Concise Review: Regeneration in Mammalian Cochlea Hair Cells: Help from Supporting Cells Transdifferentiation

Bénédicte Franco, Brigitte Malgrange

STEM CELLS

TISSUE-SPECIFIC STEM CELLS

Concise Review: Regeneration in Mammalian Cochlea Hair Cells: Help from Supporting Cells Transdifferentiation

BÉNÉDICTE FRANCO, BRIGITTE MALGRANGE

Key Words. Progenitor cells • Cellular proliferation • Cell signaling • Differentiation

Developmental Neurobiology Unit - GIGA-Neurosciences, University of Liège, Quartier Hôpital (CHU), B-4000 Liège, Belgium

Correspondence: Brigitte Malgrange, pHD, pharmD, Developmental Neurobiology Unit - GIGA-Neurosciences, University of Liège, Quartier Hôpital (CHU), Avenue Hippocrate 15 - Tour 4 - 1er étage - Bât. B36, B-4000 Liège, Belgium. Telephone: +3243665905; Fax: +3243665912; e-mail: bmalgrange@ulg.ac.be

Received September 30, 2016; accepted for publication November 27, 2016; first published online in STEM CELLS *EXPRESS* January 19, 2017.

1066-5099/2017/$30.00/0

http://dx.doi.org/10.1002/stem.2554

Available online without subscription through the open access option

ABSTRACT

It is commonly assumed that mammalian cochlear cells do not regenerate. Therefore, if hair cells are lost following an injury, no recovery could occur. However, during the first postnatal week, mice harbor some progenitor cells that retain the ability to give rise to new hair cells. These progenitor cells are in fact supporting cells. Upon hair cells loss, those cells are able to generate new hair cells both by direct transdifferentiation or following cell cycle re-entry and differentiation. However, this property of supporting cells is progressively lost after birth. Here, we review the molecular mechanisms that are involved in mammalian hair cell development and regeneration. Manipulating pathways used during development constitute good candidates for inducing hair cell regeneration after injury. Despite these promising studies, there is still no evidence for a recovery following hair cells loss in adult mammals. STEM CELLS *2017;35:551–556*

SIGNIFICANCE STATEMENT

Up to now there is no treatment to halt or replace hair cell in mammals. Recently progenitor cells have been identified and retained the capacity—at least at perinatal stages—to proliferate and/or differentiate. In this Review, we discuss recent progress in the identification of cochlear progenitor cells that could proliferate and/or differentiate into hair cells. We also review various signaling pathways that participate to cochlear development and discuss their potential use in hair cell regeneration both in vitro and in vivo.

INTRODUCTION

Neurosensory hearing loss affects a broad range of people worldwide and results mainly from an irreversible loss of cochlear hair cells. Environmental factors, ototoxic medications, and genetic predispositions are all important contributors to hair cell loss. The inner ear is an organ of exquisite organization, harboring the cochlea responsible for hearing and the vestibule responsible for balance. Both structures are organized in a sensory epithelium containing hair cells surrounded by supporting cells and neurons contacting the hair cells. In the cochlea, the hair cells and the supporting cells form the organ of Corti. In the vestibule, they are organized in different structures: saccule, utricule, and cristae. Hair cells detect mechanical stimuli, by deflection of stereocilia present at their apical surface, and then transmit the information to the neurons through their dendrites. The information is then carried by the axon to the proper region of the brain. The neurons are grouped in ganglia, either the spiral ganglion for the cochlea or the vestibular ganglion for the vestibule.

In the mammalian cochlea, it has been long admitted that hair cells do not regenerate, hampering a possible recovery. Recent studies have uncovered mechanisms in which new hair cells can be formed from the surrounding supporting cells. These cells act as hair cell progenitors either by re-entering the cell cycle and dividing to give rise to new hair cells, or by direct differentiation into hair cells, to so-called transdifferentiation. In this Review, we will focus on the mechanisms and signaling pathways involved in such recovery of hair cell loss.

