Published in final edited form as:

Curr Opin Neurobiol. 2008 August ; 18(4): 377–382. doi:10.1016/j.conb.2008.10.001.

Hair cell regeneration

Albert SB Edge[1,2,3] and **Zheng-Yi Chen**[1,2,3]

[1]Department of Otology and Laryngology, Harvard Medical School, Boston, MA 02115, United States

[2]Tillotson Unit for Cell Biology, Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA 02114, United States

[3]Program in Speech and Hearing Bioscience and Technology, Division of Health Science and Technology, Harvard & MIT, Cambridge, MA 02139, United States

Abstract

The mammalian inner ear largely lacks the capacity to regenerate hair cells, the sensory cells required for hearing and balance. Recent studies in both lower vertebrates and mammals have uncovered genes and pathways important in hair cell development and have suggested ways that the sensory epithelia could be manipulated to achieve hair cell regeneration. These approaches include the use of inner ear stem cells, transdifferentiation of nonsensory cells, and induction of a proliferative response in the cells that can become hair cells.

Introduction

A human cochlea contains about 17 000 hair cells, far fewer receptor cells than other sensory organs such as the retina or olfactory epithelium. Lacking significant redundancy, the cochlea is severely disabled by the loss of just a few thousand hair cells. The sensory epithelium of birds and fish can regenerate new hair cells after deafening whereas humans and other mammals lost this ability during evolution. Thus the loss of hair cells owing to noise trauma or ototoxic drugs is permanent, and accounts for much of the hearing loss that will plague over 30% of the elderly. The hope for the regeneration of human hair cells as a therapy for hearing loss has focused attention on hair cell development and regeneration in lower vertebrates. Here, we summarize recent work in this area, and we suggest potential routes whereby pathways of regeneration in nonmammalian species could be employed in species that have lost the capacity for the regeneration of sensory epithelia.

Regeneration in birds and fish

Birds

In avian vestibular sensory epithelia, hair cells are continuously regenerated [1], whereas in the auditory system, hair cell regeneration occurs only after hair cell damage or death [2,3]. Remarkably, after damage to hair cells that led to loss of hearing, auditory hair cell

Corresponding author: Edge, Albert SB (albert_edge@meei.harvard.edu).

regeneration brought about functional recovery [4]. In both the vestibular and auditory systems, the new hair cells could be regenerated either by cell cycle re-entry or by the transdifferentiation of supporting cells: when hair cells of posthatch chick basilar papilla (BP, the chick auditory organ) were damaged by noise followed by tritiated thymidine injection, both hair cells and supporting cells were seen labeled with thymidine, demonstrating a proliferative response leading to hair cell regeneration [2,3]. Further experiments showed that new hair cells were regenerated even when the cell cycle was partially inhibited, supporting a mechanism in which supporting cells directly transdifferentiate into hair cells [5]. The two mechanisms of hair cell regeneration appear to be elicited by different signals and at different stages. Immediately following hair cell death, hair cell regeneration occurs primarily through transdifferentiation [6]. However, most of the hair cells are subsequently regenerated by proliferation [6]. Different subsets of supporting cells may exist, some of which undergo transdifferentiation while most undergo cell cycle re-entry. It is not clear what accounts for this difference. Increases in PI3 kinase, MAPK, and mTOR activities are involved in the proliferation of supporting cells [6,7], but the signaling pathways that initiate cell cycle re-entry are still largely unknown.

How do quiescent avian BP supporting cells maintain the potential to re-enter the cell cycle? First, quiescent supporting cells may maintain expression of some progenitor genes, so that they can respond to stimuli from hair cell death. For example, Islet1 and Prox1 have been implicated in progenitor cell properties, and they are expressed in BP supporting cells [6,8]. Second, the BP supporting cells may undergo dedifferentiation (or reprogramming). In the salamander, in which tissue regeneration readily occurs, dedifferentiation of mesenchymal cells preceded cell cycle re-entry and led to the formation of a blastema which could then differentiate to specific cell types [9]. Interestingly, in an *in vitro* model in which isolated chick supporting cells could be induced to form new hair cells, the supporting cells underwent epithelial–mesenchymal–epithelial transition before hair cell formation [10]. It is tempting to speculate that supporting cell dedifferentiation may occur before cell cycle re-entry. The analysis of gene expression in regenerating chick BP and comparison with developing chick BP will help to answer these questions.

