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Inner ear supporting cells: Rethinking the silent majority

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Abstract

Sensory epithelia of the inner ear contain two major cell types: hair cells and supporting cells. It has been clear for a long time that hair cells play critical roles in mechanoreception and synaptic transmission. In contrast, until recently the more abundant supporting cells were viewed primarily as serving primarily structural and homeostatic functions. In this review we discuss the growing information about the roles that supporting cells play in the development, function and maintenance of the inner ear, their activities in pathological states, their potential for hair cell regeneration, and the mechanisms underlying these processes.

Keywords

Supporting cells; hair cells; homeostasis; regeneration; synaptogenesis; glia

1. Introduction

The sensory receptors for hearing and balance – hair cells – are highly specialized epithelial cells located within the inner ear. Hair cells convert the energy in sound and head movements into neurophysiological signals that are relayed to the brainstem. In mammals, six sensory organs contain hair-cell epithelia (Fig. 1). In the cochlear organ, which is specialized for hearing, hair cells reside within the organ of Corti, atop the basilar membrane, which vibrates in response to sound waves. Similarly, each of the five vestibular organs (the utricle, the saccule, and the three canal organs) contains sensory epithelia with hair cells that are activated by head movements and gravitational force. Hair cells are innervated by neurons whose cell bodies sit outside the sensory epithelium, either in a sensory ganglion within the temporal bone (afferent neurons) or in the hindbrain (efferent neurons). In aquatic animals, hair cells are also found within small sensory organs called

neuromasts in the lateral line system, which sense water movements and allow animals to detect predators and orient themselves relative to currents and other fish (i.e., schooling).

The development, function, and maintenance of inner ear sensory epithelia are heavily dependent upon the supporting cells, which are non-sensory cells that reside between hair cells. Unlike hair cells, which contact only the luminal surface of the epithelium, supporting cells span the entire depth of the epithelium, from the basal lamina to the lumen. Supporting cells are linked to each other and to hair cells by tight and adherens junctions; and they communicate directly with other supporting cells by gap junctions. Supporting cells serve a diverse set of functions in the sensory epithelia (Fig. 2). They have rigid cytoskeletons that maintain the structural integrity of the sensory organs during sound stimulation and head movements. Supporting cells also help to maintain an environment in the epithelium that enables hair cells to function. For instance, hair cells recycle K^+ ions, which help to maintain the driving force for generating the receptor potential. They generate components of the tectorial membrane in the organ of Corti, the otoconial membrane, and otoconial components in the macular organs, and the cupula in the cristae ampullaris. Following trauma or toxicity, supporting cells can eject injured hair cells from the epithelium, phagocytose hair cell debris, and in some cases, generate new hair cells (Fig. 3). Below, we discuss these roles of supporting cells in detail and provide an overview of supporting cells in development, function, and regeneration of sensory epithelia.

2. Embryonic derivation

The vast majority of cells that reside within the membranous labyrinth, including the hair cells, the supporting cells, and the neurons that transmit signals from the sensory epithelia to the brain, arise from ectodermal cells of the otic placode, a structure located on the lateral surface of the head. The placode closes to form the otic vesicle, and regions of the vesicle differentiate into prosensory domains that are distinguishable by their expression of genes such as *Bmp4*, *Jag1*, and *Sox2* [1–3]. Hair cells and supporting cells differentiate within these prosensory domains. In contrast, neuroblasts delaminate from the otic neuroepithelium and coalesce in the underlying mesoderm to form the cochleovestibular ganglion (CVG). Differentiating CVG neurons extend neurites to establish connections with hair cells. Through further proliferation and morphogenetic changes, the sensory organs establish their characteristic shapes, with regions between the prosensory domains differentiating into non-sensory regions. A very small number of sensory epithelial cells and neurons do not come from surface ectoderm but rather, they derive from neural crest cells that migrate into otic vesicle [4].

Within each sensory organ, hair cells and supporting cells differentiate in parallel over fairly protracted periods (e.g., [5]). For instance, in the mouse organ of Corti, cells exit the cell cycle and differentiate between embryonic day 13.5 (E13.5) and postnatal day 0 (P0) [6, 7]; in the mouse utricle, new hair cells and supporting cells are formed between E15 and P14 [7, 8]. In all organs, cell cycle exit and differentiation follow spatial patterns. In the organ of Corti, progenitor cells generate terminally mitotic cells in an apical-to-basal gradient [7, 9], and cells differentiate in an opposing gradient [10, 11]. In vestibular organs, cell cycle exit proceeds in a central-to-peripheral pattern [12], and hair cell differentiation follows a similar

pattern [13]. Although considerable research has defined details of how hair cells differentiate (reviewed in [14], relatively little is known about the timing and spatial patterns of supporting cell differentiation.

3. Supporting cell differentiation and roles in developing sensory epithelia

In the organ of Corti, cell cycle exit is easily demarcated by the upregulation of p27^{kip1} in a portion of precursor cells in the prosensory domain [15, 16]. Early on, p27^{kip1} protein is detected in differentiating hair cells and supporting cells, but is only retained in supporting cells in adulthood. Deletion of the *p27^{kip1}* gene during development extends cell cycle entry beyond the normal developmental period [15, 16] and disruption of the normal apical-to-basal gradient in cell cycle exit [17]. Further, *p27^{kip1}* knockout results in overproduction of both supporting and hair cells followed by apoptosis of some aberrant cells and loss of hearing [15].

Around the time when p27^{kip1} is upregulated in the organ of Corti, expression of the basic helix-loop-helix transcription factor, ATOH1, becomes elevated at the protein level in differentiating hair cells [18]. ATOH1 upregulation proceeds in a basal-to-apical fashion, mirroring the emergence of hair cell profiles. ATOH1 is downregulated after hair cells have differentiated [19, 20]. Loss of *Atoh1* function results in failed hair cell differentiation and abnormal supporting cell differentiation [21]. ATOH1's role in hair cells is cell-autonomous. Consistent with this, *Atoh1* misexpression in supporting cells is sufficient to trigger their conversion into hair cells, in immature [20, 22, 23] and mature [24–26] mammalian inner ear epithelia, although effects are highly reduced in the organ of Corti as animals mature [27]. Deletion of *Atoh1* likely disrupts supporting cell differentiation because critical signals derived from the normally co-developing hair cells are lacking [20]. ATOH1 could also play a cell-autonomous role in developing supporting cells, since its overexpression in embryonic and early postnatal organ of Corti triggers supporting cells to re-enter the cell cycle [28]; again, this effect is reduced as animals mature. Other transcription factors besides ATOH1 are critical for sensory epithelial differentiation, such as GATA3 and PAX2 (reviewed in [29]).

Which additional factors regulate the supporting cell fate? The notch signaling pathway diverts embryonic sensory epithelial precursors from differentiating into hair cells and consequently enables supporting cells to form. This occurs via lateral inhibition; notch ligands in nascent hair cells bind the notch receptor on neighboring undifferentiated precursor cells and drive expression of HES/HEY basic-loop-helix transcription factors [19, 30–32], which repress expression of *Atoh1* and other pro-hair cell genes. Accordingly, loss of *Hes/Hey* function during development leads to overproduction of hair cells at the expense of supporting cells [32, 33]. Inactivation of notch (and decreased HES/HEY activity) at later developmental stages using pharmaceutical inhibitors triggers presumed supporting cells to convert into hair cells [34–36], suggesting notch signaling stabilizes supporting cell fate after differentiation.

Fibroblast growth factors, or FGFs, in coordination with notch signaling, enable pillar cell development in the organ of Corti [14, 34, 37]. These effects seem to be mediated by both

FGF2 and FGF8 [38, 39]. In addition, FGF signaling regulates the stiffness of developing pillar cells [40]. FGFs also appear to maintain the supporting cell phenotype: inhibition of FGF signaling in the posthatch chicken auditory epithelium causes increased hair cell production through a non-mitotic mechanism [41], suggesting FGF signaling can inhibit activation of hair cell genetic programs in mature supporting cells.

Which roles do supporting cells play during development of the sensory epithelia? Below, we discuss three ways in which supporting cell activities help to establish mature structure and function within the sensory epithelia (Fig. 2A).

3.1. Cell patterning in the organ of Corti

In all inner ear sensory epithelia, hair cells and supporting cells are organized into a mosaic that is essential for development of normal hearing [42]. As discussed above, differentiating hair cells prevents neighboring precursor cells from becoming hair cells through notch signaling; these precursors then assume a supporting cell fate. Therefore, the invariant segregation of hair cells and supporting cells is regulated by lateral inhibition [43]. However, genetic ablation of the notch ligand, *jagged2*, results in increased numbers of hair cells in the organ of Corti but only partially disrupts cellular patterning [44]. Furthermore, some progenitor cells continue to differentiate into hair cells in the developing chicken auditory epithelium despite contacting adjacent cells forced to express another Notch ligand, delta-like 1 [45, 46]. These results suggest other mechanisms may also regulate patterning of sensory epithelia in addition to lateral inhibition. During the early development of avian basilar papilla (E8–E9), hair-hair cell contact has been observed, which disappears by E12 [47]. This process likely involves the rearrangement of hair and supporting cells because the ratio of supporting cells to hair cells remains the same and all the cells in this region are already postmitotic by E8–E9 [48]. How this mosaic pattern is maintained into the adulthood is less known, but recent study suggests that a family of cell adhesion molecules nectins may play a role [49]. Nectin-1 specifically expressed on hair cells, and nectin-3 on supporting cells form strong heterophilic interactions capable of generating an alternating pattern of the two cell types. Knockout of *nectin-3* from supporting cells results in redistribution of nectin-1 and aberrant contacts between hair cells. Thus, during development, supporting cells express *notch1* and nectin-3, which act *in trans* with hair cells to establish and maintain the mosaic structure in organ of Corti.

3.2. Planar cell polarity

One of the prominent and conserved features of the vertebrate inner ears is planar cell polarity (PCP)[50]. In auditory system, the actin-based stereociliary bundles on apical surface of hair cells are uniformly oriented towards the lateral surface of the cochlear duct. In vestibular system, hair cell bundles are oriented towards the central line of polarity reversal [51]. Such coordinated alignment of stereociliary bundles is essential for normal mechanotransduction and hearing and balance function [52, 53]. The establishment of PCP is likely mediated by core PCP genes involved in non-canonical Wnt pathway, including *Vangl2*, *Dvl1/2*, *Fz3/6*, and *Fmi (Celsr1)* [54]. *Vangl2* is enriched in the supporting cell-side of the junctions between supporting and hair cells [55]. In addition, c-FMI-1 [56] and FZ3/6 [53] have polarized localization in supporting cells as well as in hair cells that correlate with

the axis of PCP. This suggests that supporting cells may participate in PCP pathway by transmission of polarity information from cell to cell. Besides the conserved non-canonical Wnt pathway, a novel PCP regulator PTK7 is found to be critical for stereociliary bundle orientation [57]. Here, supporting cells express PTK7, which mediates the assembly of an apical myosin II network and exerts contractile tension on the medial border of neighboring hair cells. It is suggested that the contractility modulated by FZ3/6 signaling counterbalances the effect of supporting cell PTK7, facilitating the proper orientation of stereociliary bundles [57].