DEVELOPMENTAL HAIR CELL PRODUCTION

The formation of the inner ear needs several signaling pathways orchestrated in space and time during embryogenesis. The development of the mouse inner ear is initiated as early as embryonic day 7.5 (E7.5) by specification of a particular region of the anterior ectoderm, the

preplacodal region, which requires different signals [1]. From the mesoderm and the neural plate, fibroblast growth factor (FGF) signaling is important to induce otic fate [2]. FGF3 and FGF10 have been shown to be important for the formation of otic territory [3, 4]. Besides, FGF8 is also required for otic induction, as it acts to promote or maintain FGF10 expression. Moreover, otic induction requires the inhibition of Wnt and bone morphogenetic protein (BMP) signaling, which are both responsible for ectodermal formation [5, 6]. The preplacodal region is then characterized by the expression of several transcription factors such as Six1, Six4, Eya1, and Eya2 (reviewed in [7]).

Once the preplacodal region has been specified, it thickens to give rise to the otic placode at around E9. The induction of the otic placode is under the control of signaling cascades such as FGFs and Wnts [8–10]. FGF signaling induces the expression of transcription factors from the paired box (Pax) family, such as Pax2 and Pax8. Pax2-positive cells then can give rise to either otic cells or epidermis [11]. For the induction of an otic fate, activation of Wnt signaling is necessary in these Pax2-positive cells. Indeed, Wnt1, 3a, 6, and 8 are secreted by the hindbrain and rhombomeres act instructively to direct Pax2-positive cells to an otic fate [8, 12, 13]. Afterward, the placode will start to invaginate to form the otic cup [14]. Then, the otic cup closes to form the otic vesicle, which will generate nearly all cell types of the inner ear. Starting around E12, the ventral part of the developing otocyst is specified in a so-called prosensory area, expressing Sox2, that will later give rise to the organ of Corti. This territory is spatially defined by a gradient of BMPs across the cochlear duct (increasing concentrations from the Kölliker's organ or inner sulcus, the prosensory domain to the outer sulcus) allowing the specification of sensory versus non-sensory domain [15]. Notch signaling through its ligand Jagged1 is also acting for prosensory induction and the formation of sensory patches [16].

Soon after the emergence of the prosensory domain (around E13.5), a non-proliferative zone appears with the expression of the cell cycle inhibitor $p27^{kip1}$/CDKN1B [17]. The upregulation of CDNK1B could be downstream of Sox2, and in turn CDKN1B could repress Sox2 [18, 19]. Indeed, the expression of Sox2 is transient as at later stages during development Sox2 is down-regulated in mature hair cells and neurons [20, 21] and only remains in supporting cells. The reduced expression of Sox2 is essential for the induction of hair cells by activation of the transcription factor Atoh1 [22]. Between E14.5 and E15.5 a wave of differentiation is initiated from the base toward the apex of the cochlea, allowing the generation of hair cells and supporting cells upon Notch cascade by lateral inhibition [23]. This process ends between E17.5 and E18.5. At the same time, another gradient of differentiation occurs medially enabling the formation of the inner hair cells row at first, and then the three rows of outer hair cells [17, 24, 25]. During the cochlear formation, the specification of hair and supporting cells is subjected to a subtle combination of distinct signaling pathways, transcription factors expression and epigenetic regulations. Moreover, cells are rarely exposed to one stimulus at a time, and cross-talk (direct or indirect) between signaling pathways is evident and recently identified in the developing inner ear (reviewed in [26, 27]).

Atoh1

While thinking about hair cells differentiation, one consensus is the major role of Atoh1, also named Math1, a basic helix-loop-helix transcription factor related to *Drosophila melanogaster* proneural gene, *atonal* [28]. Atoh1 starts to be expressed around E12.5 in the vestibular portion of the inner ear when hair cells start to differentiate [29]. In the cochlear portion, the expression of Atoh1 is visible from E14.5, next to the emergence of the CDKN1B non-proliferative zone [30]. Paradoxically, while cells become post-mitotic in an apical-to-basal gradient, Atoh1 expression and hair cell differentiation begin in the basal turn and progress in the opposite direction [30]. Deletion of *Atoh1* gene leads to the absence of hair cells formation while its overexpression induces ectopic hair cells [31, 32]. Besides its role in early hair cells specification, Atoh1 is also important later during development by promoting their survival and maturation [33, 34].