Zebrafish

Zebrafish have been increasingly used in hair cell regeneration studies. In zebrafish larvae, lateral-line-neuromast hair cells were efficiently killed by gentamicin treatment, and hair cells regenerated fully within 72 hours of treatment [11••]. As in chick BP, most of the hair cells regenerated in zebrafish were derived from supporting cells after cell cycle re-entry. The Notch pathway was required for neuromast hair cell regeneration: blocking of the Notch pathway with a γ-secretase inhibitor led to an increase in the number of hair cells regenerated, with most cells derived from supporting cell proliferation [11••]. This differs from both chick and mouse models in which blockade of Notch resulted primarily in transdifferentiation from supporting cells to hair cells (J Stone, unpublished data). Whether transdifferentiation or proliferation is utilized for hair cell regeneration may also depend on the severity of hair cell damage. After total hair cell death in neuromasts, the proliferation of Sox2-positive supporting/progenitor cells accounted for most hair cell recovery, whereas if some hair cells survived, the new hair cells were regenerated primarily without cell division

[12]. The origin of supporting cells that can transdifferentiate into hair cells remains unknown.

Recent studies have provided further insight into the mechanism of neuromast hair cell regeneration. Using time-lapse video recording to capture the entire regeneration process after gentamicin-induced hair cell death, it was shown that two new hair cells were derived from cell division of a single supporting/progenitor cell, that is through symmetric cell division, within 24 hours [13]. The symmetric cell division in neuromasts is reminiscent of the situation in Rb1 conditional knockout mice, in which committed progenitor cells without Rb1 continue cell division to produce two new hair cells [14••]. Interestingly, a separate study showed that following gentamicin treatment, most of the cells entered S phase after 15–18 hours and completed mitosis at about 24 hours [11••]. Combined, the two studies would indicate that most hair cells are regenerated through one round of cell division within 24 hours of hair cell death.

Are supporting cells stem cells for the ear or are they replaced by another cell that can divide and replace the supporting cells that are lost by differentiation into hair cells? There is not a clear answer, although there are many clues. Clearly, for supporting cells to act as true stem cells, they must replenish themselves after differentiating to another cell type. In other tissues, this is handled by the mechanism of asymmetric division in which a progenitor cell gives rise to a partly differentiated or at least committed precursor and a new stem cell at each division. In cases in which committed progenitor cells respond to hair cell death by directly differentiating to hair cells, there must be other mechanisms involved in replenishing lost progenitor cells. It will be of great interest to identify the origin of the progenitor cells, the signals that initiate cell division to produce more progenitor cells, and the signals that lead to their differentiation to hair cells. The symmetric division appears to differ from the case of the chick BP, where asymmetric cell division of supporting cells is considered a major mechanism [15], although occasional symmetric cell division was also observed.

Why not mammals?

In contrast to lower vertebrates, mammals show little capacity for differentiation of new hair cells or proliferation of any sensory epithelial cells. There are differences between vestibular epithelia, which have some capacity for regeneration, and auditory epithelia, which do not, but the regeneration that has been demonstrated in vestibular epithelia is of limited extent [38,39].

Progenitor cells in sensory epithelia of mammalian inner ear

Whether mammalian sensory epithelia contain true stem cells is a key question. Progenitors have been shown to be present in mammalian sensory epithelia based on a number of criteria: neurosphere formation was demonstrated from both cochlear and vestibular sensory epithelia [16••] and the neurospheres were shown to be clonal and capable of self renewal [17]. The cells differentiated into cell types corresponding to all three germ layers, endoderm, mesoderm, and ectoderm, indicating that the stem cells were pluripotent. Cells in the spheres could differentiate into hair cells and neurons that had properties of inner ear

cells [16••,18]. This raised the possibility that, if properly stimulated, they could be induced to differentiate *in vivo* as the basis for future therapies to replace cells in the inner ear.