3.3. Regulation of synaptogenesis

Although the onset of hearing in rodents develops around P12, hair cells and afferent nerve terminals form immature ribbon synapses and fire calcium-based spontaneous action potentials as early as P0 [58, 59]. Similar spontaneous activity is observed in the vestibular system [60]. Supporting cells surrounding inner hair cells in the organ of Corti play a critical role in this process by releasing ATP, which activates the purinergic receptors on inner hair cells to induce Ca^{2+} release [61, 62]. ATP-induced Ca^{2+} spikes could either directly initiate action potentials [61] or modulate action potential patterning in immature inner hair cells [63]. This early activity is thought to be important for maturation of ribbon synapses and refinement of the auditory circuit.

Besides ATP, supporting cells also release the important trophic factor, neurotrophins NT-3 and BDNF, to promote the formation and/or maintenance of ribbon synapses. Both NT-3 and BDNF are highly expressed in supporting cells [64, 65]. *In vitro* study shows that they can regulate expression of both presynaptic proteins (synaptophysin, SNAP-25) and postsynaptic receptors (GluR2, GluR3) [66]. Knockout of *Bdnf* in supporting cells and spiral ganglion neurons of mature mouse cochlea using Pax2-Cre results in reduced synaptic ribbons, decreased exocytosis and impaired hearing sensitivity [67]. Reduced expression of *Bdnf* in vestibular supporting cells, either by conditional *Bdnf* knockout or by blocking Neuregulin-ErbB signaling, dramatically reduces the formation of ribbon synapses without altering sensory epithelia structure and hair cell mechanotransduction [68]. In addition, blocking Neuregulin-ErbB signaling in supporting cells results in reduction of NT-3 production and death of spiral ganglion neurons [69]. These findings clearly indicate that supporting cells secrete multiple factors that act on hair cells and/or sensory neurons through reciprocal interactions to modulate synaptic connections.

It has been well established that glial cells play critical roles in formation and maturation of synaptogenesis in both CNS and PNS (reviewed in [70]). A number of glia-derived molecules, such as TGF- β [71], thrombospondins [72], hevin [73] and glypican [74], can either directly induce the structural synapse or promote functional maturation of synapses. Inner ear supporting cells share many characteristics with the glial cells; for example, they also engulf the ribbon synaptic terminals [75] and modulate the number and activity of the synapses [61, 63, 67, 68]. Therefore, findings from glia cells may provide novel insights into the roles of supporting cells in how ribbon synapses are formed and maintained.

4. Supporting cell diversity in mature epithelia

Within the mature sensory epithelia, supporting cells share many morphological and molecular features. For instance, all supporting cells in mammalian auditory and/or vestibular epithelia express the following genes at the protein and/or transcript level: Sox2 [76], Sox9 [77], Sox10 [78], Jagged1 [76, 79], S100 α [80], and p27^{kip1} [15, 16]. However, consistent with the large range of supporting cell functions, supporting cells in a given sensory epithelium show variation with respect to their shapes and molecular profiles. This is most pronounced in the mammalian organ of Corti, which has the greatest degree of supporting cell heterogeneity (Fig. 1A). Five different types of supporting cells are organized in rows along the organ's length. From the outer edge to the inner edge of the organ, they are: 1) Hensen's cells, 2) Deiters' cells, 3) pillar cells; 4) inner phalangeal cells; and 5) border cells. These supporting cells have distinct morphologies. Hensen's cells are cuboidal or slightly oblong. Inner phalangeal cells and border cells are columnar. The remaining cells – Deiters' and pillar cells – are architecturally exquisite cells, with a strong cytoskeleton, elongated processes, and large structural demands. For instance, the inner and outer pillar cells must maintain the structure of the tunnel of Corti, despite pressure from cells located on either side of the tunnel, during acoustic stimulation.

Supporting cells in the organ of Corti also exhibit distinct expression patterns of a number of genes. For instance, in the early postnatal organ of Corti, two growth factor receptors - FGFR3 and p75^{NTR} - are expressed in pillar cells only [81, 82], and the cell surface antigen CD44 is detected only in outer pillar cells [83]. Immunolabeling for Prox1, a transcription factor, is restricted to outer pillar cells and Deiters' cells in adult mice [84]. Proteolipid protein (PLP) is confined to inner phalangeal and inner border cells [85, 86]. Glial fibrillary acid protein (GFAP) is expressed in inner border, inner phalangeal, and Deiters' cells [79, 87]. The glutamate-aspartate transporter GLAST is expressed on inner border and inner phalangeal cells [88]. Tak1 is expressed by all organ of Corti supporting cells except for Hensen's cells [89]. Aquaporin4 is found in Hensen's cells [90]. Sox21 is expressed in inner phalangeal cells, inner border cells, and Deiters' cells [91]. Although these genes and several others have been used as markers for different supporting cell populations, their physiological contributions to the functional diversity of supporting cells remain elusive. It is interesting that several of the selectively expressed markers of supporting cells (Prox1, PLP, GLAST, GFAP, Tak1, Aquaporin4 and S100 α) are also expressed in some glial cells [92–98], highlighting the similar functions of supporting cells and glia in their respective organs.

In contrast to the organ of Corti, less is known about supporting cell specialization in vestibular epithelia (Fig. 1B). Morphologically, vestibular supporting cells are considerably more homogeneous than auditory supporting cells. Several markers for vestibular supporting cells have been defined (many similar to those discussed above for the organ of Corti). Nonetheless, spatial patterns of gene expression are largely unexplored, with a few exceptions. For example, GFAP expression is higher in extrastriolar regions of the utricle than in the striolar region [87].

Lateral line neuromasts have two defined types of supporting cells with different positions and morphologies in the organ. Internal cells are located within the sensory organ and surround hair cells, and they are columnar, vertically oriented cells, similar to inner ear supporting cells [99]. Mantle cells are located peripherally, forming a ring around each neuromast to enclose the hair cells and the internal supporting cells; they are spindle-shaped, more horizontally oriented cells. To date, there are no characterized molecular markers that distinguish each supporting cell type.

5. Supporting cell functions in the mature inner ear

5.1. Structural function

In mammalian organ of Corti (Fig. 1A), the supporting cells, particularly pillar and Deiters' cells, provide a structural scaffold to enable mechanical stimulation of sensory hair cells. Individual inner hair cells are seated on inner border cells and inner phalangeal cells, at medial and lateral sides, respectively. Outer hair cells rest on the cup-shaped apical surface of Deiters' cells. The first row of outer hair cells is also in contact with outer pillar cells at the apical surface. The sensory epithelium is anchored onto the basilar lamina through the basal surfaces of pillar and Deiters' cells. Structurally, pillar and Deiters' cells contain specialized arrays of cytoskeletal filaments known as microtubule stalks, providing rigid support to hair cells [100]. Inner and outer pillar cells form a triangular-shaped tunnel of Corti, which furnishes the major structure of the organ.

At the apical surface of the sensory epithelium, the membrane of hair cells and supporting cells are interconnected by tight and adherens junctions to form the reticular lamina [42]. The cochlear duct contains two fluids with distinct ionic compositions (reviewed in [101]). The endolymph, located in the scala media and generated by the stria vascularis, has high K^+ and low Na^+ concentrations. Endolymph is in contact with the apical surfaces of hair cells and supporting cells. The perilymph, located in the scala vestibuli and the scala tympani, bathes the basolateral surfaces of hair cells and supporting cells. The perilymph is low in K^+ and high in Na^+ , similar to intracellular fluids. The resulting electrochemical gradient along the reticular lamina generates the endocochlear potential, which is required for hair cell depolarization. Thus, the reticular lamina provides a critical physical barrier between the endolymph and the perilymph [42] that both protects sensory epithelial cells from the caustic endolymph and helps maintain the endocochlear potential.

A number of proteins are involved in formation of the reticular lamina, including claudin-9 (Cldn9), claudin-14 (Cldn14), tricellulin (Tric) and vezatin [102–106]. All of these proteins are located at the cell-cell junctions at the reticular lamina. In transgenic mouse models, knockout of *Cldn9* or *Cldn14* compromises the ionic barrier of the tight junction and causes hair cell death and hearing loss [102, 103]. Knockout of *vezatin*, an adherens junction protein, results in a similar phenotype [106]. In humans, mutations of *Cldn14* and *Tric* cause non-syndromic deafness [104, 105]. Clearly, the tight seal of hair and supporting cells at the reticular lamina is essential to the structural integrity of the organ of Corti (Fig. 2B).

5.2. Homeostasis of ions and small molecules

To maintain the distinct ionic environment of endolymph and perilymph, supporting cells not only provide a physical barrier but also actively regulate the homeostasis of its ion composition (Fig. 2B). Supporting cells express a high level of epithelial Na⁺ channel (ENaC) [107]. Due to their negative resting potential, supporting cells can absorb Na⁺ from the scala media through apical ENaC, effectively reducing endolymphic Na⁺ concentration. In addition, supporting cells play a crucial role in the recycling of K⁺ from the basolateral side of hair cells back to the endolymph through K⁺/Cl⁻ co-transporters (Kcc4) and gap junctions [108, 109]. In the mature organ of Corti, Kcc4 is exclusively expressed in inner phalangeal cells and Deiters' cells. Knockout of *Kcc4* or *connexin 26* (*Cx26*) results in a similar phenotype characterized by degeneration of hair cells and deafness, possibly due to K⁺ intoxication of hair cells. It is believed that K⁺ ions that accumulate in hair cells during apical mechanotransduction are transported to supporting cells through Kcc4, then diffuse via gap junction to stria fibrocytes, and eventually through the stria vascularis into the endolymph (reviewed in [101]).

Stimulation of hair cells also results in accumulation of glutamate in the synaptic cleft, which needs to be cleared to avoid excitotoxicity. Whole-cell recordings indicate that the glutamate transporter GLAST is responsible for neurotransmitter removal at inner hair cell/afferent synapses [110]. As both cochlear and vestibular supporting cells express GLAST at their basolateral membranes [111, 112], it is likely that supporting cells regulate uptake of glutamate from both cochlear and vestibular hair cells.