Notch

Notch signaling is important for the specification of hair cells and supporting cells by lateral inhibition [35, 36]. Nascent hair cells start to express Notch ligands Jagged2 and Delta1, while surrounding cells express the Notch receptor and differentiate into supporting cells through the induction of *Hes* genes that inhibit Atoh1, an essential protein for hair cells. The acquired supporting cell fate is not solely due to absence of Atoh1, but is also dependent on activation of transcriptional signature upon Notch activation [37].

microRNAs

Regulation of transcript expression through microRNAs (miRNAs) is also involved in the specification of hair cells versus supporting cells. miR-183 family members, that include miR-183, miR-182, and miR-96, are expressed in hair cells but not in supporting cells [38]. Down-regulation of these miRNAs results in the production of fewer hair cells [39]. Moreover, mutations within the miR-96 gene have been associated with human hereditary hearing loss [40, 41]. More recently, miR-124 has also been involved in cochlear development. Indeed, by targeting secreted frizzled-related protein 4 (Sfrp4) and Sfrp5, two inhibitors of the Wnt pathway, miR-124 controls the Wnt pathways that contribute to hair cells differentiation and polarization in the organ of Corti [42].

Wnt Pathway

Canonical Wnt pathway activation is responsible for cell proliferation in the prosensory domain [43]. Indeed, continuous Wnt/β-catenin activation using TCF/Lef/H2B-GFP reporter mouse cochleae upregulates Sox2 prosensory cell numbers and confers a more progenitor-like character. This increased proliferation is observed both within the early E12 proliferative and the late E13.5–E14.5 post-mitotic prosensory domain. Subsequently, Wnt/β-catenin pathway is also required for hair cells differentiation. Inhibition of Wnt signaling through use of pharmacological agents or loss of β-catenin results in a failure of hair cells to differentiate [43, 44]. At that developmental stage, the canonical Wnt/β-catenin pathway controls the expression of Atoh1 [45]. Once specified, Atoh1-positive hair cells are not any more dependent on Wnt/β-catenin pathway [44].

FGFs

FGFs signaling has essential functions at several stages of inner ear development. Early during development, that is, at E9–E10 in mouse, FGF signaling is important for the specification of otic territory [4]. FGFs are also required for cell fate decision in the

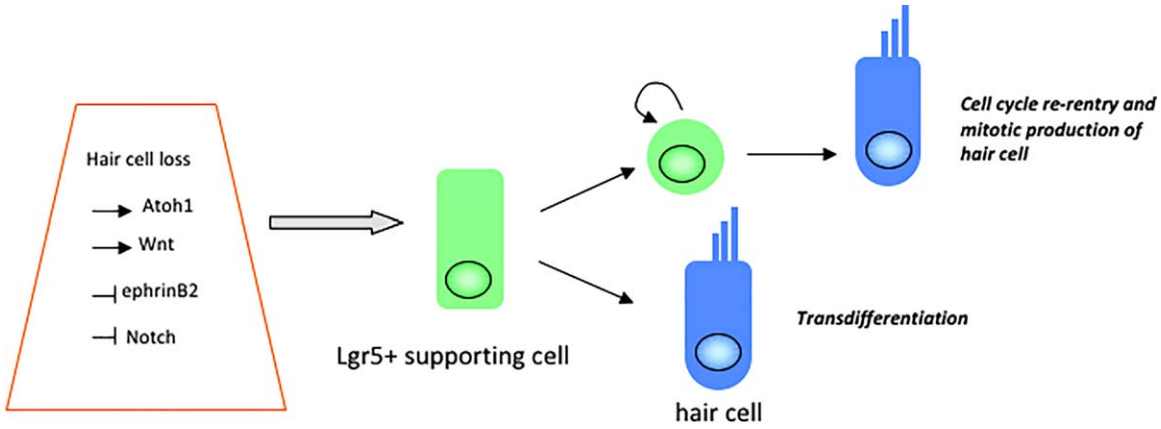

Figure 1. Examples of signaling pathways that induce hair cell formation from supporting cells. The Lgr5-positive cells represent the most potent pool of progenitor cells. Different stimuli such as hair call loss, inhibition of Notch pathway, forced expression of Atoh1, activation of Wnt canonical pathway, or inhibition of EphrinB2 signaling can trigger supporting cells proliferation and/or their transdifferentiation.

organ of Corti (after E15 in mouse). Indeed, loss of FGF receptor type 3 leads to an increased number of hair cells [46], while deletion of FGF8 induces a loss of Pillar cells, a particular type of supporting cells [47].