The cells obtained by neurosphere formation could be residual embryonic progenitor cells or could be a true stem cell compartment. In the case of the heart [19] and visual system [20], proliferating cells observed in newborns were found to decrease in number with age and have been proposed to represent embryonic progenitors. Like the ear, these tissues show limited regenerative capacity. Despite low or undetectable proliferation by their resident progenitor cells, these tissues do have stem cell compartments with the capacity to enter the cell cycle. In the heart, for example, where tissue damage does not result in a repair process, resident stem cells have been isolated and shown to divide *in vitro* [19], and even in tissues such as blood a slow rate of division is characteristic of the stem cell compartment. Expression of markers is one useful tool for the identification of these cells and identification of marker genes will be important in studies of the inner ear.

Pathways of development as targets for gene manipulation

Regeneration can be seen as recapitulating development; thus many of the genes involved in hair cell development are being investigated both to gain insights and to use as tools for initiating *de novo* formation of hair cells.

The basic helix–loop–helix transcription factor, Atoh1, is a key gene in hair cell development. First, there was a complete lack of hair cells in an Atoh1-knockout animal [21]. Second, expression of Atoh1 converted supporting cells as well as epithelial cells outside the organ of Corti to hair cells [22,23••,24,25]. The hair cells formed were functional [22].

The Notch pathway is also important for laying down the alternating hair cell–supporting cell–hair cell pattern of the cochlear and vestibular epithelia [26,27]; specifically, Notch activation in supporting cells appeared to suppress a hair cell phenotype. Disruption of Notch signaling by the deletion of Notch ligands appeared to release the inhibition of hair cell phenotype so that extra hair cells were formed in the sensory epithelium [28]. The new cells arose from supporting cells, which underwent further cell division in the developing cochlea [28]. The inhibition of Notch signaling with a γ-secretase inhibitor also resulted in the formation of new hair cells in organ of Corti explants from newborn animals [29]. The conversion of progenitors to hair cells after γ-secretase inhibition was dependent on Atoh1 [30], which was increased by the treatment with a γ-secretase inhibitor.

Several other pathways involved in the development of hair cells are also potential targets for manipulations to regenerate hair cells. The BMP pathway has an effect on the number of hair cells in developing chick otocyst, and BMP inhibitors such as noggin may have an effect on their development [31,32]. The hedgehog (HH) pathway is also involved in hair cell development and again disruption can have an effect on the generation of hair cells. A mutation in GLI3, a downstream mediator of HH, increased the size of the sensory epithelium and increased the number of hair cells that developed [33]. The Wnt pathway helps to determine the balance between stem cell proliferation and stem cell differentiation. Wnt signaling modulated hair cell formation in the developing chick through the action of

the canonical Wnt pathway mediated by β-catenin [34]. Preliminary data (F Shi, and A Edge, unpublished) show that overexpressing β-catenin in inner ear progenitor cells increases the number of hair cells that differentiate.

Cell cycle exit and establishment of postmitotic quiescence in the mature inner ear is controlled by the function of multiple negative cell-growth genes, whose suppression may lead to strategies for producing new hair cells. For instance, p27Kip is expressed in supporting cells and was responsible for their exit from the cell cycle at E14 [35]. Extra hair cells formed by the disruption of p27 expression resulted from the division of committed progenitor cells that differentiate into hair cells. In the p27Kip knockout animal, the disruption of the normal morphology of the organ of Corti by additional cells resulted in loss of hearing. Similarly, the deletion of Rb led to direct proliferation of progenitor cells, as well as differentiating hair cells and supporting cells [14••], indicating that the differentiation of the sensory epithelia and cell division are not mutually exclusive. This may also lead to apoptosis of cochlear hair cells [36], but in vestibular epithelia the new hair cells appeared to survive and were functional [37].