Supporting cell gap junctions not only allow ion diffusion among connected cells, such as during K⁺ recycling, but they also provide biochemical coupling for intercellular signaling. In organotypic cochlear cultures, gap junctions mediate the intercellular diffusion of inositol 1,4,5-trisphosphate (IP3) to allow propagation of Ca²⁺ signaling in coupled supporting cells [113–115]. Consistent with the finding, *Cx26* mutations that cause reduced permeability of large molecules, including IP3, result in hearing loss that is independent of K⁺ toxicity [114, 115]. This intercellular Ca²⁺ signaling is dependent on ATP activation of purinergic receptors on supporting cells [113]. Intriguingly, when the intracellular Ca²⁺ is low, supporting cells can release ATP through connexin hemichannels [113, 116]. The released ATP can therefore activate Ca²⁺ signaling even in uncoupled supporting cells over a long distance. Additionally, the ATP is sufficient to activate P2 receptors on outer hair cells to affect their electromotility and control hearing sensitivity. Supporting cells thus exert a dual mechanism in mediating intercellular Ca²⁺ signaling by direct diffusion of IP3 to adjacent cells through gap junctions and release of ATP to extracellular space through hemichannels.

5.3. Modulation of extracellular matrices

The apical surfaces of inner ear sensory epithelia are covered by acellular structures crucial for hair cell mechanotransduction. In mammals, these structures include the tectorial membrane of the organ of Corti, the otoconial membrane of the utricle and saccule, and the cupulae of the canal cristae. Formation and maintenance of these structures requires a concert of proteins expressed on or secreted from inner ear epithelial cells, such as otogelin, tectorin, otoancorin and otopenin (Fig. 2B). During development, secretion of otogelin from

microvilli of the Deiters' cells is important for the organization of fibrillar network during maturation of the tectorial membrane [117, 118]. In the vestibule, otogelin is expressed exclusively in supporting cells and required for the anchoring of otoconial membranes and cupulae to the sensory epithelium [118, 119]. Otogelin continues to be secreted from adult vestibular supporting cells, suggesting that otogelin may be continuously renewed in the otoconial membrane and cupulae [118]. Otoancorin, a GPI-anchored protein highly expressed in interdental cells (which are very medial epithelial cells in the organ of Corti), is essential for attachment of the tectorial membrane to the spiral limbus [120]. Otoancorin is also expressed at the apical surfaces of cochlear border cells and vestibular supporting cells [120, 121]. Supporting cell-derived otoancorin may help maintain the anchorage of acellular gels in the adult inner ear.

In the utricle and saccule, otoconia are calcium carbonate crystals critical for transducing motion to hair cell stereociliary deflection. In a mouse model of a vestibular disorder, mutations in the transmembrane protein otopetrin-1 are associated with impaired otoconial morphogenesis [122]. Interestingly, otopetrin-1 is predominantly expressed on the apical microvilli of supporting cells [123, 124]. Expression of otopetrin-1 inhibits P2Y receptor-mediated intracellular Ca^{2+} release, leading to increased intracellular Ca^{2+} in supporting cells. This process is important for mediating nucleation, growth, and maintenance of otoconia in a low Ca^{2+} environment [124].

In the organ of Corti, the integrity and rigidity of the sensory epithelium are dependent on the extracellular adhesion between supporting cells and the basilar membrane. A number of extracellular matrix (ECM) proteins are highly enriched in basilar lamina and basement membrane, such as collagen, tenascin, chondroitin sulfate proteoglycans, and integrins [125]. Supporting cells, particularly pillar and Deiters' cells, express high levels of discoidin domain receptor 1 (DDR1), a collagen-activated receptor tyrosine kinase involved in matrix homeostasis [126]. Knockout of *Ddr1* results in elevated hearing thresholds with abnormal morphology of the supporting cells and detachment of sensory epithelium from the basilar membrane. Therefore, supporting cells may regulate the deposition and turnover of ECM underlying the basilar lamina, which is required for normal morphology and function of the organ of Corti.

5.4. Mutations in supporting cells affecting hearing and balance function

To date, more than 60 genes have been identified that cause non-syndromic hearing loss, the most common form of genetic deafness [127]. Some of these genes are active in supporting cells (also see discussion of *Cldn*'s and *Ddr1* above). Since gap junctions and hemichannels formed by connexins are critical for supporting cell-mediated ionic and biochemical coupling in inner ear, it is not surprising that many mutations in *connexin* genes (*Cx26*, *Cx30*) are responsible for hearing loss and in some cases balance disorders. *Cx26* (GJB2) mutations are the most common cause of hereditary deafness (DFNB1/DFNA3). More than 100 sequence variants have been reported [128]. *In vitro* experiments suggest that the majority of *Cx26* mutations disrupt formation or permeability of gap junctions [114, 115]. These mutations may affect a variety of processes, including translation, stability, transport, and membrane targeting, connexon assembly, and pore permeability. Loss of *Cx26* function

causes failure in recycling of K^+ to endolymph, leading to imbalanced endocochlear potential, hair cell degeneration, and hearing loss. A subset of deafness-linked *Cx26* mutations (V84L, V95M, and A88S) specifically disrupts the permeability of IP3 with normal intercellular ion and electric conductance [114, 115]. These mutations indicate that intercellular Ca^{2+} signaling propagation through IP3 and possibly other second messengers is involved in processing of auditory signals in the periphery. T5M, a missense mutation in *Cx30* (GJB6), results in mild hearing loss (DFNB1a) and may also involve impaired intercellular Ca^{2+} signaling in a mechanism dependent on ATP release from supporting cell hemichannels [129]. Vestibular disorders resulting from gap junction mutations are less common, possibly due to compensatory effects of other connexins and/or central vestibular pathways [130]. Both *Cx26* and *Cx30* mutations cause saccular dysfunction [131], possibly due to degeneration of hair cells [132].

Formation of the reticular lamina requires assembly of both bicellular and tricellular tight junctions by claudins (Cldn) and tricellulin (Tric). Two mutations of *claudin-14* (*Cldn14*), 398delT and V85D, have been found to cause an autosomal recessive form of deafness DFNB29 [105]. Loss of *Cldn14* function may increase the cation permeability, leading to influx of K^+ from the endolymph to the tunnel of Corti and progressive hair cell degeneration [102]. Tric, another tight junction protein, is enriched at tricellular junctions of hair and supporting cells. Several splice-site and premature truncation mutations of *Tric* co-segregate with moderate-to-profound deafness DFNB49 [104]. It is suggested that these mutations compromise the rigidity of the reticular lamina, causing abnormalities in stereocilia deflections or vulnerability of the reticular lamina to outer hair cell mechanical stress.

6. Supporting cell roles in damaged states

6.1. Maintaining sensory epithelial integrity after hair cell trauma

Hair cells are highly sensitive cells, and they undergo necrotic and/or apoptotic cell death in response to some conditions, including acoustic overstimulation, ototoxic drugs, or changes in the inner ear associated with normal aging (reviewed in [133]). Supporting cells can also die as a result of the same traumas that injure hair cells (e.g., noise [134] and the ototoxin cisplatin [135]). However, in most cases of hair cell injury, supporting cells survive and are integral to maintaining the health and structure of the sensory epithelium.

Supporting cells show a range of responses to hair cell injury that depend upon the degree and type of damage, the sensory organ, and the type of species involved. Supporting cells can converge as hair cells are being expelled from the epithelium, which prevents the toxic, high- K^+ endolymph from coming into contact with the basolateral surfaces of supporting cells and surviving hair cells (Fig. 3A)(e.g., [136]). In the avian auditory organ, the corpses of hair cells are readily detected on the underside of the tectorial membrane within a few days of treatment with ototoxic drug [137, 138]. More dramatically, supporting cell movements can result in bisection of damaged hair cells *in situ*, with the apical (luminal) half subsequently getting extruded into the scala media and the basal half remaining in the epithelium [139]. Cells in the macrophage lineage have phagocytic activities after hair cell damage (e.g., [140, 141]). In the avian utricle, adjacent supporting cells also contribute to

clearance, converging to surround and phagocytose dying hair cells [139]. Similarly, in the guinea pig organ of Corti, the outer hair cell-specific protein prestin, has been detected in supporting cells after noise damage, suggesting supporting cells consumed the debris remaining after hair cell death [142].

6.2. Forming new hair cells to replace injured ones

Supporting cells have another essential role in repair of sensory epithelia after hair cell damage in non-mammals: they regenerate hair cells in both the auditory and vestibular epithelia of birds and reptiles as well as vestibular and lateral line hair cells in amphibians and fish (reviewed in [143]). In birds, regenerated hair cells restore hearing and balance function in a couple of months after damage [144]. Further, in vestibular epithelia and in the lateral line neuromasts of fish, hair cells undergo continual death and replacement (turnover) in adulthood [145]. In the case where supporting cells continually form hair cells throughout life, they are thought to have some stem cell-like characteristics.

In mammals, the degree of hair cell regeneration that occurs after damage is considerably more limited. No hair cells are replaced in the adult organ of Corti after damage [146, 147]. By contrast, a low level of hair cell replacement occurs in the adult mammalian vestibular system following hair cell loss [146, 148].

Non-mammals utilize two mechanisms to replace hair cells (Fig. 3B). In some cases, supporting cells divide, and daughter cells differentiate into either hair cells or supporting cells. In other cases, supporting cells transdifferentiate into hair cells without dividing, a process termed “direct transdifferentiation” or “direct conversion”. In the avian inner ear, supporting cells readily use either mitotic regeneration [149, 150] or non-mitotic regeneration [151, 152] to replace hair cells lost after damage. Studies suggest that zebrafish preferentially replace lost lateral line hair cells via supporting cell mitosis [140, 153]. In contrast, mammalian vestibular epithelia utilize primarily direct transdifferentiation to replace lost hair cells [146], and accordingly, only very low rates of supporting cell division occur after even very severe hair cell lesions [154].

6.2.1. Mitotic hair cell production in mature sensory epithelia—How are these different regenerative behaviors in mature supporting cells regulated? It has been postulated that healthy hair cells emit signals that maintain supporting cell mitotic quiescence and hair cell loss removes this tonic inhibition [149]. Consistent with this, supporting cell division in non-mammals is correlated temporally and spatially with the loss of hair cells (e.g., [155, 156]). Further, application of bFGF, presumably expressed in hair cells, inhibits supporting cell division when added to cultured chicken auditory organs [157]. Further, expression of FGF receptor is downregulated in areas of the chicken auditory epithelium where supporting cell division occurs [158].