Generation of New Hair Cells After Loss: Regeneration

In non-mammalian vertebrates, such as birds, replacement of hair cell loss occurs spontaneously for a long period after birth (for review see [48]). The formation of new hair cells can occur via two modes: mitotic regeneration, in which surrounding supporting cells re-enter the cell cycle and divide to give rise to new hair cells, and direct transdifferentiation in which supporting cells change their fate to become hair cells, even in the presence of antimitotic drugs [49–52] (Fig. 1).

Although the cochlea is assumed to be unable to regenerate in mammals, some evidences showed that during the first 2 weeks after birth, cochlear and vestibular cells retain the capacity of sphere formation, a stem cell-like behavior [53, 54]. If sphere formation can be achieved in the mammalian inner ear, it might reflect that some cells have the ability to proliferate or differentiate.

As in birds, these cells have been identified as the supporting cells. Indeed, CDKN1B-GFP supporting cells from neonatal mice isolated and placed in culture have been shown to divide and differentiate into hair cell-like cells [55]. In the same line, using different cell surface markers and fluorescence-activated cell sorting (FACS), dissociated cell from the perinatal (postnatal day 3, P3) cochlea can be separated into four different populations of non-sensory cells. Among these, numerous are able to re-enter the cell cycle and proliferate. However, only supporting cells are able to give rise to new hair cells [56]. More recently, studies have shown that Lgr5-positive supporting cells are the progenitors that can regenerate hair cells [57, 58]. Besides their ability to differentiate in vitro, supporting cells can also generate new hair cells in vivo after ablation of hair cells [59, 60]. This mitotic hair cell regeneration occurs only at neonatal stages. Indeed, when hair cell ablation occurs at 1 week of age, no regeneration is observed. Such a limited ability of regeneration with a short time-window is currently being unraveled. It is already clear that both cell intrinsic (such as senescence, cell cycle alterations) as well as extrinsic factors (such as alterations in the regenerative environment) play significant roles [61].

The neonatal mouse cochlea is pre-hearing and not mature in respect to its anatomy and physiology. For example, the tunnel of Corti is not formed, and all the cells of the greater epithelial ridge (the future inner sulcus) are still present. With maturation, the organ of Corti is no longer able to regenerate, the opening of the tunnel of Corti could be responsible of the loss of this ability by separating the pool of progenitors from the hair cells. In addition, cell-cell junctions can also act to inhibit regenerative processes during postnatal development. Indeed, there is a changing expression of connexins corresponding to a maturation of gap junctions between supporting cells contributing to antagonizing proliferation (reviewed in [62]). In parallel to this cochlear anatomy modification, many changes in cell signaling occur within the postnatal organ of Corti and contribute to a decreased regenerative capacity.

Molecular Pathways Involved in Hair Cell Regeneration

During cochlea formation, as mentioned above, Notch signaling is very important for the specification of supporting cells versus hair cells. Similarly, Notch inhibition, either in conditional knockout mice or by treatment with γ-secretase inhibitors, enhances supporting cells proliferation and formation of new hair cells in the perinatal cochlea [63]. Moreover, treatment with γ-secretase inhibitors in vivo induces new hair cells and can cause partial recovery of hearing following noise trauma [64, 65]. However, a recent study showed that the response of supporting cells to Notch inhibition drops dramatically during the first postnatal week in mice, concomitant with a down-regulation of many components of the Notch signaling pathway [66]. This suggests that manipulating Notch pathway alone is unlikely to promote significant hair cell regeneration in the postnatal/adult organ of Corti, and that supplementary interventions should be considered. Indeed, recent studies are much more focused on manipulation of at least two crucial signaling pathways involved in cochlear development.