Hair cell regeneration in adult inner ear is necessary for any therapeutic intervention to be applied to deaf patients. Thus far, only Atoh1 overexpression has been shown to be effective in converting adult supporting cells to hair cells [23••,24,25].Most of the signaling pathways described here are only active during early inner ear development and their activation in adult inner ear will be necessary to assess their potential for hair cell regeneration.

Are supporting cells hair cell progenitors?

While neurospheres could be obtained from vestibular sensory epithelia throughout life, the capacity to form neurospheres from the cochlea decreased in the first few weeks after birth [16••]. The loss of cochlear sphereforming capacity correlated with the complete lack of regenerative potential in adult cochlea. The persistence of stem cells in the vestibular system correlated with the small degree of cell cycle re-entry and hair cell differentiation that had previously been seen after hair cell damage in the adult utricle [38,39]. Recent studies have shown that supporting cells from early postnatal mouse cochlea had the potential to re-enter the cell cycle *in vitro* [40••]. However, the capacity for cell division by the supporting cells was lost during the first two postnatal weeks, indicating a similar course for the decline in sphere formation and proliferation by supporting cells.

The data from fish and birds as well as more recent data from mammals suggest that supporting cells or a subset of supporting cells can act as precursors for hair cells, and several studies suggest that supporting cells have stem cell characteristics. These characteristics may vary even among the different supporting cell types, which have distinct morphologies and gene expression profiles, although delineating the exact differences will require improved reporters and gene profiling of single cell types. Stem cell markers such as Sox2 [41], Nestin [42], Musashi [43], Notch [26], Prox1 [44], and islet1 [8] were expressed in postnatal supporting cells; the cells are normally quiescent but the cell cycle can be initiated. Supporting cells undergo cell division in what appears to be an asymmetric fashion; Notch signaling appears to keep supporting cells in a nondifferentiated state. Similarly, the central-nervous-system radial glia act as stem cells which are also quiescent

but can be recruited for cell repair, and so supporting cells may be similar to radial glia both in their normal physiological (supporting) role and in their ability to become progenitors on demand. Are the progenitors isolated in neurospheres derivatives of supporting cells? A definitive answer will come from lineage tracing to mark the cells that form spheres.

Regeneration of inner ear using stem cells from other sources

Attempts to make both auditory neurons and hair cells using embryonic stem (ES) cells have been partially successful. Hair cells were obtained from mouse ES cells by timed incubation in the growth factors EGF, bFGF, and IGF-1 [45]. The cells thusly obtained expressed markers of developing and mature hair cells, Atoh1, Brn3c, and myosin VIIa, and had stereociliary bundles that could be labeled for espin and F-actin. Auditory neurons have been produced from ES cells [46,47]. Human ES cells could be converted to progenitors that had markers of embryonic precursors in the neural crest and sensory placodes, and these cells could be differentiated to neurons that had many of the characteristic markers of neurons of the auditory system [48].

In recent reports, other adult stem cells have been used as a source of hair cells. Cells with markers of hair cell progenitors were generated by the application of growth factors to mesenchymal stem cells from bone marrow [49]. Expression of *Atoh1* in these progenitor cells resulted in their conversion to cells that expressed hair cell markers. Olfactory progenitors cocultured with supernatants from cochlear cultures also expressed hair cell markers [50].

It has been difficult to replace hair cells with transplanted cells derived from sources other than the ear. There has been little integration or function by cells transplanted into sensory epithelia, despite reports of cells recovered in the organ of Corti, but advances in ways to obtain hair cells from stem cells should lead to progress [49,50]. Cell transplantation may enable cell replacement in the inner ear, but critical issues would have to be addressed, including the choice of cell type and stage of differentiation, and the timing of infusion relative to the damage. The timing of transplantation could affect the ability of hair cell progenitors to interact with the proper cells in the sensory epithelium to regenerate the correct cell–cell contacts. In the retina, precursors had the capacity to replace photoreceptors if the optimal developmental stage was employed for transplantation [51].