It is also likely that damage triggers release of positive regulators. This hypothesis is supported by studies in non-mammals demonstrating changes in growth factor receptor gene expression after hair cell damage (e.g., [159]) and mitogenic effects in supporting cells of factors such as insulin-like growth factor 1 [160], transforming growth factor alpha [161], tumor necrosis factor alpha [161], and epidermal growth factor [162]. Some growth factors

also promote cell cycle entry of supporting cells in adult mammalian vestibular epithelia [163], but effects are very weak. This is likely due to processes in mammalian supporting cells that block their cell cycle reentry and progression or promote mitotically active cells to undergo cell death. Adult supporting cells express cyclin-dependent kinase inhibitors and pocket proteins that block their transition from G1 to S phase (e.g. [15, 16, 164, 165]). Deletion of *p27^{kip1}* or *Rbl2* in adult mice enables some supporting cells to enter the cell cycle [15, 16, 165]. Forced misexpression of *cyclinD1* [77] or *c-Myc* [166] promotes adult mammalian vestibular supporting cells to re-enter the cell cycle. Following forced cyclinD1 expression, supporting cells fail to complete the cell cycle and undergo apoptosis. By contrast, supporting cells divide in response to *c-Myc* misexpression, and some post-mitotic cells survive for long periods and differentiate hair cell features. It is likely that similar and perhaps more potent brakes on cell division are in place in the adult organ of Corti, where mitogens have limited or no impact on supporting cell quiescence (e.g., [167]).

6.2.2. Direct transdifferentiation of supporting cells into hair cells in adults—

Regulation of direct transdifferentiation, the non-mitotic form of hair cell regeneration, has been less well studied. Forced expression of the prosensory transcription factor ATOH1 in adult mammalian auditory [24] and vestibular supporting cells [25, 26] forces them to acquire adult hair cell-like features without an apparent intervening cell division. Similar effects are achieved with *Atoh1* overexpression in the post-hatch avian auditory epithelium [168]. Inhibition of notch activity, which antagonizes *Atoh1* transcription, results in direct conversion of supporting cells into hair cells in the damaged posthatch chicken auditory epithelium [169] and the adult mouse utricle [170]. However, notch inactivation is not sufficient to promote hair cell regeneration in the adult mammalian auditory epithelium [171]. It is not clear why supporting cells in the organ of Corti fail to respond or why only some supporting cells in the chicken auditory epithelium and mouse utricle overproduce hair cells. Recent studies suggest that transcription of hair cell-specific genes in supporting cells (or embryonic precursor cells) requires the coordinated activity of several transcription factors [172] as well as appropriate epigenetic regulation [173].

7. Supporting cells: Specialization and plasticity

During normal homeostatic conditions, supporting cells serve a large variety of functions. In injured organs of non-mammals, supporting cells have the additional tasks of dividing or converting into hair cells, cleaning up the environment via phagocytosis, and making new matrices, while maintaining the structural and ionic integrity of the epithelium, as well as their connections with the underlying substrate and adjacent cells.

How do supporting cells do it all? Is each individual supporting cell capable of each and every task, or are there subsets of supporting cells specialized for each purpose? The answer to this question is not known, but studies clearly show that different supporting cell groups have distinct potentials for hair cell regeneration. In the organ of Corti, a subset of supporting cells – those that express the G protein-coupled receptor *Lgr5* [174] – are more prone to proliferative activity and hair cell formation than other supporting cells [174, 175]. Further, the internal supporting cells of lateral line neuromasts are the direct progenitors to new hair cells [140] but mantle cells may serve as stem cells in the hair cell lineage [153].

Further, supporting cells in the neural region of the chicken auditory epithelium more commonly form hair cells by cell division than direct transdifferentiation, and supporting cells in the abneural region show reciprocal tendencies [176].

The potentials of mammalian supporting cells to form new hair cells become restricted over the earliest stages of postnatal development. Supporting cells in the embryonic organ of Corti readily convert into hair cells upon hair cell loss, thus preserving developmental patterning [177]. However, this does not occur in maturing mammals (e.g., [146]), even after experimental manipulations such as growth factor treatment, cellular dissociation, or notch inactivation [34, 178–180]. These developmental changes may occur because cells with the capacity to form new hair cells are abundant until a certain point but then degenerate or terminally differentiate. Consistent with this, the capacity for colony formation amongst inner ear supporting cells upon dissociation progressively decreases over the postnatal period [181]. Another idea recently put forth is that the apical cytoskeleton of mammalian supporting cells becomes more restrictive to cell spreading upon hair cell loss and thus prevents them from dividing or transdifferentiating [182, 183]. Another confounding factor is the more dramatic restriction of mammalian auditory supporting cells that occurs after hair cell loss, which is evident in both their morphology and proliferative potential [184, 185]. Mechanisms restricting regenerative behaviors in supporting cells will be a large topic of investigation in future studies. Of particular consideration should be the properties that enable adult vestibular supporting cells to convert into hair cells after damage.

8. Conclusions

As has occurred with the non-neuronal cells of the central and peripheral nervous system, the glia, we are gaining a greater appreciation of the important roles that supporting cells play in the inner ear. These findings not only provide insights into the cellular and molecular mechanisms of inner ear development and function, but also show that supporting cells and molecules expressed by them are important therapeutic targets for the treatment of hearing loss and balance disorders. A better understanding of these cells and their biological roles could be eventually translated into methods to better protect the ear from insults, to enhance the survival of hair cells and sensory neurons in disease states, or even to induce regenerative processes that could restore function after hair cell loss.

References

1. Kiernan AE, Pelling AL, Leung KK, Tang AS, Bell DM, Tease C, et al. Sox2 is required for sensory organ development in the mammalian inner ear. *Nature*. 2005; 434:1031–1035. [PubMed: 15846349]
2. Morrison A, Hodgetts C, Gossler A, Hrabe de Angelis M, Lewis J. Expression of Delta1 and Serrate1 (Jagged1) in the mouse inner ear. *Mech Dev*. 1999; 84:169–172. [PubMed: 10473135]
3. Oh SH, Johnson R, Wu DK. Differential expression of bone morphogenetic proteins in the developing vestibular and auditory sensory organs. *J Neurosci*. 1996; 16:6463–6475. [PubMed: 8815925]
4. Freyer L, Aggarwal V, Morrow BE. Dual embryonic origin of the mammalian otic vesicle forming the inner ear. *Development*. 2011; 138:5403–5414. [PubMed: 22110056]
5. Goodyear R, Holley M, Richardson G. Hair and supporting-cell differentiation during the development of the avian inner ear. *J Comp Neurol*. 1995; 351:81–93. [PubMed: 7896941]

6. Pujol, R.; Lavigne-Rebillard, M.; Lenoir, M. Development of sensory and neural structures in the mammalian cochlea. In: Rubel, EW.; Popper, AN.; Fay, RR., editors. Development of the auditory system. New York: Springer; 1998. p. 146-192.
7. Ruben RJ. Development of the inner ear of the mouse: a radioautographic study of terminal mitoses. *Acta Otolaryngol.* 1967; 220(Suppl):1-44. [PubMed: 6067797]
8. Burns JC, On D, Baker W, Collado MS, Corwin JT. Over half the hair cells in the mouse utricle first appear after birth, with significant numbers originating from early postnatal mitotic production in peripheral and striolar growth zones. *J Assoc Res Otolaryngol.* 2012; 13:609-627. [PubMed: 22752453]
9. Matei V, Pauley S, Kaing S, Rowitch D, Beisel KW, Morris K, et al. Smaller inner ear sensory epithelia in *Neurog 1* null mice are related to earlier hair cell cycle exit. *Dev Dyn.* 2005; 234:633-650. [PubMed: 16145671]
10. Lim DJ, Anniko M. Developmental morphology of the mouse inner ear. A scanning electron microscopic observation. *Acta Otolaryngol Suppl.* 1985; 422:1-69. [PubMed: 3877398]
11. Sher AE. The embryonic and postnatal development of the inner ear of the mouse. *Acta Otolaryngol Suppl.* 1971; 285:1-77. [PubMed: 4334052]
12. Sans A, Chat M. Analysis of temporal and spatial patterns of rat vestibular hair cell differentiation by tritiated thymidine radioautography. *J Comp Neurol.* 1982; 206:1-8. [PubMed: 6124561]
13. Rusch A, Lysakowski A, Eatock RA. Postnatal development of type I and type II hair cells in the mouse utricle: acquisition of voltage-gated conductances and differentiated morphology. *J Neurosci.* 1998; 18:7487-7501. [PubMed: 9736667]
14. Kelley MW. Regulation of cell fate in the sensory epithelia of the inner ear. *Nat Rev Neurosci.* 2006; 7:837-849. [PubMed: 17053809]
15. Chen P, Segil N. p27(Kip1) links cell proliferation to morphogenesis in the developing organ of Corti. *Development.* 1999; 126:1581-1590. [PubMed: 10079221]
16. Lowenheim H, Furness DN, Kil J, Zinn C, Gultig K, Fero ML, et al. Gene disruption of p27(Kip1) allows cell proliferation in the postnatal and adult organ of corti. *Proc Natl Acad Sci U S A.* 1999; 96:4084-4088. [PubMed: 10097167]
17. Lee YS, Liu F, Segil N. A morphogenetic wave of p27Kip1 transcription directs cell cycle exit during organ of Corti development. *Development.* 2006; 133:2817-2826. [PubMed: 16790479]
18. Chen P, Johnson JE, Zoghbi HY, Segil N. The role of *Math1* in inner ear development: Uncoupling the establishment of the sensory primordium from hair cell fate determination. *Development.* 2002; 129:2495-2505. [PubMed: 11973280]
19. Lanford PJ, Shailam R, Norton CR, Gridley T, Kelley MW. Expression of *Math1* and *HES5* in the cochleae of wildtype and *Jag2* mutant mice. *J Assoc Res Otolaryngol.* 2000; 1:161-171. [PubMed: 11545143]
20. Woods C, Montcouquiol M, Kelley MW. *Math1* regulates development of the sensory epithelium in the mammalian cochlea. *Nat Neurosci.* 2004; 7:1310-1318. [PubMed: 15543141]
21. Bermingham NA, Hassan BA, Price SD, Vollrath MA, Ben-Arie N, Eatock RA, et al. *Math1*: an essential gene for the generation of inner ear hair cells. *Science.* 1999; 284:1837-1841. [PubMed: 10364557]
22. Zhao LD, Guo WW, Lin C, Li LX, Sun JH, Wu N, et al. Effects of DAPT and *Atoh1* overexpression on hair cell production and hair bundle orientation in cultured Organ of Corti from neonatal rats. *PLoS One.* 2011; 6:e23729. [PubMed: 22028767]
23. Zheng JL, Gao WQ. Overexpression of *Math1* induces robust production of extra hair cells in postnatal rat inner ears. *Nat Neurosci.* 2000; 3:580-586. [PubMed: 10816314]
24. Kawamoto K, Ishimoto S, Minoda R, Brough DE, Raphael Y. *Math1* gene transfer generates new cochlear hair cells in mature guinea pigs in vivo. *J Neurosci.* 2003; 23:4395-4400. [PubMed: 12805278]
25. Shou J, Zheng JL, Gao WQ. Robust generation of new hair cells in the mature mammalian inner ear by adenoviral expression of *Hath1*. *Mol Cell Neurosci.* 2003; 23:169-179. [PubMed: 12812751]