Activation of Wnt canonical pathway is also an interesting strategy for hair cell regeneration. Indeed, upon genetic or chemical (using GSK3β inhibitor) β-catenin stabilization, Lgr5-positive progenitor cells are able to re-enter in proliferation and

generate new hair cells [67, 68]. Wnt activation followed by Notch inhibition strongly promotes the mitotic regeneration of new hair cells in both normal and neomycin-damaged cochleae [69]. However, the newly generated hair cells still underwent incomplete maturation. A combined activation of Wnt pathway, through β-catenin overexpression, with Notch knock-down and forced expression of Atoh1 in Lgr5-positive cells enhances greatly the formation of hair cells and the expression of genes implicated in hair cells maturation [70]. The proliferative state of Lgr5+ cells could be due to the activation of Wnt canonical pathway and the inhibition of Notch pathway, while the differentiation into hair cells is triggered by ectopic expression of Atoh1. Indeed, ectopic activation of Atoh1 induces new hair cells in young postnatal mice [71, 72]. Moreover, in the young adult deafened guinea pig, forced expression of Atoh1 is able to induce hair cell regeneration and hearing threshold [73]. However, only a subset of these cells and at early postnatal stages is able to give rise to new hair cells, unraveling a more complex genetic regulation, and the cells produced do not always reach terminal differentiation, as Atoh1 should be lowered at the end of the differentiation process [74]. Nevertheless, the reactivation of proliferation and differentiation cues is able to form hair cells from surrounding supporting cells, but it appears that there is a tight interplay between different signaling molecules.

Ephrins and their receptors Eph also contribute to supporting cell differentiation into hair cells. Indeed, EphA4 receptor is present in hair cells while Ephrin-B2 is present in supporting cells [75]. This complementary pattern of expression is necessary for the establishment of compartment boundary between hair cells and supporting cells. When this Ephrin signaling is disrupted, using either Ephrin-B2 conditional knockout mice, shRNA-mediated gene silencing or soluble inhibitors, the organ of Corti harbors supernumerary hair cells that are generated from direct supporting cells transdifferentiation. Further studies using lineage tracing experiments are needed to rigorously validate this hypothesis. Importantly, those new hair cells directly integrate the hair cell layer and, therefore, could be more rapidly able to fit into functional circuitry. Whether Ephrin signaling acts in isolation or as part of a complex network of regulatory pathways remains to be determined. Interestingly, Ephrin-B2 and Notch are expressed in similar supporting cell types throughout the development [35]. Ephrin-B2 is a direct Notch target whose expression is induced by Notch signaling [76]. Therefore, following Notch lateral inhibition, Ephrin-B2 could be required to segregate the supporting cells from adjacent hair cells.

Conclusion

In mammals, it has been described that hair cells do not regenerate, impairing the ability to restore hearing. However, growing body of evidence has demonstrated that in particular cases, some regenerative properties can be encountered in the inner ear. Although during adulthood, few example of restoration have been found. Different signaling pathways have been characterized to have the capacity of inducing supporting cells proliferation and differentiation into hair cells. The newly formed cells express markers for hair cells and are also reached by spiral ganglion fibers, but are not yet mature and synapses are not perfectly formed. Knowing in details how the formation of hair cells is achieved is the starting point to discover new mechanisms that could help to identify the molecules that could be induced in supporting cells to allow them to transdifferentiate; and how the maturation of the organ of Corti is correlated to an inability to spontaneously regenerate. These lines of investigation could enable the discovery of regeneration in more mature cochlea.

Acknowledgments

B.M. is a Research Director from the Belgian National Funds for Scientific Research (FNRS). This work was supported by grants from the FSR-FNRS, the Fonds Léon Fredericq, the Fondation Médicale Reine Elisabeth, and the Belgian Science Policy (IAP-VII network P7/07).

