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Review

Molecular mechanisms and potentials for differentiating inner ear stem cells into sensory hair cells

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ABSTRACT

In mammals, hair cells may be damaged or lost due to genetic mutation, infectious disease, chemical ototoxicity, noise and other factors, causing permanent sensorineural deafness. Regeneration of hair cells is a basic pre-requisite for recovery of hearing in deaf animals. The inner ear stem cells in the organ of Corti and vestibular utricle are the most ideal precursors for regeneration of inner ear hair cells. This review highlights some recent findings concerning the proliferation and differentiation of inner ear stem cells. The differentiation of inner ear stem cells into hair cells involves a series of signaling pathways and regulatory factors. This paper offers a comprehensive analysis of the related studies.

Introduction

Cochlear hair cells are terminally differentiated cells that serve as mechanosensory receptors converting sound stimuli into electric signals (Savary et al., 2008). These hair cells are susceptible to damage from noise trauma, aging and aminoglycoside ototoxicity, while their non-sensory supporting cell counterparts are substantially more resistant to these factors (Batts et al., 2009). Loss of hair cells in higher vertebrates appears to be non-reversible and leads to permanent hearing loss. The terminal mitosis of hair cells and supporting cells in the mammalian cochlea occurs during middle embryonic development (Lou et al., 2007). After birth, the regenerative capacity of mammalian hair cells is very limited, with little capacity for differentiation into new hair cells after auditory hair cell injury (Bermingham-McDonogh and Reh, 2011). In contrast, fishes, amphibians and birds have an incredible ability to regenerate damaged hair cells through direct and indirect trans-differentiation of supporting cells (Bodson et al., 2010). This huge difference prompts a strong interest in the study of the mechanisms of hair cell generation, which provides a basis for hearing recovery.

In mammals, although the damaged auditory hair cells are not spontaneously replaced, transplantation of inner ear stem cells into the otic vesicles can generate new hair cells (Li et al., 2003). In addition, some recent studies showed that the surviving supporting cells in mammals could be forced to trans-differentiate into new hair cells (Batts et al., 2009; Sinkkonen et al., 2011). The differentiation of inner ear stem cells and trans-differentiation of supporting cells are regulated by a series of specific genes and signaling pathways (Magarinos et al., 2012; Okano and Kelley, 2012), such as cell cycle inhibitors, the pro-hair cell gene Atoh1 (used to be referred to as Math1), the Notch signaling pathway. Modification of regulatory genes and signaling pathways in the cochlea of a deaf animal may be an effective way to regenerate hair cells.

Inner ear stem cells can be isolated from the cochlear organ of Corti, as well as the vestibular sensory epithelia (Li et al., 2003; Oshima et al., 2007; Savary et al., 2007, 2008; Wang et al., 2006). These cells are capable of self-renewal and have multipotentials of differentiation. A hallmark of these multipotent stem cells is their ability to form spheres (Oshima et al., 2007). The spheres can be passaged and give rise to hair cell-like cells with functional features that are distinctively similar to nascent hair cells in vivo and in vitro. The differentiation mechanism of inner ear stem cells into hair cells is becoming increasingly clear, making it possible to treat sensorineural deafness.

Tissue origin of inner ear stem cells

Stem cells are a class of cells with the capacity for self-renewal and differentiation into a variety of cell types. Stem cells have been found in a number of organs, including the central nervous system, blood, skin and eye (McKay, 1997; Osawa et al., 1996; Toma et al., 2001; Tropepe et al., 2000). In 2003, inner ear stem cells were

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isolated from adult mouse utricle maculae sensory epithelium (Li et al., 2003) (Fig. 1B and F). These cells were cultured in a plastic Petri dish for 8 days with serum-free DMEM/high-glucose and F12 medium (mixed 1:1) supplemented with N2, B27 and growth factors, and formed spheres. These spheres expressed Pax-2, bone morphogenetic protein (BMP)-4, BMP-7 and Nestin, a combination of markers for the developing inner ear (Oshima et al., 2007). To determine whether sphere cells were generated by mitosis, Li et al. (2003) used the thymidine analog 5-bromo-2′-deoxyuridine (BrdU) to label mitotically active cells during sphere formation. However, BrdU labeling of all cells in the spheres does not necessarily imply clonal relationship, unless the sphere started out as a single cell. Therefore, they also plated a single inner ear stem cell into a well in 96-well plate and monitored each well microscopically for the presence of a single cell, which confirmed that the spheres were generated by a single cell proliferation. Therefore, these cells were termed inner ear stem cells. Inner ear stem cells were also isolated from the organ of Corti (Oshima et al., 2007), greater epithelial ridge (GER) (Zhang et al., 2007), lesser epithelial ridge (LER) (Zhai et al., 2005) (Fig. 1D), vestibular sensory epithelium (Hu et al., 2012; Li et al., 2003) (Fig. 1F) and the cristae of the three semicircular canals (Li et al., 2003; Oshima et al., 2007) (Fig. 1A and B). In Fig. 1, we point out the location of inner ear stem cell in the cochlea (Fig. 1). These stem cells are capable of self-renewal and have multiple potential routes of differentiation. Inner ear stem cells derived from different species and tissues express specific markers, such as Table 1. In mice, the sphere formation ability in the cochlea decreases by ~100-fold during the second and third postnatal weeks. The inner ear stem cells of the cochlea can only be isolated in the first week.