26. Staecker H, Praetorius M, Baker K, Brough DE. Vestibular hair cell regeneration and restoration of balance function induced by math1 gene transfer. *Otol Neurotol.* 2007; 28:223–231. [PubMed: 17255891]
27. Liu Z, Dearman JA, Cox BC, Walters BJ, Zhang L, Ayrault O, et al. Age-dependent in vivo conversion of mouse cochlear pillar and Deiters' cells to immature hair cells by Atoh1 ectopic expression. *J Neurosci.* 2012; 32:6600–6610. [PubMed: 22573682]
28. Kelly MC, Chang Q, Pan A, Lin X, Chen P. Atoh1 directs the formation of sensory mosaics and induces cell proliferation in the postnatal mammalian cochlea in vivo. *J Neurosci.* 2012; 32:6699–6710. [PubMed: 22573692]
29. Schimmang T. Transcription factors that control inner ear development and their potential for transdifferentiation and reprogramming. *Hear Res.* 2012
30. Li S, Mark S, Radde-Gallwitz K, Schlisner R, Chin MT, Chen P. Hey2 functions in parallel with Hes1 and Hes5 for mammalian auditory sensory organ development. *BMC Dev Biol.* 2008; 8:20. [PubMed: 18302773]
31. Murata J, Ohtsuka T, Tokunaga A, Nishiike S, Inohara H, Okano H, et al. Notch-Hes1 pathway contributes to the cochlear prosensory formation potentially through the transcriptional downregulation of p27Kip1. *J Neurosci Res.* 2009; 87:3521–3534. [PubMed: 19598246]
32. Zine A, Aubert A, Qiu J, Therianos S, Guillemot F, Kageyama R, et al. Hes1 and Hes5 activities are required for the normal development of the hair cells in the mammalian inner ear. *J Neurosci.* 2001; 21:4712–4720. [PubMed: 11425898]
33. Zheng JL, Shou J, Guillemot F, Kageyama R, Gao WQ. Hes1 is a negative regulator of inner ear hair cell differentiation. *Development.* 2000; 127:4551–4560. [PubMed: 11023859]
34. Doetzlhofer A, Basch ML, Ohshima T, Gessler M, Groves AK, Segil N. Hey2 regulation by FGF provides a Notch-independent mechanism for maintaining pillar cell fate in the organ of Corti. *Dev Cell.* 2009; 16:58–69. [PubMed: 19154718]
35. Hayashi T, Kokubo H, Hartman BH, Ray CA, Reh TA, Bermingham-McDonogh O. Hes1 and Hes2 may act as early effectors of Notch signaling in the developing cochlea. *Dev Biol.* 2008; 316:87–99. [PubMed: 18291358]
36. Takebayashi S, Yamamoto N, Yabe D, Fukuda H, Kojima K, Ito J, et al. Multiple roles of Notch signaling in cochlear development. *Dev Biol.* 2007; 307:165–178. [PubMed: 17531970]
37. Pickles JO, Chir B. Roles of fibroblast growth factors in the inner ear. *Audiol Neurotol.* 2002; 7:36–39. [PubMed: 11914524]
38. Jacques BE, Montcouquiol ME, Layman EM, Lewandoski M, Kelley MW. Fgf8 induces pillar cell fate and regulates cellular patterning in the mammalian cochlea. *Development.* 2007; 134:3021–3029. [PubMed: 17634195]
39. Mueller KL, Jacques BE, Kelley MW. Fibroblast growth factor signaling regulates pillar cell development in the organ of Corti. *J Neurosci.* 2002; 22:9368–9377. [PubMed: 12417662]
40. Szarama KB, Stepanyan R, Petralia RS, Gavara N, Frolenkov GI, Kelley MW, et al. Fibroblast growth factor receptor 3 regulates microtubule formation and cell surface mechanical properties in the developing organ of Corti. *Bioarchitecture.* 2012; 2
41. Jacques BE, Dabdoub A, Kelley MW. Fgf signaling regulates development and transdifferentiation of hair cells and supporting cells in the basilar papilla. *Hear Res.* 2012; 289:27–39. [PubMed: 22575790]
42. Gulley RL, Reese TS. Intercellular junctions in the reticular lamina of the organ of Corti. *J Neurocytol.* 1976; 5:479–507. [PubMed: 993823]
43. Kelley MW. Cell adhesion molecules during inner ear and hair cell development, including notch and its ligands. *Curr Top Dev Biol.* 2003; 57:321–356. [PubMed: 14674486]
44. Lanford PJ, Lan Y, Jiang R, Lindsell C, Weinmaster G, Gridley T, et al. Notch signalling pathway mediates hair cell development in mammalian cochlea. *Nat Genet.* 1999; 21:289–292. [PubMed: 10080181]
45. Chrysostomou E, Gale JE, Daudet N. Delta-like 1 and lateral inhibition during hair cell formation in the chicken inner ear: evidence against cis-inhibition. *Development.* 2012; 139:3764–3774. [PubMed: 22991441]

46. Eddison M, Le Roux I, Lewis J. Notch signaling in the development of the inner ear: lessons from *Drosophila*. *Proc Natl Acad Sci U S A*. 2000; 97:11692–11699. [PubMed: 11050197]
47. Goodyear R, Richardson G. Pattern formation in the basilar papilla: evidence for cell rearrangement. *J Neurosci*. 1997; 17:6289–6301. [PubMed: 9236239]
48. Katayama A, Corwin JT. Cell production in the chicken cochlea. *J Comp Neurol*. 1989; 281:129–135. [PubMed: 2925897]
49. Togashi H, Kominami K, Waseda M, Komura H, Miyoshi J, Takeichi M, et al. Nectins establish a checkerboard-like cellular pattern in the auditory epithelium. *Science*. 2011; 333:1144–1147. [PubMed: 21798896]
50. Lewis J, Davies A. Planar cell polarity in the inner ear: how do hair cells acquire their oriented structure? *J Neurobiol*. 2002; 53:190–201. [PubMed: 12382275]
51. McNeill H. Planar cell polarity: keeping hairs straight is not so simple. *Cold Spring Harb Perspect Biol*. 2010; 2:a003376. [PubMed: 20182624]
52. Montcouquiol M, Rachel RA, Lanford PJ, Copeland NG, Jenkins NA, Kelley MW. Identification of *Vangl2* and *Scrb1* as planar polarity genes in mammals. *Nature*. 2003; 423:173–177. [PubMed: 12724779]
53. Wang Y, Guo N, Nathans J. The role of *Frizzled3* and *Frizzled6* in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *J Neurosci*. 2006; 26:2147–2156. [PubMed: 16495441]
54. May-Simera H, Kelley MW. Planar cell polarity in the inner ear. *Curr Top Dev Biol*. 2012; 101:111–140. [PubMed: 23140627]
55. Giese AP, Ezan J, Wang L, Lasvaux L, Lembo F, Mazzocco C, et al. *Gipc1* has a dual role in *Vangl2* trafficking and hair bundle integrity in the inner ear. *Development*. 2012; 139:3775–3785. [PubMed: 22991442]
56. Davies A, Formstone C, Mason I, Lewis J. Planar polarity of hair cells in the chick inner ear is correlated with polarized distribution of c-flamingo-1 protein. *Dev Dyn*. 2005; 233:998–1005. [PubMed: 15830377]
57. Lee J, Andreeva A, Sipe CW, Liu L, Cheng A, Lu X. *PTK7* regulates myosin II activity to orient planar polarity in the mammalian auditory epithelium. *Curr Biol*. 2012; 22:956–966. [PubMed: 22560610]
58. Beutner D, Moser T. The presynaptic function of mouse cochlear inner hair cells during development of hearing. *J Neurosci*. 2001; 21:4593–4599. [PubMed: 11425887]
59. Johnson SL, Marcotti W, Kros CJ. Increase in efficiency and reduction in Ca^{2+} dependence of exocytosis during development of mouse inner hair cells. *J Physiol*. 2005; 563:177–191. [PubMed: 15613377]
60. Desmadryl G, Raymond J, Sans A. In vitro electrophysiological study of spontaneous activity in neonatal mouse vestibular ganglion neurons during development. *Brain Res*. 1986; 390:133–136. [PubMed: 3948026]
61. Tritsch NX, Bergles DE. Developmental regulation of spontaneous activity in the Mammalian cochlea. *J Neurosci*. 2010; 30:1539–1550. [PubMed: 20107081]
62. Tritsch NX, Yi E, Gale JE, Glowatzki E, Bergles DE. The origin of spontaneous activity in the developing auditory system. *Nature*. 2007; 450:50–55. [PubMed: 17972875]
63. Johnson SL, Eckrich T, Kuhn S, Zampini V, Franz C, Ranatunga KM, et al. Position-dependent patterning of spontaneous action potentials in immature cochlear inner hair cells. *Nat Neurosci*. 2011; 14:711–717. [PubMed: 21572434]
64. Sugawara M, Murtie JC, Stankovic KM, Liberman MC, Corfas G. Dynamic patterns of neurotrophin 3 expression in the postnatal mouse inner ear. *J Comp Neurol*. 2007; 501:30–37. [PubMed: 17206617]
65. Pirvola U, Ylikoski J, Palgi J, Lehtonen E, Arumae U, Saarma M. Brain-derived neurotrophic factor and neurotrophin 3 mRNAs in the peripheral target fields of developing inner ear ganglia. *Proc Natl Acad Sci U S A*. 1992; 89:9915–9919. [PubMed: 1409719]
66. Flores-Otero J, Xue HZ, Davis RL. Reciprocal regulation of presynaptic and postsynaptic proteins in bipolar spiral ganglion neurons by neurotrophins. *J Neurosci*. 2007; 27:14023–14034. [PubMed: 18094241]