Authors Contributions

B.F. and B.M. wrote the manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

References

1 Schlosser G. Induction and specification of cranial placodes. Dev Biol 2006;294:303–351.
2 Schimmang T. Expression and functions of FGF ligands during early otic development. Int J Dev Biol 2007;51:473–481.
3 Alvarez Y, Alonso MT, Vendrell V et al. Requirements for FGF3 and FGF10 during inner ear formation. Development 2003;130:6329–6338.
4 Wright TJ, Mansour SL. Fgf3 and Fgf10 are required for mouse otic placode induction. Development 2003;130:3379–3390.
5 Pieper M, Ahrens K, Rink E et al. Differential distribution of competence for panplacodal and neural crest induction to non-neural and neural ectoderm. Development 2012;139:1175–1187.
6 Reichert S, Randall RA, Hill CS. A BMP regulatory network controls ectodermal cell fate decisions at the neural plate border. Development 2013;140:4435–4444.
7 Chen J, Streit A. Induction of the inner ear: Stepwise specification of otic fate from multipotent progenitors. Hear Res 2013;297:3–12.
8 Ohyama T, Mohamed OA, Taketo MM et al. Wnt signals mediate a fate decision between otic placode and epidermis. Development 2006;133:865–875.
9 Ladher RK, O'Neill P, Begbie J. From shared lineage to distinct functions: The development of the inner ear and epibranchial placodes. Development 2010;137:1777–1785.
10 Wright KD, Mahoney Rogers AA, Zhang J et al. Cooperative and independent functions of FGF and Wnt signaling during early inner ear development. BMC Dev Biol 2015;15:33.
11 Streit A. Extensive cell movements accompany formation of the otic placode. Dev Biol 2002;249:237–254.
12 Riccomagno MM, Takada S, Epstein DJ. Wnt-dependent regulation of inner ear morphogenesis is balanced by the opposing and supporting roles of Shh. Genes Dev 2005;19:1612–1623.
13 Urness LD, Paxton CN, Wang X et al. FGF signaling regulates otic placode induction and refinement by controlling both ectodermal target genes and hindbrain Wnt8a. Dev Biol 2010;340:595–604.
14 Ohyama T, Groves AK, Martin K. The first steps towards hearing: Mechanisms of otic placode induction. Int J Dev Biol 2007;51:463–472.
15 Ohyama T, Basch ML, Mishina Y et al. BMP signaling is necessary for patterning the sensory and nonsensory regions of the

