identified because the SC-derived HCs exhibited terminal markers of mature HCs. Electrophysiological experiments have been further designed to verify the functional maturation of the IHCs, which showed that they acquired steady K^+ and Ca^{2+} currents and exhibited exocytosis in a manner resembling the process of ribbon synapse refinement in IHCs. Functional maturation of the IHCs represents a significant step forward in sensory regeneration based on SC reprogramming. However, progression was observed only in the neonatal cochlea. Iyer et al. (2022) compared the effects of the same combination strategy in cochlear SCs at birth and at 1 week of age and found that IHCs in the latter stage were less mature. ScRNA-seq and ATAC-seq analyses indicated that the reduction in the reprogramming ability in SCs was related to at least two impediments. HC gene loci in SCs become less epigenetically accessible over the course of post-natal development, and SCs in older mice received negative signals from HCs and thus were prevented from reprogramming.

Other strategies not involving the key transcription factor Atoh1 have also recently been investigated. Follistatin was found to enhance LIN28B-induced SC reprogramming and HC regeneration in the cochleae of neonatal mice via a mechanism in which Follistatin counterbalanced the abnormal activation of TGF-β by LIN28B (Li et al., 2022).

5. Conclusions and future outlooks

This review summarised the literature concerning the important roles played by SCs following the occurrence of inner ear damage, including initial structural repair and the reprogramming process for HC regeneration, and discussed approaches to enhance this process. Although SC-originated HC regeneration occurring either spontaneously or having been induced increases the number of HCs to a large extent, full functional restoration of the inner ear has seldom been reported (Chen et al., 2021; Guo et al., 2021; Iyer et al., 2022; Shu et al., 2019). Attempts to promote SC programming and explore the related mechanisms highlight the need for future research to make a more detailed analysis of transcription factors in favour of HC development and SC epigenetic regulators inhibiting SC-specific genes in order to induce better functional HC maturity. It is also notable that the regenerative features of the cochlear and vestibular epithelium differ a lot. Studies illuminating the mechanisms underlying the diversity will help with solutions promoting HC regeneration further.

Epigenetic modifications are considered to be related to a rapid decrease in inner ear SC plasticity in the post-natal stage, and several studies have investigated the importance of epigenetic regulation in the development, protection, and regeneration of the inner ear (Deng and Hu, 2020; Ma et al., 2022; Nguyen et al., 2023; Tao et al., 2021; Wang et al., 2023). DNA demethylation (Deng and Hu, 2020) and H3K4me3 histone modification (Ma et al., 2022) are indispensable for SC dedifferentiation and HC regeneration. The extent of Sox2 enhancer influence has been postulated to govern the reprogramming potential of SCs into stem cells (Waldhaus et al., 2012). The hypothesis that epigenetic modification has the potential to enhance SC plasticity has been validated in an experiment conducted with an isolated neonatal cochlea, which showed that Wnt activation was related to Lgr5+ SCs expansion, and the SC-originated clonal colonies increased significantly in the presence of the histone deacetylase inhibitor valproic acid (McLean et al., 2017). Future exploration involving epigenetic manipulation of SCs may provide better targeted approaches toward full functional recovery of the inner ear.

As SCs play essential roles in the inner ear, it is important to maintain the HC-to-SC ratio during regeneration. Ensuring SC proliferation while direct transdifferentiation occurs is another prerequisite. While several studies have shown SC proliferation using different approaches, such as Sox2 haploinsufficiency (Atkinson et al., 2018) and cell cycle manipulation (Oesterle et al., 2011; Walters et al., 2014) in the neonatal inner ear, this capability remains limited in the mature cochlea (Atkinson

et al., 2018; Oesterle et al., 2011). Determining mechanisms that restrict the proliferative behaviour of mature SCs is likely to be a key area in future research.

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