Of course, hair cell regeneration would not be useful if the new hair cells did not form synapses with auditory or vestibular neurons to bring the signals to the brain. Several studies that looked for hair cell replacement also demonstrated reinnervation of the regenerated hair cells [14••,22,23••,24]. Conversely, other kinds of hearing loss result from the degeneration of the eighth nerve neurons rather than hair cells. Exogenous stem cells have been used to replace degenerated auditory nerve; in an animal model of neuronal degeneration, these neurons grew fibers in an apical direction from the nerve trunk and through the osseous spiral lamina into the organ of Corti where they contacted hair cells [47]. The central fibers grew into the brainstem [48]. Still to be determined is whether these neurons can restore function.

Conclusions

Several avenues can be explored to achieve mammalian hair cell regeneration for the purpose of restoring hearing and balance. *Transplantation of inner ear stem cells* has great promise provided that they can be expanded for directional differentiation *in vivo*, which requires identification and characterization of inner ear stem cell genes and an understanding of the cascades required for differentiation. The use of cells from an exogenous source for cell replacement is a promising avenue because of the progress in deriving hair cells *in vitro*, but cell transplantation must first overcome a number of hurdles. *Cell cycle re-entry* by endogenous hair cells or supporting cells for regeneration has distinct advantages, as the cells are produced *in situ* and are likely to attract neurons to forma synapse, but control of cell cycle remains a challenge. *Transdifferentiation of supporting cells into hair cells* occurs in lower vertebrates and may be possible in mammalian cochlea. Relatively normal hair cells and supporting cells can be regenerated during early development by blockade of pRb and p27kip1 function, but this cannot currently be done in the adult. The activation of a group of stem cell genes can reprogram fully mature postmitotic cells to become induced pluripotent stem cells (iPS) [52], and a subset of stem/progenitor genes might be recruited to reprogram mature hair cells or supporting cells to an embryonic or immature phenotype that can re-enter the cell cycle while remaining differentiated. For all these strategies, the vestibular system may be an easier initial target for regenerative therapies since the epithelial architecture is not so elaborated as the cochlea and function may not be severely affected by the conversion of supporting cells to hair cells. And in all cases, an additional challenge is proper spatial integration and innervation of new hair cells.

Acknowledgments

Research in the authors' laboratories was supported by grants DC006908, DC007174 and DC05209 from the National Institute on Deafness and other Communicative Disorders (NIDCD), by Robert Boucai, and by the Hamilton H. Kellogg and Mildred H. Kellogg Charitable Trust.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

•• of outstanding interest

1. 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.
2. Corwin JT, Cotanche DA. Regeneration of sensory hair cells after acoustic trauma. Science. 1988; 240:1772–1774. [PubMed: 3381100]
3. Ryals BM, Rubel EW. Hair cell regeneration after acoustic trauma in adult Coturnix quail. Science. 1988; 240:1774–1776. [PubMed: 3381101]
4. Marean GC, Burt JM, Beecher MD, Rubel EW. Hair cell regeneration in the European starling (*Sturnus vulgaris*): recovery of pure-tone detection thresholds. Hear Res. 1993; 71:125–136. [PubMed: 8113131]
5. 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]
6. Stone JS, Cotanche DA. Hair cell regeneration in the avian auditory epithelium. Int J Dev Biol. 2007; 51:633–647. [PubMed: 17891722]

7. Witte MC, Montcouquiol M, Corwin JT. Regeneration in avian hair cell epithelia: identification of intracellular signals required for S-phase entry. Eur J Neurosci. 2001; 14:829–838. [PubMed: 11576187]

8. Li H, Liu H, Sage C, Huang M, Chen ZY, Heller S. Islet-1 expression in the developing chicken inner ear. J Comp Neurol. 2004; 477:1–10. [PubMed: 15281076]