developing mammalian cochlea. J Neurosci 2010;30:15044–15051.
16 Hartman BH, Reh TA, Bermingham-McDonogh O. Notch signaling specifies prosensory domains via lateral induction in the developing mammalian inner ear. Proc Natl Acad Sci USA 2010;107:15792–15797.
17 Chen P, Segil N. p27(Kip1) links cell proliferation to morphogenesis in the developing organ of Corti. Development 1999;126:1581–1590.
18 Liu Z, Walters BJ, Owen T et al. Regulation of p27Kip1 by Sox2 maintains quiescence of inner pillar cells in the murine auditory sensory epithelium. J Neurosci 2012; 32:10530–10540.
19 Li H, Collado M, Villasante A et al. p27(Kip1) directly represses Sox2 during embryonic stem cell differentiation. Cell Stem Cell 2012;11:845–852.
20 Dabdoub A, Puligilla C, Jones JM et al. Sox2 signaling in prosensory domain specification and subsequent hair cell differentiation in the developing cochlea. Proc Natl Acad Sci USA 2008;105:18396–18401.
21 Puligilla C, Dabdoub A, Brenowitz SD et al. Sox2 induces neuronal formation in the developing mammalian cochlea. J Neurosci 2010;30:714–722.
22 Puligilla C, Kelley MW. Dual role for Sox2 in specification of sensory competence and regulation of Atoh1 function. Dev Neurobiol 2017;77.
23 Bryant J, Goodyear RJ, Richardson GP. Sensory organ development in the inner ear: Molecular and cellular mechanisms. Br Med Bull 2002;63:39–57.
24 Sher AE. The embryonic and postnatal development of the inner ear of the mouse. Acta Otolaryngol Suppl 1971;285:1–77.
25 Lim DJ, Anniko M. Developmental morphology of the mouse inner ear. A scanning electron microscopic observation. Acta Otolaryngol Suppl 1985;422:1–69.
26 Zak M, Klis SF, Grolman W. The Wnt and Notch signalling pathways in the developing cochlea: Formation of hair cells and induction of regenerative potential. Int J Dev Neurosci 2015;47:247–258.
27 Waqas M, Zhang S, He Z et al. Role of Wnt and Notch signaling in regulating hair cell regeneration in the cochlea. Front Med 2016;10:237–249.
28 Jahan I, Pan N, Fritzsch B. Opportunities and limits of the one gene approach: The ability of Atoh1 to differentiate and maintain hair cells depends on the molecular context. Front Cell Neurosci 2015;9:26.
29 Bermingham NA, Hassan BA, Price SD et al. Math1: An essential gene for the generation of inner ear hair cells. Science 1999; 284:1837–1841.
30 Chen P, Johnson JE, Zoghbi HY et al. 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.
31 Pan N, Jahan I, Kersigo J et al. Conditional deletion of Atoh1 using Pax2-Cre results in viable mice without differentiated cochlear hair cells that have lost most of the organ of Corti. Hear Res 2011;275:66–80.
32 Gubbels SP, Woessner DW, Mitchell JC et al. Functional auditory hair cells produced in the mammalian cochlea by in utero gene transfer. Nature 2008;455:537–541.
33 Chonko KT, Jahan I, Stone J et al. Atoh1 directs hair cell differentiation and survival in the late embryonic mouse inner ear. Dev Biol 2013;381:401–410.
34 Cai T, Seymour ML, Zhang H et al. Conditional deletion of Atoh1 reveals distinct critical periods for survival and function of hair cells in the organ of Corti. J Neurosci 2013;33:10110–10122.
35 Lanford PJ, Lan Y, Jiang R et al. Notch signalling pathway mediates hair cell development in mammalian cochlea. Nat Genet 1999;21:289–292.
36 Zine A, Van De Water TR, de Ribaupierre F. Notch signaling regulates the pattern of auditory hair cell differentiation in mammals. Development 2000;127:3373–3383.
37 Campbell DP, Chrysostomou E, Doetzlhofer A. Canonical Notch signaling plays an instructive role in auditory supporting cell development. Sci Rep 2016;6:19484.
38 Weston MD, Pierce ML, Rocha-Sanchez S et al. MicroRNA gene expression in the mouse inner ear. Brain Res 2006;1111:95–104.
39 Li H, Kloosterman W, Fekete DM. MicroRNA-183 family members regulate sensorineural fates in the inner ear. J Neurosci 2010;30:3254–3263.
40 Mencia A, Modamio-Hoybjor S, Redshaw N et al. Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. Nat Genet 2009;41:609–613.
41 Solda G, Robusto M, Primignani P et al. A novel mutation within the MIR96 gene causes non-syndromic inherited hearing loss in an Italian family by altering pre-miRNA processing. Hum Mol Genet 2012;21:577–585.
42 Huyghe A, Van den Ackerveken P, Sacheli R et al. MicroRNA-124 regulates cell specification in the cochlea through modulation of Sfrp4/5. Cell Rep 2015;13:31–42.
43 Jacques BE, Puligilla C, Weichert RM et al. A dual function for canonical Wnt/beta-catenin signaling in the developing mammalian cochlea. Development 2012;139: 4395–4404.
44 Shi F, Hu L, Jacques BE et al. β-*Cat*enin is required for hair-cell differentiation in the cochlea. J Neurosci 2014;34:6470–6479.
45 Shi F, Cheng YF, Wang XL et al. Beta-catenin up-regulates Atoh1 expression in neural progenitor cells by interaction with an Atoh1 3′ enhancer. J Biol Chem 2010;285:392–400.
46 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.
47 Jacques BE, Montcouquiol ME, Layman EM et al. Fgf8 induces pillar cell fate and regulates cellular patterning in the mammalian cochlea. Development 2007;134:3021–3029.
48 Brignull HR, Raible DW, Stone JS. Feathers and fins: Non-mammalian models for hair cell regeneration. Brain Res 2009;1277:12–23.
49 Duncan LJ, Mangiardi DA, Matsui JI et al. Differential expression of unconventional myosins in apoptotic and regenerating chick hair cells confirms two regeneration mechanisms. J Comp Neurol 2006;499:691–701.
50 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.
51 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.
52 Shang J, Cafaro J, Nehmer R et al. Supporting cell division is not required for regeneration of auditory hair cells after ototoxic injury in vitro. J Assoc Res Otolaryngol 2010; 11:203–222.
53 Oshima K, Grimm CM, Corrales CE 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.
54 Malgrange B, Belachew S, Thiry M et al. Proliferative generation of mammalian auditory hair cells in culture. Mech Dev 2002; 112:79–88.
55 White PM, Doetzlhofer A, Lee YS et al. Mammalian cochlear supporting cells can divide and trans-differentiate into hair cells. Nature 2006;441:984–987.
56 Sinkkonen ST, Chai R, Jan TA et al. Intrinsic regenerative potential of murine cochlear supporting cells. Sci Rep 2011;1:26.
57 Chai R, Kuo B, Wang T et al. Wnt signaling induces proliferation of sensory precursors in the postnatal mouse cochlea. Proc Natl Acad Sci USA 2012;109:8167–8172.
58 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.
59 Cox BC, Chai R, Lenoir A et al. Spontaneous hair cell regeneration in the neonatal mouse cochlea in vivo. Development 2014; 141:816–829.
60 Ni W, Lin C, Guo L et al. Extensive supporting cell proliferation and mitotic hair cell generation by in vivo genetic reprogramming in the neonatal mouse cochlea. J Neurosci 2016;36:8734–8745.
61 Yun MH. Changes in regenerative capacity through lifespan. Int J Mol Sci 2015;16: 25392–25432.
62 Walters BJ, Zuo J. Postnatal development, maturation and aging in the mouse cochlea and their effects on hair cell regeneration. Hear Res 2013;297:68–83.
63 Li W, Wu J, Yang J et al. Notch inhibition induces mitotically generated hair cells in mammalian cochleae via activating the Wnt pathway. Proc Natl Acad Sci USA 2015;112: 166–171.
64 Mizutari K, Fujioka M, Hosoya M et al. Notch inhibition induces cochlear hair cell regeneration and recovery of hearing after acoustic trauma. Neuron 2013;77:58–69.
65 Tona Y, Hamaguchi K, Ishikawa M et al. Therapeutic potential of a gamma-secretase inhibitor for hearing restoration in a guinea pig model with noise-induced hearing loss. BMC Neurosci 2014;15:66.
66 Maass JC, Gu R, Basch ML et al. Changes in the regulation of the Notch signaling pathway are temporally correlated with regenerative failure in the mouse cochlea. Front Cell Neurosci 2015;9:110.

