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GENES ASSOCIATED WITH REGENERATION OF UTRICLE HAIR CELLS IN MAMMALIANS: A REVIEW

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ABSTRACT

Death of mechanosensory cells in the inner ear leads to hearing loss and balance disorders. Fish and birds have ability to form new hair cells after embryogenesis while mammals lack the capacity to regenerate hair cells. Researches have been done on determining genes contributing to hair cell protection or susceptibility. It is assumed these gens might targets for gene therapy in the future (Lang et al., 2006; Owens et al., 2008). So, the aim of the current literature review paper was to determine genes associated with regeneration of utricle hair cells in mammalians using the PubMed and Medline database English literature by the terms: "Utricle hair cells", "Zirconia", "Genes" and "framework design" and "Mammals".

Keywords: Utricle hair cells, Genes, Mammals

INTRODUCTION

Individuals older then 60 years old suffer hearing loss because of aging, genetic predisposition or environmental exposure to noise or ototoxic drugs (Beisel et al., 2008). Hearing and balance deficiencies spring from damage or loss of sensory hair cells, which are highly specialized cells with elaborate microvillar arrays called hair bundles (Brignull et al. 2009). The hair bundle is responsible for transducing sound energy or head movements into the neural signals and transmits to the auditory and vestibular nervous system. Strategies enrolled to treat hair cell loss are including prevention and replacement. Furthermore, pharmacological approaches for preventing hair cell loss have been identified (Guthrie, 2008 and Cotanche, 2008). Researches have been done on determining genes contributing to hair cell protection or

susceptibility. It is assumed these gens might targets for gene therapy in the future (Lang et al., 2006; Owens et al., 2008). So, the aim of the current literature review paper was to determine genes associated with regeneration of utricle hair cells in mammalians using the PubMed and Medline database English literature by the terms: "Utricle hair cells", "Zirconia", "Genes" and "framework design" and "Mammals".

Progenitor cells in sensory epithelia of mammalian inner ear

The mammalian inner ear possesses just a few thousand sensory cells that are encased in several layers of bone and show little proliferative activity in vitro. Thus there has been some effort to establish mammalian cell lines that will enable more controlled studies of the mechanisms of cell differentiation and regeneration (Zheng et al. 1998).

Genetic expression of mammalian hair cell differentiation

Comparing gene expression between different end organs may highlight potential therapeutic targets that may help guide mammalian cochlear sensory epithelia into a proliferative state, allowing for potential hair cell regeneration. Hawkins *et al.* (2003) found 20 different inner ear genes and 80 transcription factors (TF) that were significantly different between the avian cochlea and utricle. *Bmp4*, *Gata3*, *Gsn*, *Foxf1* and *Prdm7* were some of the genes that were upregulated in the cochlea, while *Smad2*, *Kit*, *β-amyloid*, *Loc51637*, *Hmg20b* and *Crip2* are examples of genes that were upregulated in the utricle. While some of these genes are well known to be involved in the development of the inner ear, some of them were novel TF, like *Loc51637* and *Hmg20b*, about which little was previously known. comparing gene expression between different end organs may highlight potential therapeutic targets that may help guide mammalian cochlear sensory epithelia into a proliferative state, allowing for potential hair cell regeneration. Hawkins *et al.* (2003) found 20 different inner ear genes and 80 transcription factors (TF) that were significantly different between the avian cochlea and utricle. *Bmp4*, *Gata3*, *Gsn*, *Foxf1* and *Prdm7* were some of the genes that were upregulated in the cochlea, while *Smad2*, *Kit*, *β-amyloid*, *Loc51637*, *Hmg20b* and *Crip2* are examples of genes that were upregulated in the utricle. While some of these genes are well known to be involved in the development of the inner ear e.g., Gata3, some of them were novel TF, like *Loc51637* and *Hmg20b*, about which little was previously known (Smith and Gopinath Rajadinakaran, 2013).

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 (Jeon et al. 2007). 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 (Shi et al. 2007). Here we provided gens responsible in regeneration of utricle hair cells in mammalians.

DLL_1 and JAG_2

Expression studies have shown that the genes encoding the Notch ligands JAG_2 and DLL_1 are both expressed in nascent hair cells as they begin to differentiate (Morrison et al., 1999). Several different Notch receptors are also expressed in the ear, supporting a role for Notch-mediated lateral inhibition in the inner ear (Lanford et al., 1999). Direct functional evidence for lateral inhibition Expression studies have shown that the genes encoding the Notch ligands JAG_2 and DLL_1 are both expressed in nascent hair cells as they begin to differentiate (Lanford et al., 1999; Morrison et al., 1999). Several different Notch receptors are also expressed in the ear, supporting a role for Notch-mediated lateral inhibition in the inner ear (Lanford et al., 1999).