9. Brockes JP, Kumar A. Appendage regeneration in adult vertebrates and implications for regenerative medicine. Science. 2005; 310:1919–1923. [PubMed: 16373567]

10. Hu Z, Corwin JT. Inner ear hair cells produced in vitro by a mesenchymal-to-epithelial transition. Proc Natl Acad Sci U S A. 2007; 104:16675–16680. [PubMed: 17895386]

11••. 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. In a detailed study on zebrafish neuromast hair cell regeneration after neomycin induced hair cell death, blockade of the Notch pathway by γ-secretase inhibitor DAPT resulted in an increase in the number of hair cells regenerated. In contrast to the mammalian inner ear, most of the regenerated neuromast hair cells were derived from the proliferation of supporting cells. [PubMed: 18305259]

12. Hernandez PP, Olivari FA, Sarrazin AF, Sandoval PC, Allende ML. Regeneration in zebrafish lateral line neuromasts: expression of the neural progenitor cell marker sox2 and proliferation-dependent and -independent mechanisms of hair cell renewal. Dev Neurobiol. 2007; 67:637–654. [PubMed: 17443814]

13. Lopez-Schier H, Hudspeth AJ. A two-step mechanism underlies the planar polarization of regenerating sensory hair cells. Proc Natl Acad Sci U S A. 2006; 103:18615–18620. [PubMed: 17124170]

14••. Sage C, Huang M, Karimi K, Gutierrez G, Vollrath MA, Zhang DS, Garcia-Anoveros J, Hinds PW, Corwin JT, Corey DP, et al. Proliferation of functional hair cells in vivo in the absence of the retinoblastoma protein. Science. 2005; 307:1114–1118. We used a conditional knockout mouse model to demonstrate that Rb1 is required for cell cycle exit of inner ear sensory progenitor cells, and establishment of the postmitotic status of hair cells and supporting cells. We showed that hair cells without Rb1 could undergo differentiation and proliferation concurrently and the mitosis-derived hair cells were functional. This study suggested a strategy to achieve hair cell regeneration by blocking cell cycle inhibitors. [PubMed: 15653467]

15. Stone JS, Rubel EW. Temporal, spatial, and morphologic features of hair cell regeneration in the avian basilar papilla. J Comp Neurol. 2000; 417:1–16. [PubMed: 10660884]

16••. Oshima K, Grimm CM, Corrales CE, Senn P, Martinez Monedero R, Geleoc GS, Edge A, Holt JR, Heller S. Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. J Assoc Res Otolaryngol. 2007; 8:18–31. The authors comprehensively quantified stem cells from a range of inner ear tissues throughout development. They showed that all the tissues sampled harbored stem cells that could differentiate into inner ear cell types such as hair cells and ganglion neurons. However, the number of stem cells decreased rapidly in the adult cochlea, in contrast to relatively abundant stem cells in the adult vestibular system. This observation may help to explain the lack of hair cell regeneration in the cochlea compared to the utricle. [PubMed: 17171473]

17. Li H, Liu H, Heller S. Pluripotent stem cells from the adult mouse inner ear. Nat Med. 2003; 9:1293–1299. [PubMed: 12949502]

18. Martinez-Monedero R, Yi E, Oshima K, Glowatzki E, Edge AS. Differentiation of inner ear stem cells to functional sensory neurons. Dev Neurobiol. 2008; 68:669–684. [PubMed: 18278797]

19. Qyang Y, Martin-Puig S, Chiravuri M, Chen S, Xu H, Bu L, Jiang X, Lin L, Granger A, Moretti A, et al. The renewal and differentiation of Isl1+ cardiovascular progenitors are controlled by a Wnt/beta-catenin pathway. Cell Stem Cell. 2007; 1:165–179. [PubMed: 18371348]

20. Lamba D, Karl M, Reh T. Neural regeneration and cell replacement: a view from the eye. Cell Stem Cell. 2008; 2:538–549. [PubMed: 18522847]