67 Shi F, Hu L, Edge AS. Generation of hair cells in neonatal mice by beta-catenin overexpression in Lgr5-positive cochlear progenitors. Proc Natl Acad Sci USA 2013;110: 13851–13856.
68 Roccio M, Hahnewald S, Perny M et al. Cell cycle reactivation of cochlear progenitor cells in neonatal FUCCI mice by a GSK3 small molecule inhibitor. Sci Rep 2015;5:17886.
69 Ni W, Zeng S, Li W et al. Wnt activation followed by Notch inhibition promotes mitotic hair cell regeneration in the postnatal mouse cochlea. Oncotarget 2016;7.
70 Waqas M, Guo L, Zhang S et al. Characterization of Lgr5+ progenitor cell transcriptomes in the apical and basal turns of the mouse cochlea. Oncotarget 2016:7.
71 Liu Z, Dearman JA, Cox BC 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.
72 Kelly MC, Chang Q, Pan A et al. Atoh1 directs the formation of sensory mosaics and induces cell proliferation in the postnatal mammalian cochlea in vivo. J Neurosci 2012; 32:6699–6710.
73 Izumikawa M, Minoda R, Kawamoto K et al. Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nat Med 2005;11:271–276.
74 Kuo BR, Baldwin EM, Layman WS et al. In vivo cochlear hair cell generation and survival by coactivation of beta-Catenin and Atoh1. J Neurosci 2015;35:10786–10798.
75 Defourny J, Mateo Sanchez S, Schoonaert L et al. Cochlear supporting cell transdifferentiation and integration into hair cell layers by inhibition of ephrin-B2 signalling. Nat Commun 2015;6:7017.
76 Grego-Bessa J, Luna-Zurita L, del Monte G et al. Notch signaling is essential for ventricular chamber development. Dev Cell 2007;12:415–429.