Both *Dll1/Jag2* double mutant cochleae and *Foxg1-Cre Notch1flox/–* cochleae displayed severely disorganized hair cell rows, and loss of organization and polarity of the hair cell stereociliary bundles. It is not clear whether this disorganization is a direct consequence of reduced Notch signaling, or whether it is a secondary event resulting from the abnormal cellular composition of the cochlea. Recent work has shown that an evolutionarily conserved mechanism for generating cell polarity within epithelial cell layers, termed planar cell polarity (PCP), is involved in regulating the polarity of inner ear hair cells and the orientation of their stereocilia bundles (Barald and Kelley, 2004). A role for Notch signaling in PCP has not been reported in any vertebrate system. However, a role for the Notch pathway in planar polarity has been shown during eye development in *Drosophila*, where Notch signaling specifies the R4 photoreceptor cell fate (McNeill, 2002).

E-cadherin

E-cadherin is expressed in vestibular, mechanosensory epithelia during early embryonic development. During late embryonic and neonatal stages it is expressed in supporting cells but down-regulated in differentiating sensory hair cells (Kussel-Andermann et al. 2000).

Atoh1

Atoh1 is the mammalian homolog of the Drosophila gene atonal, which was first described as a proneural gene involved in regulating the formation of mechanoreceptors and photoreceptors in Drosophila (Atkinson et al. 2015). Atoh1 (the gene formerly known as Math1) has been identified as the earliest hair cell specific gene required for definitive hair cell development, because the loss of Atoh1 results in the failure of any hair cell differentiation in the mouse cochlea (Bermingham et al., 1999). Although the deletion of Sox2 or Eya1 also leads to a lack of hair cell differentiation, this is thought to involve a loss of the progenitor cell population in general and not a specific block in hair cell differentiation (Kiernan et al., 2005; Kalatzis et al., 1998; Zou et al., 2008). At E13.5, Atoh1 mRNA is broadly expressed in cells throughout the presumptive sensory epithelium, as determined by in situ hybridization (Matei et al., 2005). These Atoh1-positive cells are the progenitor cells that will subsequently differentiate further into presumptive hair cells and supporting cells. Interestingly, a single row of cells within the Atoh1-positive progenitor cells labels with antibodies to myosin VI at this time. These myosin VI-positive cells will give rise to the single row of inner hair cells in the organ of Corti. One day later, at E14.5, the presumptive inner hair cells begin to express higher levels of Atoh1 than their neighbors, as determined by Atoh1-GFP reporter expression (Bermingham-McDonogh et al., 2006). In addition, these same cells begin to express Delta1 and Jagged2 mRNA (Hartman et al., 2007). At E15.5 the three rows of OHC can be identified with the Atoh1- GFP reporter mouse (Bermingham-McDonogh et al., 2006) and by E17 only the four rows of hair cells express Atoh1 mRNA (Lanford et al., 2000). At E16.5-E18 Delta1 and Jagged2 mRNA can be localized within the three rows of OHC, as well (Murata et al., 2006; Kelley, 2007).

Notch

The Notch pathway mediates short-range cell-cell communication and controls diverse cellular processes, including proliferation, differentiation and cell death in a context-dependent manner. Upon ligand activation, the Notch receptor is enzymatically cleaved, resulting in the release of the Notch intracellular domain

(NICD). NICD then translocates into the nucleus, where it interacts with the DNA-binding protein and core effector of the canonical Notch pathway, RBPjk. Readers are referred to the following reviews for a more in-depth discussion of Notch signaling (Kiernan, 2013; Kopan, 2012; Louvi and Artavanis-Tsakonas, 2012). Notch signaling, acting via the process of lateral induction, is sufficient to specify prosensory cells. First, the Notch ligand jagged1 (Jag1) is expressed prior to and during specification of the prosensory region, which is identified by Sox2 expression (Brooker et al., 2006; Kiernan et al., 2006). Second, conditional deletion of Jag1 leads to the downregulation of this prosensory marker, resulting in a malformed cochlear duct that contains few hair cells and supporting cells (Brooker et al., 2006; Kiernan et al., 2006).