21. Bermingham NA, Hassan BA, Price SD, Vollrath MA, Ben-Arie N, Eatock RA, Bellen HJ, Lysakowski A, Zoghbi HY. Math1: an essential gene for the generation of inner ear hair cells. Science. 1999; 284:1837–1841. [PubMed: 10364557]

22. Gubbels SP, Woessner DW, Mitchell JC, Ricci AJ, Brigande JV. Functional auditory hair cells produced in the mammalian cochlea by in utero gene transfer. Nature. 2008; 455:537–541. [PubMed: 18754012]

23••. Izumikawa M, Minoda R, Kawamoto K, Abrashkin KA, Swiderski DL, Dolan DF, Brough DE, Raphael Y. Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nat Med. 2005; 11:271–276. The authors overexpressed Atoh1 in deafened guinea pigs and observed the production of new hair cells as a consequence of the transdifferentiation of supporting cells to hair cells. Limited improvement in auditory thresholds was also observed in the ears overexpressing Atoh1. [PubMed: 15711559]

24. Kelley MW. Regulation of cell fate in the sensory epithelia of the inner ear. Nat Rev Neurosci. 2006; 7:837–849. [PubMed: 17053809]

25. 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]

26. Lanford PJ, Lan Y, Jiang R, Lindsell C, Weinmaster G, Gridley T, Kelley MW. Notch signalling pathway mediates hair cell development in mammalian cochlea. Nat Genet. 1999; 21:289–292. [PubMed: 10080181]

27. Zine A, Aubert A, Qiu J, Therianos S, Guillemot F, Kageyama R, de Ribaupierre F. 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]

28. Kiernan AE, Cordes R, Kopan R, Gossler A, Gridley T. The Notch ligands DLL1 and JAG2 act synergistically to regulate hair cell development in the mammalian inner ear. Development. 2005; 132:4353–4362. [PubMed: 16141228]

29. Yamamoto N, Tanigaki K, Tsuji M, Yabe D, Ito J, Honjo T. Inhibition of Notch/RBP-J signaling induces hair cell formation in neonate mouse cochleas. J Mol Med. 2006; 84:37–45. [PubMed: 16283144]

30. Jeon SJ, Fujioka M, Edge AS. Inhibition of Notch signaling increases differentiation of stem cells to hair cells through upregulation of Math1. Association for Research in Otolaryngology Thirty-First Midwinter Meeting. 2008 Abstract 570.

31. Li H, Corrales CE, Wang Z, Zhao Y, Wang Y, Liu H, Heller S. BMP4 signaling is involved in the generation of inner ear sensory epithelia. BMC Dev Biol. 2005; 5:16. [PubMed: 16107213]

32. Pujades C, Kamaid A, Alsina B, Giraldez F. BMP-signaling regulates the generation of hair-cells. Dev Biol. 2006; 292:55–67. [PubMed: 16458882]

33. Driver EC, Pryor SP, Hill P, Turner J, Ruther U, Biesecker LG, Griffith AJ, Kelley MW. Hedgehog signaling regulates sensory cell formation and auditory function in mice and humans. J Neurosci. 2008; 28:7350–7358. [PubMed: 18632939]

34. Stevens CB, Davies AL, Battista S, Lewis JH, Fekete DM. Forced activation of Wnt signaling alters morphogenesis and sensory organ identity in the chicken inner ear. Dev Biol. 2003; 261:149–164. [PubMed: 12941626]

35. Chen P, Segil N. p27(Kip1) links cell proliferation to morphogenesis in the developing organ of Corti. Development. 1999; 126:1581–1590. [PubMed: 10079221]

36. Mantela J, Jiang Z, Ylikoski J, Fritzsch B, Zacksenhaus E, Pirvola U. The retinoblastoma gene pathway regulates the postmitotic state of hair cells of the mouse inner ear. Development. 2005; 132:2377–2388. [PubMed: 15843406]