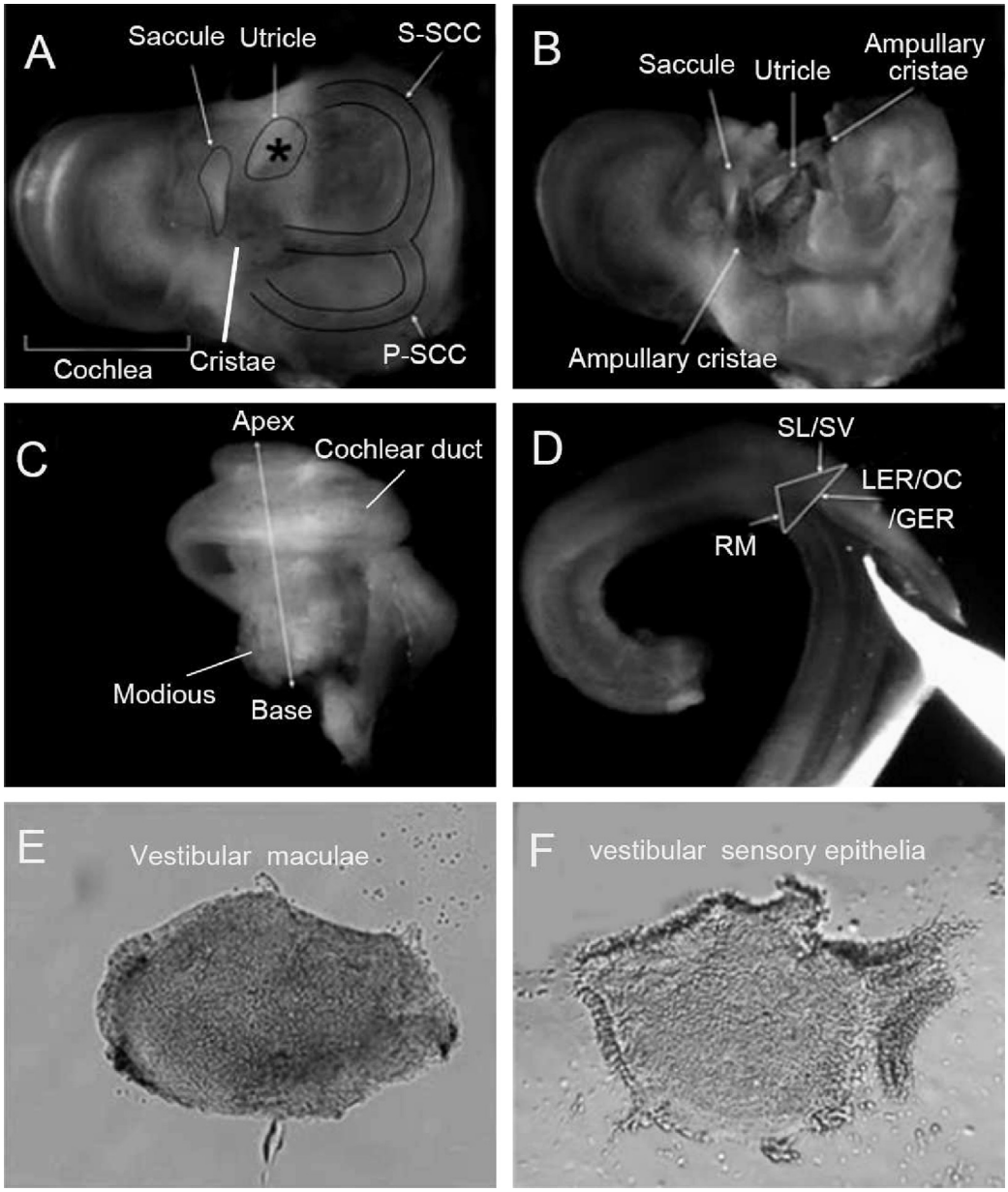

Fig. 1. Demonstrated sources of inner ear stem cells in the inner ear. (A) The inner ear after removal of the bulla and surrounding tissue. The asterisk (*) indicates the cartilage overlying the utricle. S-SCC=superior semicircular canal, P-SCC=posterior semicircular canal. (B) The utricle is exposed by fenestration of the overlying cartilaginous plate. (C) The entire membranous labyrinth of the cochlea. (D) The triangle illustrates the cross-section of the cochlear duct. RM= Reissner's membrane, SL/SV=spiral ligament with stria vascularis, and OC/GER=the organ of Corti (OC) with the greater epithelial ridge (GER) and lesser epithelial ridge (LER). (E) The vestibular maccula (utricle and saccule). (E) The vestibular sensory epithelia (Utricle and saccule sensory epithelium). The inner ear stem cells are harbored in the cochlea (organ of Corti, LER and GER), the vestibular sensory epithelia and the cristae of the three semicircular canals. All pictures were quoted from the paper of Oshima et al. (2009).