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ScienceDirect
Hearing Research 236 (2008) 1–10
Hearing Research
www.elsevier.com/locate/heares

Research paper

Strategies to regenerate hair cells: Identification of progenitors and critical genes

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Received 5 February 2007; received in revised form 3 August 2007; accepted 12 August 2007
Available online 6 September 2007

Abstract

Deafness commonly results from a lesion of the sensory cells and/or of the neurons of the auditory part of the inner ear. There are currently no treatments designed to halt or reverse the progression of hearing loss. A key goal in developing therapy for sensorineural deafness is the identification of strategies to replace lost hair cells. In amphibians and birds, a spontaneous post-injury regeneration of all inner ear sensory hair cells occurs. In contrast, in the mammalian cochlea, hair cells are only produced during embryogenesis.

Many studies have been carried out in order to demonstrate the persistence of endogenous progenitors. The present review is first focused on the occurrence of spontaneous supernumerary hair cells and on nestin positive precursors found in the organ of Corti. A second approach to regenerating hair cells would be to find genes essential for their differentiation. This review will also focus on critical genes for embryonic hair cell formation such as the cell cycle related proteins, the *Atoh1* gene and the Notch signaling pathway. Understanding mechanisms that underlie hair cell production is an essential prerequisite to defining therapeutic strategies to regenerate hair cells in the mature inner ear.

Keywords: Inner ear; Cochlea; Development; Regeneration

1. Introduction

In mammals, permanent acquired hearing loss is commonly caused by the loss of sensory hair cells as a consequence of aging and environmental stresses, e.g. acoustic trauma or exposure to ototoxic drugs (cisplatin, aminoglycosides...) (Reviewed in (Hawkins et al., 1976; Saunders et al., 1985; Schacht, 1986)). In contrast, damaged vestibular organs retain some capacity for self-repair (Forge et al., 1993). After HC loss, limited numbers of supporting cells (SCs) divide in these sensory epithelia and several progenies appear to differentiate as HCs (Kuntz and Oesterle, 1998; Lambert et al., 1997; Warchol et al., 1993). The development of the organ of Corti involves the differentiation of placodal tissue into sensory HCs and non-sensory SCs. The mammalian organ of Corti is a complex structure containing a single row of inner HCs and three rows of outer HCs, supported by one row of phalangeal cells and three rows of Deiters cells (Fig. 1). Additional SC types can be distinguished including pillar cells, Hensen cells,

Abbreviations: HC, hair cell; SC, supporting cell; E, embryonic day; P, postnatal day; GER, greater epithelial ridge; LER, lesser epithelial ridge; DIV, day *in vitro*; EGF, epidermal growth factor; FGF2, fibroblast growth factor 2; GFP, green fluorescent protein; CDK, cyclin-dependent kinase; CDKI, CDK inhibitors; pRb, retinoblastoma protein; bHLH, basic helix-loop-helix

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doi:10.1016/j.heares.2007.08.007

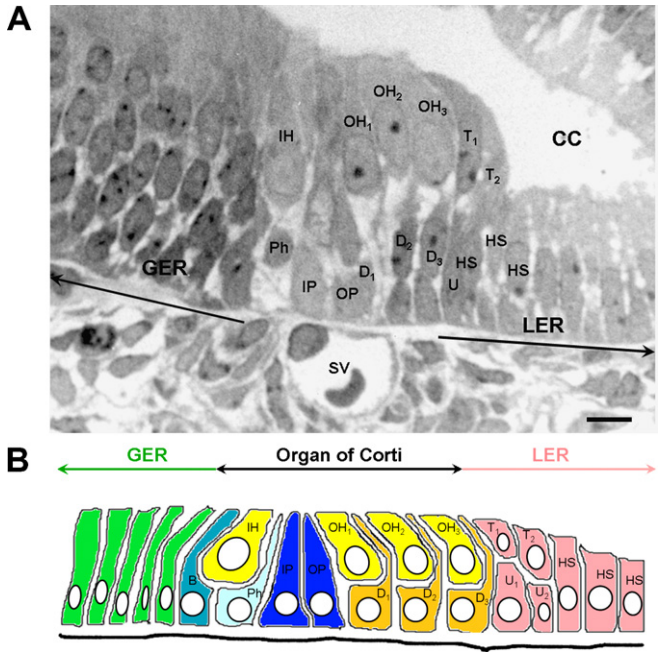

Fig. 1. The developing organ of Corti. (A) Cross-section through the organ of Corti in a E19 rat. (B) Schematic representation of the corresponding structure illustrating the organization of the organ of Corti and the adjacent structures, i.e. the GER and the LER. B = border cell; Ph = phalangeal cell; IP = inner pillar cell; OP = outer pillar cell; IH = inner hair cell; OH = outer hair cells; D = Deiters'cells; T = tectal cells; U = undertectal cells; HS = Hensen's cells. Scale bar in A represents 0.2 μm.