37. Sage C, Huang M, Vollrath MA, Brown MC, Hinds PW, Corey DP, Vetter DE, Chen ZY. Essential role of retinoblastoma protein in mammalian hair cell development and hearing. Proc Natl Acad Sci U S A. 2006; 103:7345–7350. [PubMed: 16648263]

38. 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]

39. Warchol ME, Lambert PR, Goldstein BJ, Forge A, Corwin JT. Regenerative proliferation in inner ear sensory epithelia from adult guinea pigs and humans. Science. 1993; 259:1619–1622. [PubMed: 8456285]

40••. 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. The authors used a p27kip1 promoter-driven GFP transgenic mouse model and showed that purified early postnatal

cochlear supporting cells have the potential to re-enter the cell cycle and differentiate into hair cells *in vitro*. However, this capacity was rapidly lost postnatally. Deletion of p27kip1 led to enhanced proliferation in early postnatal supporting cells. [PubMed: 16791196]

41. Hume CR, Bratt DL, Oesterle EC. Expression of LHX3 and SOX2 during mouse inner ear development. Gene Expr Patterns. 2007; 7:798–807. [PubMed: 17604700]
42. Lopez IA, Zhao PM, Yamaguchi M, de Vellis J, Espinosa-Jeffrey A. Stem/progenitor cells in the postnatal inner ear of the GFP-nestin transgenic mouse. Int J Dev Neurosci. 2004; 22:205–213. [PubMed: 15245756]
43. Sakaguchi H, Yaoi T, Suzuki T, Okano H, Hisa Y, Fushiki S. Spatiotemporal patterns of Musashi1 expression during inner ear development. Neuroreport. 2004; 15:997–1001. [PubMed: 15076722]
44. Stone JS, Shang JL, Tomarev S. cProx1 immunoreactivity distinguishes progenitor cells and predicts hair cell fate during avian hair cell regeneration. Dev Dyn. 2004; 230:597–614. [PubMed: 15254895]
45. Li H, Roblin G, Liu H, Heller S. Generation of hair cells by stepwise differentiation of embryonic stem cells. Proc Natl Acad Sci U S A. 2003; 100:13495–13500. [PubMed: 14593207]
46. Coleman B, Fallon JB, Pettingill LN, de Silva MG, Shepherd RK. Auditory hair cell explant co-cultures promote the differentiation of stem cells into bipolar neurons. Exp Cell Res. 2007; 313:232–243. [PubMed: 17112512]
47. Corrales CE, Pan L, Li H, Liberman MC, Heller S, Edge AS. Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti. J Neurobiol. 2006; 66:1489–1500. [PubMed: 17013931]
48. Shi F, Corrales CE, Liberman MC, Edge AS. BMP4 induction of sensory neurons from human embryonic stem cells and reinnervation of sensory epithelium. Eur J Neurosci. 2007; 26:3016–3023. [PubMed: 18005071]
49. Jeon SJ, Oshima K, Heller S, Edge AS. Bone marrow mesenchymal stem cells are progenitors in vitro for inner ear hair cells. Mol Cell Neurosci. 2007; 34:59–68. [PubMed: 17113786]
50. Doyle KL, Kazda A, Hort Y, McKay SM, Oleskevich S. Differentiation of adult mouse olfactory precursor cells into hair cells in vitro. Stem Cells. 2007; 25:621–627. [PubMed: 17110620]
51. MacLaren RE, Pearson RA, MacNeil A, Douglas RH, Salt TE, Akimoto M, Swaroop A, Sowden JC, Ali RR. Retinal repair by transplantation of photoreceptor precursors. Nature. 2006; 444:203–207. [PubMed: 17093405]
52. Hanna J, Markoulaki S, Schorderet P, Carey BW, Beard C, Wernig M, Creyghton MP, Steine EJ, Cassady JP, Foreman R, et al. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. Cell. 2008; 133:250–264. [PubMed: 18423197]