67. Zuccotti A, Kuhn S, Johnson SL, Franz C, Singer W, Hecker D, et al. Lack of brain-derived neurotrophic factor hampers inner hair cell synapse physiology, but protects against noise-induced hearing loss. *J Neurosci.* 2012; 32:8545–8553. [PubMed: 22723694]
68. Gomez-Casati ME, Murtie JC, Rio C, Stankovic K, Liberman MC, Corfas G. Nonneuronal cells regulate synapse formation in the vestibular sensory epithelium via erbB-dependent BDNF expression. *Proc Natl Acad Sci U S A.* 2010; 107:17005–17010. [PubMed: 20837532]
69. Stankovic K, Rio C, Xia A, Sugawara M, Adams JC, Liberman MC, et al. Survival of adult spiral ganglion neurons requires erbB receptor signaling in the inner ear. *J Neurosci.* 2004; 24:8651–8661. [PubMed: 15470130]
70. Pfrieger FW. Role of glial cells in the formation and maintenance of synapses. *Brain research reviews.* 2010; 63:39–46. [PubMed: 19931561]
71. Fuentes-Medel Y, Ashley J, Barria R, Maloney R, Freeman M, Budnik V. Integration of a retrograde signal during synapse formation by glia-secreted TGF-beta ligand. *Curr Biol.* 2012; 22:1831–1838. [PubMed: 22959350]
72. Christopherson KS, Ullian EM, Stokes CC, Mallowney CE, Hell JW, Agah A, et al. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell.* 2005; 120:421–433. [PubMed: 15707899]
73. Kucukdereli H, Allen NJ, Lee AT, Feng A, Ozlu MI, Conatser LM, et al. Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC. *Proc Natl Acad Sci U S A.* 2011; 108:E440–E449. [PubMed: 21788491]
74. Allen NJ, Bennett ML, Foo LC, Wang GX, Chakraborty C, Smith SJ, et al. Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. *Nature.* 2012; 486:410–414. [PubMed: 22722203]
75. Whitehead MC, Morest DK. The growth of cochlear fibers and the formation of their synaptic endings in the avian inner ear: a study with the electron microscope. *Neuroscience.* 1985; 14:277–300. [PubMed: 3974882]
76. Oesterle EC, Campbell S, Taylor RR, Forge A, Hume CR. Sox2 and JAGGED1 expression in normal and drug-damaged adult mouse inner ear. *J Assoc Res Otolaryngol.* 2008; 9:65–89. [PubMed: 18157569]
77. Loponen H, Ylikoski J, Albrecht JH, Pirvola U. Restrictions in cell cycle progression of adult vestibular supporting cells in response to ectopic cyclin D1 expression. *PLoS One.* 2011; 6:e27360. [PubMed: 22073316]
78. Watanabe K, Takeda K, Katori Y, Ikeda K, Oshima T, Yasumoto K, et al. Expression of the Sox10 gene during mouse inner ear development. *Brain Res Mol Brain Res.* 2000; 84:141–145. [PubMed: 11113541]
79. Smeti I, Savary E, Capelle V, Hugnot JP, Uziel A, Zine A. Expression of candidate markers for stem/progenitor cells in the inner ears of developing and adult GFAP and nestin promoter-GFP transgenic mice. *Gene Expr Patterns.* 2011; 11:22–32. [PubMed: 20817025]
80. Coppens AG, Kiss R, Heizmann CW, Schafer BW, Poncelet L. Immunolocalization of the calcium binding S100A1, S100A5 and S100A6 proteins in the dog cochlea during postnatal development. *Brain Res Dev Brain Res.* 2001; 126:191–199.
81. Hayashi T, Cunningham D, Bermingham-McDonogh O. Loss of Fgfr3 leads to excess hair cell development in the mouse organ of Corti. *Dev Dyn.* 2007; 236:525–533. [PubMed: 17117437]
82. Sano H, Mukai J, Monoo K, Close LG, Sato TA. Expression of p75NTR and its associated protein NADE in the rat cochlea. *Laryngoscope.* 2001; 111:535–538. [PubMed: 11224788]
83. Hertzano R, Puligilla C, Chan SL, Timothy C, Depireux DA, Ahmed Z, et al. CD44 is a marker for the outer pillar cells in the early postnatal mouse inner ear. *J Assoc Res Otolaryngol.* 2010; 11:407–418. [PubMed: 20386946]
84. Bermingham-McDonogh O, Oesterle EC, Stone JS, Hume CR, Huynh HM, Hayashi T. Expression of Prox1 during mouse cochlear development. *J Comp Neurol.* 2006; 496:172–186. [PubMed: 16538679]
85. Gomez-Casati ME, Murtie J, Taylor B, Corfas G. Cell-specific inducible gene recombination in postnatal inner ear supporting cells and glia. *J Assoc Res Otolaryngol.* 2010; 11:19–26. [PubMed: 19820996]

86. Morris JK, Maklad A, Hansen LA, Feng F, Sorensen C, Lee KF, et al. A disorganized innervation of the inner ear persists in the absence of ErbB2. *Brain Res.* 2006; 1091:186–199. [PubMed: 16630588]
87. Rio C, Dikkes P, Liberman MC, Corfas G. Glial fibrillary acidic protein expression and promoter activity in the inner ear of developing and adult mice. *J Comp Neurol.* 2002; 442:156–162. [PubMed: 11754168]
88. Zilberstein Y, Liberman MC, Corfas G. Inner hair cells are not required for survival of spiral ganglion neurons in the adult cochlea. *J Neurosci.* 2012; 32:405–410. [PubMed: 22238076]
89. Parker MA, Jiang K, Kempfle JS, Mizutani K, Simmons CL, Bieber R, et al. TAK1 expression in the cochlea: a specific marker for adult supporting cells. *J Assoc Res Otolaryngol.* 2011; 12:471–483. [PubMed: 21472480]
90. Hirt B, Gleiser C, Eckhard A, Mack AF, Muller M, Wolburg H, et al. All functional aquaporin-4 isoforms are expressed in the rat cochlea and contribute to the formation of orthogonal arrays of particles. *Neuroscience.* 2011; 189:79–92. [PubMed: 21621589]
91. Hosoya M, Fujioka M, Matsuda S, Ohba H, Shibata S, Nakagawa F, et al. Expression and function of Sox21 during mouse cochlea development. *Neurochem Res.* 2011; 36:1261–1299. [PubMed: 21287267]
92. Cid E, Santos-Ledo A, Parrilla-Monge M, Lillo C, Arevalo R, Lara JM, et al. Prox1 expression in rod precursors and Muller cells. *Experimental eye research.* 2010; 90:267–276. [PubMed: 19895810]
93. Wight PA, Duchala CS, Readhead C, Macklin WB. A myelin proteolipid protein-LacZ fusion protein is developmentally regulated and targeted to the myelin membrane in transgenic mice. *J Cell Biol.* 1993; 123:443–454. [PubMed: 8408224]
94. Storck T, Schulte S, Hofmann K, Stoffel W. Structure, expression, and functional analysis of a Na(+)-dependent glutamate/aspartate transporter from rat brain. *Proc Natl Acad Sci U S A.* 1992; 89:10955–10959. [PubMed: 1279699]
95. Choi BH, Kim RC. Expression of glial fibrillary acidic protein in immature oligodendroglia. *Science.* 1984; 223:407–409. [PubMed: 6197755]
96. Katsura H, Obata K, Miyoshi K, Kondo T, Yamanaka H, Kobayashi K, et al. Transforming growth factor-activated kinase 1 induced in spinal astrocytes contributes to mechanical hypersensitivity after nerve injury. *Glia.* 2008; 56:723–733. [PubMed: 18293403]
97. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP. Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci.* 1997; 17:171–180. [PubMed: 8987746]
98. Mata M, Alessi D, Fink DJ. S100 is preferentially distributed in myelin-forming Schwann cells. *J Neurocytol.* 1990; 19:432–442. [PubMed: 2391542]
99. Hama K, Yamada Y. Fine structure of the ordinary lateral line organ. II. The lateral line canal organ of spotted shark, *Mustelus manazo*. *Cell Tissue Res.* 1977; 176:23–36. [PubMed: 837393]
100. Slepecky NB, Henderson CG, Saha S. Post-translational modifications of tubulin suggest that dynamic microtubules are present in sensory cells and stable microtubules are present in supporting cells of the mammalian cochlea. *Hear Res.* 1995; 91:136–147. [PubMed: 8647715]
101. Wangemann P. Supporting sensory transduction: cochlear fluid homeostasis and the endocochlear potential. *J Physiol.* 2006; 576:11–21. [PubMed: 16857713]
102. Ben-Yosef T, Belyantseva IA, Saunders TL, Hughes ED, Kawamoto K, Van Itallie CM, et al. Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration. *Hum Mol Genet.* 2003; 12:2049–2061. [PubMed: 12913076]
103. Nakano Y, Kim SH, Kim HM, Sanneman JD, Zhang Y, Smith RJ, et al. A claudin-9-based ion permeability barrier is essential for hearing. *PLoS Genet.* 2009; 5:e1000610. [PubMed: 19696885]
104. Riazuddin S, Ahmed ZM, Fanning AS, Lagziel A, Kitajiri S, Ramzan K, et al. Tricellulin is a tight-junction protein necessary for hearing. *Am J Hum Genet.* 2006; 79:1040–1051. [PubMed: 17186462]