Wnt

Wnt signaling pathway is indispensable for various modes of operation during development and adult life including proliferation, determining the cell fate, maintaining progenitors and participation in cellular polarization. Wnt pathway triggers upon binding of wnt ligands to their respective Frizzled trans membrane receptor and generally categorized into distinct intracellular canonical and non-canonical signaling pathways (Jansson et al. 2015). Our primary focus is to discuss the role of canonical Wnt pathway in hair cells regeneration. Non-mammals such as fish and birds retain the ability to regenerate hair cells and restore hearing spontaneously after damage (Niehrs, 2012). However, adult mammals lack the competence to regenerate hair cells after ototoxic insult (Ryals and Rubel, 1988). The β-catenin dependent canonical pathway regulates the cell fate and proliferation in sensory epithelium [97]; the nuclear translocation and the binding of β-catenin to TCF/LEF transcription factor complex activates the downstream expression of wnt target genes, including Lgr5, Axin2. Multiple studies, in recent years have reported that the canonical Wnt pathway is involved in hair cell regeneration. The expression of Wnt target gene Lgr5, Lgr6 and Axin2 in cochlear supporting cells indicate the existence of active Wnt signaling pathway in neonatal cochlea (Jacques et al, 2012). Lgr^{5+} cells have been demonstrated as an enriched population of hair cell progenitors both *in vitro* and *in vivo* (Jan et al. 2013). Wnt agonist enhanced the proliferation and hair cell regeneration efficiency of Lgr^{5+} progenitor cells in the inner ear (Chai et al. 2012). Moreover, the forced expression of β-catenin triggers the Atoh1 expression in colonies derived from isolated supporting

cells and promotes the hair cells formation *in vivo* and vitro (Jacques et al, 2012). Similarly, the conditional overexpression of β-catenin in Lgr5+ progenitors expands the proliferation and regeneration capability of supporting cells to generate hair cells (Shi et al. 2010).

PAX–EYA–SIX–DACH

The Pax, Eya, Six and Dach gene families are important in otic development, but defining how they fit together has proved difficult. The recent derivation of Six1 null mice is one step towards determining the complex epistatic relationships between these TF gene families (Ozaki et al. 2003). Six1 expression is lost in Eya1 nulls, which appears to place it downstream of Eya1 (Zheng et al. 2003). However, the relationship of Pax genes to Six and Eya expression is less clear. This may reflect functional redundancy in Pax genes. Likewise, Dach1 does not appear to be simply downstream of Eya1 or Pax2, unlike the situation in Drosophila. Defining these connections and functional redundancies is a continuing challenge (Heanue et al. 2002).

p27Kip1

The p27Kip1 is a molecule that blocks progenitor cells (or supporting cells) in the organ of Corti of mice from dividing during embryonic and postnatal development. Embryonic deletion of the gene encoding p27Kip1 causes an excess of cells to be formed in the organ of Corti, including hair cells (Chen and Segil, 1999; Lowenheim et al., 1999). In mature mice, blocking the synthesis of p27Kip1 causes a small but significant increase in cell division in some types of supporting cells in the organ of Corti (Oesterle et al., 2011). Inhibition of p27Kip1 and similar molecules is under investigation as a way to promote mammalian hair cell regeneration. Activity of p27Kip1 and other regulators of cell division is controlled by extracellular signaling molecules.

Factors inhibiting hair cell regeneration in vestibular epithelia

Attempts to make both auditory neurons and hair cells using embryonic stem cells have been partially successful. Hair cells were obtained from mouse embryonic stem cells by timed incubation in the growth factors EGF, bFGF, and IGF-1 (Jeon et al. 2007). 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. 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 (Jeon et al. 2007).

Age-dependent changes to regenerative capacity

The age-related declines in the early and robust proliferation responses observed here in neomycin-treated utricle cultures and *in vivo* are reminiscent of responses observed in vestibular epithelia that were delaminated from rodent utricles and cultured with Glial Growth Factor 2 (rhGGF2), insulin, or serum. For both humans and mice, the decrease in spreading could stem from postnatal thickening of F-actin belts that bracket the apical junctions between neighboring vestibular supporting cells (Meyers and Corwin, 2007; Burns et al., 2008). Differences between mammals and the birds, sharks, bony fish, and amphibians that all exhibit rapid mitotic hair cell regeneration also extend to the cadherins at intercellular junctions of supporting cells (Hackett et al., 2002; Warchol, 2007). Cell cycle regulatory proteins that are expressed in many tissues, such as the retinoblastoma protein (pRb) and the cyclin dependent kinase inhibitors, p27Kip1, p21Cip1, and p19INK4d, have established influences on the proliferation and postmitotic state of cells during the embryonic development of the mammalian cochlea, so it seems likely that such proteins may have roles in limiting vestibular regeneration (Chen and Segil, 1999).

CONCLUSION

In many non-mammalian vertebrates, continuous proliferation of glial and sustentacular stem cells gives rise to new neurons and sensory cells and is paralleled by life-long regeneration. Based on the reports there are differences in gen regulating utricle hair cells in mammalians compared to the other animals.

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