tectal cells, undertectal cells and Claudius cells (Lim and Rueda, 1992; Lim, 1986; Malgrange et al., 2002b). In mammals, the HCs and SCs of the organ of Corti undergo their final round of division between embryonic day 12 (E12) and E16 (Ruben, 1967). After this critical period, mammalian HC generation no longer occurs. Potential strategies to replace lost sensory HCs include: (1) replacement of lost cochlear cells with transplanted stem cells in the damaged inner ear and (2) recruitment of inner ear endogenous stem cells or progenitor cells to generate new HCs. Embryonic stem cells (Li et al., 2003, 2004; Rivolta et al., 2006) or adult stem cells from various tissues (Doyle et al., 2006; Jeon et al., 2007) can be induced to differentiate into new HCs. However, these approaches are not yet possible in the adult mammalian organ of Corti *in vivo*. In addition, this strategy is technically challenging, since it requires (1) the design of surgical procedures to implant progenitor cells in the damaged cochlea without causing additional damage to the inner ear, (2) the isolation of precursor cells competent to integrate and ultimately to differentiate into functional HCs in the mature mammalian organ of Corti *in vivo* and (3) the recovery of functional HCs in the organ of Corti even if the cochlea cytoarchitecture is not restored. Recruiting cochlear precursor cells capable of dividing and further differentiating into specific cochlear end-phenotypes, such as HCs or SCs, represents the most elegant strategy for replacing HCs lost as a result of injury or disease. This review will focus on this approach for HC regeneration.

1.1. Identification of HC progenitors: focus on endogenous cells for replacing lost HCs

During rodent embryonic morphogenesis, the cochlear epithelial progenitor cells are formed in the otic vesicle at E11.5. The epithelial progenitor cells proliferate and expand to form two distinct regions in the dorsal epithelium of the cochlear canal, the greater epithelial ridge (GER) and the lesser epithelial ridge (LER), which contribute to the formation of HCs and SCs in the primitive organ of Corti from E15-P0 (postnatal day 0). Although disappearing gradually, the GER and LER, which can still be observed at birth, will give rise to inner spiral sulcus, outer spiral sulcus (primitive Hensen and Claudius cells) and other non-hair cell epithelial cells (Lim and Rueda, 1992). In the present review, we define the epithelium bordering on the organ of Corti at the most distant edge from the modiolus as LER and the epithelium bordering on the organ of Corti at the nearest edge from the modiolus as GER in the neonatal rat (Fig. 1). Alternative nomenclature for these regions includes outer spiral sulcus and inner spiral sulcus cells, respectively (Sobkowicz et al., 1975, 1990, 1993).

In order to generate new HCs, we have to develop strategies to change the fate of non-sensory cells (i.e. precursor cells) and to transdifferentiate them into new HCs with or without a mitotic cycle. Immature cells must be present and must retain the potential to undergo differentiation into HCs. These immature cells may arise from the GER and/or the LER. But SCs can also be a good candidate for HC progenitors. They can generate new HCs by transdifferentiation (conversion of the phenotype without cell division) (Baird et al., 2000; Minoda et al., 2004). Therefore, the SCs are attractive targets for interventions designed to produce new HCs.

1.1.1. Mammalian vestibular progenitors

Stem cells are remarkable in their capacity to self-renew and to differentiate into a multiplicity of cell types (Raff, 2003). Such cells have been identified and isolated from several adult tissues. Taking advantage of some well-known characteristics of stem cells growing *ex-vivo*, Li et al. (2003) isolated cells from adult mouse utricular macula. After dissociation, these cells proliferated and expanded as free floating clonal spheres. After 8 days *in vitro* (DIV), these cells progressed to a progenitor cell state characterized by the expression of nestin. Nestin is an intermediate filament protein expressed by stem cells and progenitors early in development and throughout the early postnatal period in the central and peripheral nervous systems. Nestin is considered a neural stem cell marker (Lendahl et al., 1990). Li et al. (2003) found that some nestin positive cells amplified in culture from the utricular macula could be differentiated into HCs characterized by the expression of myosin VIIa, *Brn3c* and espin but not