105. Wilcox ER, Burton QL, Naz S, Riazuddin S, Smith TN, Ploplis B, et al. Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. *Cell*. 2001; 104:165–172. [PubMed: 11163249]
106. Bahloul A, Simmler MC, Michel V, Leibovici M, Perfettini I, Roux I, et al. Vezatin, an integral membrane protein of adherens junctions, is required for the sound resilience of cochlear hair cells. *EMBO Mol Med*. 2009; 1:125–138. [PubMed: 20049712]
107. Grunder S, Muller A, Ruppersberg JP. Developmental and cellular expression pattern of epithelial sodium channel alpha, beta and gamma subunits in the inner ear of the rat. *Eur J Neurosci*. 2001; 13:641–648. [PubMed: 11207799]
108. Boettger T, Hubner CA, Maier H, Rust MB, Beck FX, Jentsch TJ. Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter Kcc4. *Nature*. 2002; 416:874–878. [PubMed: 11976689]
109. Cohen-Salmon M, Ott T, Michel V, Hardelin JP, Perfettini I, Eybalin M, et al. Targeted ablation of connexin26 in the inner ear epithelial gap junction network causes hearing impairment and cell death. *Curr Biol*. 2002; 12:1106–1111. [PubMed: 12121617]
110. Glowatzki E, Cheng N, Hiel H, Yi E, Tanaka K, Ellis-Davies GC, et al. The glutamate-aspartate transporter GLAST mediates glutamate uptake at inner hair cell afferent synapses in the mammalian cochlea. *J Neurosci*. 2006; 26:7659–7664. [PubMed: 16855093]
111. Takumi Y, Matsubara A, Danbolt NC, Laake JH, Storm-Mathisen J, Usami S, et al. Discrete cellular and subcellular localization of glutamine synthetase and the glutamate transporter GLAST in the rat vestibular end organ. *Neuroscience*. 1997; 79:1137–1144. [PubMed: 9219972]
112. Furness DN, Lehre KP. Immunocytochemical localization of a high-affinity glutamate-aspartate transporter, GLAST, in the rat and guinea-pig cochlea. *Eur J Neurosci*. 1997; 9:1961–1969. [PubMed: 9383219]
113. Anselmi F, Hernandez VH, Crispino G, Seydel A, Ortolano S, Roper SD, et al. ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca²⁺ signals across the inner ear. *Proc Natl Acad Sci U S A*. 2008; 105:18770–18775. [PubMed: 19047635]
114. Zhang Y, Tang W, Ahmad S, Sipp JA, Chen P, Lin X. Gap junction-mediated intercellular biochemical coupling in cochlear supporting cells is required for normal cochlear functions. *Proc Natl Acad Sci U S A*. 2005; 102:15201–15206. [PubMed: 16217030]
115. Beltramello M, Piazza V, Bukauskas FF, Pozzan T, Mammano F. Impaired permeability to Ins(1,4,5)P₃ in a mutant connexin underlies recessive hereditary deafness. *Nat Cell Biol*. 2005; 7:63–69. [PubMed: 15592461]
116. Zhao HB, Yu N, Fleming CR. Gap junctional hemichannel-mediated ATP release and hearing controls in the inner ear. *Proc Natl Acad Sci U S A*. 2005; 102:18724–18729. [PubMed: 16344488]
117. Kaltenbach JA, Falzarano PR. The development of the reticular lamina in the hamster: an examination of transitory features and their functional roles. *Int J Dev Neurosci*. 1997; 15:509–521. [PubMed: 9263029]
118. El-Amraoui A, Cohen-Salmon M, Petit C, Simmler MC. Spatiotemporal expression of otogelin in the developing and adult mouse inner ear. *Hear Res*. 2001; 158:151–159. [PubMed: 11506947]
119. Simmler MC, Cohen-Salmon M, El-Amraoui A, Guillaud L, Benichou JC, Petit C, et al. Targeted disruption of otog results in deafness and severe imbalance. *Nat Genet*. 2000; 24:139–143. [PubMed: 10655058]
120. Lukashkin AN, Legan PK, Weddell TD, Lukashkina VA, Goodyear RJ, Welstead LJ, et al. A mouse model for human deafness DFNB22 reveals that hearing impairment is due to a loss of inner hair cell stimulation. *Proc Natl Acad Sci U S A*. 2012; 109:19351–19356. [PubMed: 23129639]
121. Zwaenepoel I, Mustapha M, Leibovici M, Verpy E, Goodyear R, Liu XZ, et al. Otoancorin, an inner ear protein restricted to the interface between the apical surface of sensory epithelia and their overlying acellular gels, is defective in autosomal recessive deafness DFNB22. *Proc Natl Acad Sci U S A*. 2002; 99:6240–6245. [PubMed: 11972037]

122. Hurle B, Ignatova E, Massironi SM, Mashimo T, Rios X, Thalmann I, et al. Non-syndromic vestibular disorder with otoconial agenesis in tilted/mergulhador mice caused by mutations in otopenin 1. *Hum Mol Genet.* 2003; 12:777–789. [PubMed: 12651873]
123. Kim E, Hyrc KL, Speck J, Salles FT, Lundberg YW, Goldberg MP, et al. Missense mutations in Otopenin 1 affect subcellular localization and inhibition of purinergic signaling in vestibular supporting cells. *Mol Cell Neurosci.* 2011; 46:655–661. [PubMed: 21236346]
124. Kim E, Hyrc KL, Speck J, Lundberg YW, Salles FT, Kachar B, et al. Regulation of cellular calcium in vestibular supporting cells by otopenin 1. *J Neurophysiol.* 2010; 104:3439–3450. [PubMed: 20554841]
125. Tsuprun V, Santi P. Ultrastructure and immunohistochemical identification of the extracellular matrix of the chinchilla cochlea. *Hear Res.* 1999; 129:35–49. [PubMed: 10190750]
126. Meyer zum Gottesberge AM, Gross O, Becker-Lendzian U, Massing T, Vogel WF. Inner ear defects and hearing loss in mice lacking the collagen receptor DDR1. *Lab Invest.* 2008; 88:27–37. [PubMed: 18026164]
127. Safieddine S, El-Amraoui A, Petit C. The auditory hair cell ribbon synapse: from assembly to function. *Annu Rev Neurosci.* 2012; 35:509–528. [PubMed: 22715884]
128. Chang EH, Van Camp G, Smith RJ. The role of connexins in human disease. *Ear Hear.* 2003; 24:314–323. [PubMed: 12923422]
129. Schutz M, Scimemi P, Majumder P, De Siati RD, Crispino G, Rodriguez L, et al. The human deafness-associated connexin 30 T5M mutation causes mild hearing loss and reduces biochemical coupling among cochlear non-sensory cells in knock-in mice. *Hum Mol Genet.* 2010; 19:4759–4773. [PubMed: 20858605]
130. Darlington CL, Smith PF. Molecular mechanisms of recovery from vestibular damage in mammals: recent advances. *Prog Neurobiol.* 2000; 62:313–325. [PubMed: 10840152]
131. Todt I, Hennies HC, Basta D, Ernst A. Vestibular dysfunction of patients with mutations of Connexin 26. *Neuroreport.* 2005; 16:1179–1181. [PubMed: 16012344]
132. Qu Y, Tang W, Dahlke I, Ding D, Salvi R, Sohl G, et al. Analysis of connexin subunits required for the survival of vestibular hair cells. *J Comp Neurol.* 2007; 504:499–507. [PubMed: 17702002]
133. Op de Beeck K, Schacht J, Van Camp G. Apoptosis in acquired and genetic hearing impairment: the programmed death of the hair cell. *Hear Res.* 2011; 281:18–27. [PubMed: 21782914]
134. Sugawara M, Corfas G, Liberman MC. Influence of supporting cells on neuronal degeneration after hair cell loss. *J Assoc Res Otolaryngol.* 2005; 6:136–147. [PubMed: 15952050]
135. Slatery EL, Warchol ME. Cisplatin ototoxicity blocks sensory regeneration in the avian inner ear. *J Neurosci.* 2010; 30:3473–3481. [PubMed: 20203207]
136. Leonova EV, Raphael Y. Organization of cell junctions and cytoskeleton in the reticular lamina in normal and ototoxically damaged organ of Corti. *Hear Res.* 1997; 113:14–28. [PubMed: 9387983]
137. Mangiardi DA, McLaughlin-Williamson K, May KE, Messana EP, Mountain DC, Cotanche DA. Progression of hair cell ejection and molecular markers of apoptosis in the avian cochlea following gentamicin treatment. *J Comp Neurol.* 2004; 475:1–18. [PubMed: 15176081]
138. Duncan LJ, Mangiardi DA, Matsui JI, Anderson JK, McLaughlin-Williamson K, Cotanche DA. Differential expression of unconventional myosins in apoptotic and regenerating chick hair cells confirms two regeneration mechanisms. *J Comp Neurol.* 2006; 499:691–701. [PubMed: 17048225]
139. Bird JE, Daudet N, Warchol ME, Gale JE. Supporting cells eliminate dying sensory hair cells to maintain epithelial integrity in the avian inner ear. *J Neurosci.* 2010; 30:12545–12556. [PubMed: 20844149]
140. Balak KJ, Corwin JT, Jones JE. Regenerated hair cells can originate from supporting cell progeny: evidence from phototoxicity and laser ablation experiments in the lateral line system. *J Neurosci.* 1990; 10:2502–2512. [PubMed: 2388077]
141. Fredelius L, Rask-Andersen H. The role of macrophages in the disposal of degeneration products within the organ of Corti after acoustic overstimulation. *Acta Otolaryngol.* 1990; 109:76–82. [PubMed: 2309562]

142. Abrashkin KA, Izumikawa M, Miyazawa T, Wang CH, Crumling MA, Swiderski DL, et al. The fate of outer hair cells after acoustic or ototoxic insults. *Hear Res.* 2006; 218:20–29. [PubMed: 16777363]
143. Warchol ME. Sensory regeneration in the vertebrate inner ear: differences at the levels of cells and species. *Hear Res.* 2011; 273:72–79. [PubMed: 20488231]
144. Bermingham-McDonogh O, Rubel EW. Hair cell regeneration: winging our way towards a sound future. *Curr Opin Neurobiol.* 2003; 13:119–126. [PubMed: 12593990]
145. Jorgensen JM, Mathiesen C. The avian inner ear. Continuous production of hair cells in vestibular sensory organs, but not in the auditory papilla. *Naturwissenschaften.* 1988; 75:319–320. [PubMed: 3205314]
146. Forge A, Li L, Nevill G. Hair cell recovery in the vestibular sensory epithelia of mature guinea pigs. *J Comp Neurol.* 1998; 397:69–88. [PubMed: 9671280]
147. Roberson DW, Rubel EW. Cell division in the gerbil cochlea after acoustic trauma. *Am J Otol.* 1994; 15:28–34. [PubMed: 8109626]
148. Forge A, Li L, Corwin JT, Nevill G. Ultrastructural evidence for hair cell regeneration in the mammalian inner ear. *Science.* 1993; 259:1616–1619. [PubMed: 8456284]
149. Corwin JT, Cotanche DA. Regeneration of sensory hair cells after acoustic trauma. *Science.* 1988; 240:1772–1774. [PubMed: 3381100]
150. Ryals BM, Rubel EW. Hair cell regeneration after acoustic trauma in adult Coturnix quail. *Science.* 1988; 240:1774–1776. [PubMed: 3381101]
151. Adler HJ, Raphael Y. New hair cells arise from supporting cell conversion in the acoustically damaged chick inner ear. *Neurosci Lett.* 1996; 205:17–20. [PubMed: 8867010]
152. Roberson DW, Alosi JA, Cotanche DA. Direct transdifferentiation gives rise to the earliest new hair cells in regenerating avian auditory epithelium. *J Neurosci Res.* 2004; 78:461–471. [PubMed: 15372572]
153. Ma EY, Rubel EW, Raible DW. Notch signaling regulates the extent of hair cell regeneration in the zebrafish lateral line. *J Neurosci.* 2008; 28:2261–2273. [PubMed: 18305259]
154. Rubel EW, Dew LA, Roberson DW. Mammalian vestibular hair cell regeneration. *Science.* 1995; 267:701–707. [PubMed: 7839150]
155. Stone JS, Cotanche DA. Synchronization of hair cell regeneration in the chick cochlea following noise damage. *J Cell Sci.* 1992; 102(Pt 4):671–680. [PubMed: 1429885]
156. Stone JS, Cotanche DA. Identification of the timing of S phase and the patterns of cell proliferation during hair cell regeneration in the chick cochlea. *J Comp Neurol.* 1994; 341:50–67. [PubMed: 8006223]
157. Oesterle EC, Bhav SA, Coltrera MD. Basic fibroblast growth factor inhibits cell proliferation in cultured avian inner ear sensory epithelia. *J Comp Neurol.* 2000; 424:307–326. [PubMed: 10906705]
158. Bermingham-McDonogh O, Stone JS, Reh TA, Rubel EW. FGFR3 expression during development and regeneration of the chick inner ear sensory epithelia. *Dev Biol.* 2001; 238:247–259. [PubMed: 11784008]
159. Saffer LD, Gu R, Corwin JT. An RT-PCR analysis of mRNA for growth factor receptors in damaged and control sensory epithelia of rat utricles. *Hear Res.* 1996; 94:14–23. [PubMed: 8789807]
160. Oesterle EC, Tsue TT, Rubel EW. Induction of cell proliferation in avian inner ear sensory epithelia by insulin-like growth factor-I and insulin. *J Comp Neurol.* 1997; 380:262–274. [PubMed: 9100136]
161. Warchol ME. Immune cytokines and dexamethasone influence sensory regeneration in the avian vestibular periphery. *J Neurocytol.* 1999; 28:889–900. [PubMed: 10900092]
162. White PM, Stone JS, Groves AK, Segil N. EGFR signaling is required for regenerative proliferation in the cochlea: conservation in birds and mammals. *Dev Biol.* 2012; 363:191–200. [PubMed: 22230616]
163. Yamashita H, Oesterle EC. Induction of cell proliferation in mammalian inner-ear sensory epithelia by transforming growth factor alpha and epidermal growth factor. *Proc Natl Acad Sci U S A.* 1995; 92:3152–3155. [PubMed: 7724532]

164. Laine H, Sulg M, Kirjavainen A, Pirvola U. Cell cycle regulation in the inner ear sensory epithelia: role of cyclin D1 and cyclin-dependent kinase inhibitors. *Dev Biol.* 2010; 337:134–146. [PubMed: 19854167]
165. Rocha-Sanchez SM, Scheetz LR, Contreras M, Weston MD, Korte M, McGee J, et al. Mature mice lacking Rbl2/p130 gene have supernumerary inner ear hair cells and supporting cells. *J Neurosci.* 2011; 31:8883–8893. [PubMed: 21677172]
166. Burns JC, Yoo JJ, Atala A, Jackson JD. MYC Gene Delivery to Adult Mouse Utricles Stimulates Proliferation of Postmitotic Supporting Cells In Vitro. *PLoS One.* 2012; 7:e48704. [PubMed: 23119091]
167. Daudet N, Ripoll C, Lenoir M. Transforming growth factor- α -induced cellular changes in organotypic cultures of juvenile, amikacin-treated rat organ of corti. *J Comp Neurol.* 2002; 442:6–22. [PubMed: 11754363]
168. Lewis RM, Hume CR, Stone JS. Atoh1 expression and function during auditory hair cell regeneration in post-hatch chickens. *Hear Res.* 2012; 289:74–85. [PubMed: 22543087]
169. Daudet N, Gibson R, Shang J, Bernard A, Lewis J, Stone J. Notch regulation of progenitor cell behavior in quiescent and regenerating auditory epithelium of mature birds. *Dev Biol.* 2009; 326:86–100. [PubMed: 19013445]
170. Lin V, Golub JS, Nguyen TB, Hume CR, Oesterle EC, Stone JS. Inhibition of Notch activity promotes nonmitotic regeneration of hair cells in the adult mouse utricles. *J Neurosci.* 2011; 31:15329–15339. [PubMed: 22031879]
171. Hori R, Nakagawa T, Sakamoto T, Matsuoka Y, Takebayashi S, Ito J. Pharmacological inhibition of Notch signaling in the mature guinea pig cochlea. *Neuroreport.* 2007; 18:1911–1914. [PubMed: 18007185]
172. Ahmed M, Wong EY, Sun J, Xu J, Wang F, Xu PX. Eya1-Six1 interaction is sufficient to induce hair cell fate in the cochlea by activating Atoh1 expression in cooperation with Sox2. *Dev Cell.* 2012; 22:377–390. [PubMed: 22340499]
173. Slattery EL, Speck JD, Warchol ME. Epigenetic influences on sensory regeneration: histone deacetylases regulate supporting cell proliferation in the avian utricle. *J Assoc Res Otolaryngol.* 2009; 10:341–353. [PubMed: 19340485]
174. Chai R, Xia A, Wang T, Jan TA, Hayashi T, Bermingham-McDonogh O, et al. Dynamic expression of Lgr5, a Wnt target gene, in the developing and mature mouse cochlea. *J Assoc Res Otolaryngol.* 2011; 12:455–469. [PubMed: 21472479]
175. Shi F, Kempfle JS, Edge AS. Wnt-responsive Lgr5-expressing stem cells are hair cell progenitors in the cochlea. *J Neurosci.* 2012; 32:9639–9648. [PubMed: 22787049]
176. Cafaro J, Lee GS, Stone JS. Atoh1 expression defines activated progenitors and differentiating hair cells during avian hair cell regeneration. *Dev Dyn.* 2007; 236:156–170. [PubMed: 17096404]
177. Kelley MW, Talreja DR, Corwin JT. Replacement of hair cells after laser microbeam irradiation in cultured organs of corti from embryonic and neonatal mice. *J Neurosci.* 1995; 15:3013–3026. [PubMed: 7722642]
178. Gu R, Montcouquiol M, Marchionni M, Corwin JT. Proliferative responses to growth factors decline rapidly during postnatal maturation of mammalian hair cell epithelia. *Eur J Neurosci.* 2007; 25:1363–1372. [PubMed: 17425563]
179. Hume CR, Kirkegaard M, Oesterle EC. ErbB expression: the mouse inner ear and maturation of the mitogenic response to heregulin. *J Assoc Res Otolaryngol.* 2003; 4:422–443. [PubMed: 14690060]
180. White PM, Doetzlhofer A, Lee YS, Groves AK, Segil N. Mammalian cochlear supporting cells can divide and trans-differentiate into hair cells. *Nature.* 2006; 441:984–987. [PubMed: 16791196]
181. Oshima K, Grimm CM, Corrales CE, Senn P, Martinez Monedero R, Geleoc GS, et al. Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. *J Assoc Res Otolaryngol.* 2007; 8:18–31. [PubMed: 17171473]

182. Collado MS, Burns JC, Meyers JR, Corwin JT. Variations in shape-sensitive restriction points mirror differences in the regeneration capacities of avian and mammalian ears. *PLoS One*. 2011; 6:e23861. [PubMed: 21909368]
183. Meyers JR, Corwin JT. Shape change controls supporting cell proliferation in lesioned mammalian balance epithelium. *J Neurosci*. 2007; 27:4313–4325. [PubMed: 17442815]
184. Izumikawa M, Batts SA, Miyazawa T, Swiderski DL, Raphael Y. Response of the flat cochlear epithelium to forced expression of Atoh1. *Hear Res*. 2008; 240:52–56. [PubMed: 18430530]
185. Raphael Y, Kim YH, Osumi Y, Izumikawa M. Non-sensory cells in the deafened organ of Corti: approaches for repair. *Int J Dev Biol*. 2007; 51:649–654. [PubMed: 17891723]

Highlights

- Supporting cells are required for cell patterning, planar cell polarity, and synaptogenesis in developing sensory epithelia
- In mature sensory epithelia, supporting cells preserve the structural integrity of the sensory organs, modulate ion and small molecule homeostasis, and maintain the accessory extracellular matrices that enable hair cell mechanotransduction
- Supporting cells clear damaged hair cells and regenerate hair cells in injured sensory epithelia

Fig. 1.

Structural and cellular organizations of the mammalian cochlear (A) and vestibular (B) sensory epithelia. (A) The functional unit of cochlea, organ of Corti, consists of one row of inner hair cells (IHC) and three rows of outer hair cells (OHC) that are surrounded by various supporting cells identifiable by structural features and expression of markers. The organ of Corti is covered by tectorial membrane and seated onto the basilar membrane. Both inner and outer hair cells are innervated by cochlear nerves. The apical and basolateral surfaces of the cochlear sensory epithelia are immersed in endolymphic and perilymphic media, respectively. (B) Hair cells (HC) in vestibular organs are ensheathed and separated by more homogenous supporting cells (SC). Type II hair cells form typical ribbon synapses with vestibular nerve endings, whereas type I hair cells have their entire cell body wrapped by calyx nerve terminals. In utricle and saccule, the sensory epithelia are covered by otoconia which stimulate hair cells upon head motion.

Fig. 2.

Diverse roles of supporting cells in developing (A) and mature (B) sensory epithelia. (A) During development, supporting cells (SC) form mosaic cellular pattern with hair cells (HC) through Notch signaling and Nectin heterophilic interactions. Supporting cells express Ptk7 which collaborate with Fz3/6 signaling to regulate planar cell polarity of hair cells. Production of ATP and neurotrophins (NTs) by supporting cells are involved in ribbon synaptogenesis. (B) In mature sensory epithelia, the apical surfaces of hair and supporting cells are sealed by connexin (Cx) tight junctions to form reticular lamina. Supporting cells mediate homeostasis of cations (Na⁺, K⁺) and small molecules (Glu, IP3) through transporters and gap junctions. Supporting cells also modulate extracellular matrices (ECM) by production of ECM components.

Fig. 3.

Roles of supporting cells in injured sensory epithelia. (A) Upon hair cells (HC) damage, supporting cells (SC) may clear the damaged hair cells by expelling them to the scala media and/or phagocytose the debris of injured hair cell. The supporting cells surrounding injury site converge to re-seal the sensory epithelia to prevent further damage. (B) The damaged hair cells can be regenerated by direct transdifferentiation or mitotic proliferation of supporting cells.