Cytotherapy, 2017; 19: 909–915

International Society for Cellular Therapy
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Regenerative medicine in hearing recovery

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Abstract

Hearing loss, or deafness, affects 360 million people worldwide of which about 32 million are children. Deafness is irreversible when it involves sensory hair cell death because the regenerative ability of these cells is lost in mammals after embryo development. The therapeutic strategies for deafness include hearing aids and/or implantable devices. However, not all patients are eligible or truly benefit from these medical devices. Regenerative medicine based on stem cell application could play a role in both improvement of extant medical devices and *in vivo* recovery of auditory function by regeneration of inner ear cells and neurons. A review of recent literature on the subject indicates that two promising approaches to renewal and differentiation of cochlear tissues are transplantation of stem cells and *in situ* administration of growth factors. Rather than directly regenerating dead cells, these procedures apparently induce, through various pathways, differentiation of resident cochlear cells. More studies on the possible adverse effects of transplanted cells and the recovery of tonotopic sensorineural activity or required. To date, no reliable clinical results have been obtained in the field of cochlear regeneration.

Key Words: *Cochlear stem cells, endogenous stem cells, growth factor supplements, hearing loss, hearing recovery, inner ear, stem cell transplantation*

Introduction

Worldwide, the majority of hearing disabilities (approximately 90%) result from the death of sensory cells, either hair cells (HCs) or spiral ganglion neurons (SGNs), thus leading to sensorial and/or neural hearing loss (SNHL). The etiology of SNHL includes mainly ototoxicity, deafening noise and presbyacusis [1,2]. Impairment due to SNHL has significant social and economic impact because it affects the ability to interact with people and the surrounding environment and, when it occurs early in life, causes language development delays and social integration problems [3,4].

The cochlear implant is the unique surgical option for people with severe-to-profound SNHL. This electronic high-technology device transforms sound waves into an electric stimulus, bypassing the damaged cochlear cells to directly stimulate the acoustic nerve. However, even with recent advances in engineering, surgery and pharmaceutical treatments that have improved the efficacy of cochlear implants and reduced electrode insertion trauma, normal auditory function cannot be completely restored [5,6]. New therapeutic strategies based on molecular, cellular and nanotechnological tools are aimed at regenerating and/or preserving sensory cells in the cochlea, contributing to improved cochlear implant outcomes [7–9]. Studies on nanotechnological tools involve the development of nanostructured electrodes and the improvement of drug delivery systems based on nanoparticles [10–12]. Regenerative medicine, an intriguing therapeutic strategy, has been successful in several research and clinical fields, such as dermatology, cardiovascular medicine and orthopedics [13]. Among regenerative medicine strategies, the use of stem cells (SCs) to restore damaged tissues is one of the most studied cell-based applications [14]. In otology, for example, SCs transplanted on synthetic scaffolds have recently been applied in tissue engineering for reconstruction of the human auricle [15,16]. The aim of SC-based therapy in SNHL is to replace lost HCs or SGNs, and the major challenge is to achieve this without affecting the complex cytoarchitecture of the cochlea and any residual hearing function [17,18].

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(Received 20 January 2017; accepted 21 April 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.04.008

This review highlights the state of the art in regenerative medicine of inner-ear cells, discussing recent applications in hearing disabilities. Two general approaches are commonly used for tissue regeneration based on SCs: tissue-resident SCs or transplanted allogeneic SCs. The review focuses first on cochlear-resident putative SCs and then on *in vitro* production of HCs and SGNs. Finally, it considers *in vivo* tissue regeneration by transplantation of allogeneic SCs and/or growth factor supplementation for neuronal regrowth and protection.

Cochlear SCs

The identification of neural SCs (NSCs) in the adult central nervous system [19] and the well-known capability of injured non-mammalian adult vertebrates to restore damaged auditory sensory epithelium [20] encouraged researchers to investigate whether SCs could also be located in the mammalian inner ear and whether their regenerative ability could also be exploited in mammalian tissues.

In inner ear, a small number of SCs were isolated from adult mouse utricles, amounting to 0.025% of utricular cells [21]. Later, several studies identified and isolated SCs from mammalian cochlear sensory epithelium, spiral ganglion and stria vascularis of early postnatal mice, rats, guinea pigs and human fetuses [22–26]. These cells form self-renewing spheres in non-adherent cultures, but this ability weakens after several *ex vivo* passages [27]. When isolated from the cochlear sensory epithelium, these SCs are also able to differentiate into inner ear cell lineages, such as HC-like cells and sensory neurons, and into mature neurons and glia cells when isolated from the spiral ganglion [28,29].

In mice, the ability of sensory epithelial SCs to form spheres decreases about 100-fold during the second and third postnatal week, together with the expression of developmental and progenitor cell markers in the cochlea [21]. In contrast, utricle SCs maintain these characteristics into adulthood [20,21]. There is only one report concerning isolation of NSCs from adult human and guinea pig spiral ganglion [30].

Over the past few years, there has been evidence indicating that postnatal cochlear supporting cells also maintain SC-like characteristics in mammals [31] and are able to divide and trans-differentiate *in vitro* into HCs [32]. These abilities decline with age [33]: in cochlear SCs forming spheres, this decline is partially caused by changes in the expression of the cyclin-dependent kinase inhibitor (Cdkn1b), which plays a central role in regulating cell proliferation. After HC formation, the expression of Cdkn1b in the organ of Corti is restricted to non-sensory cells, preventing further divisions of HCs [33,34]. Moreover, p27-deficient mice exhibit hearing damage due to the over-proliferation and irregular positioning of both hair and supporting cells [35]. Another key gene involved in HC differentiation is *ATOH1* (atonal bHLH transcription factor 1), also known as *MATH1* (mouse atonal homolog 1) [36,37]. It has been shown that transfection of *ATOH1 in vivo* after acoustic trauma induces its over-expression in supporting cells, promoting their differentiation in HCs and hearing recovery [36,37]. A known marker of adult SC, Lgr5, has been used to verify that supporting cells are the progenitors of HC: $Lgr5^+$ supporting cells isolated from neonatal mice were able to form self-renewing neurospheres and differentiate into $myo7a^+$ HCs both *in vitro* and *in vivo* [38]. These observations are supported by those obtained on avian models, where adult supporting cells maintain their ability to differentiate into HCs, replacing them after a cochlear damage [39,40]. Other authors maintain that putative cochlear SCs may derive from the mesenchymal SCs (MSCs) reservoir located in the inner ear stroma underlying the epithelial tissue [41]. Although it remains unclear, some studies have shown that MSCs are able to differentiate *in vitro* in both HC-like cells and neurons [42,43].

In vitro regeneration by SCs

Transplantation of SCs to restore damaged inner ear cells and restore hearing function is an emerging field of research because mammalian HCs and SGNs are unable to regenerate after cell death resulting from trauma, disease or genetic mutation [7,44].

HCs and SGNs have been obtained *in vitro* from several types of SCs, including bone marrow MSCs (BM-MSCs), adipose-derived MSC (ASCs), olfactory precursor cells, embryonic SCs and adult brain germinal zone–derived cells (NSCs) from mice, rats and humans [45–52]. Despite these data, the ability of MSCs to differentiate into HCs and neurons is still controversial. Induced pluripotent SCs (iPSs) from mice were induced to become otic progenitors by exposure to growth factors, producing functionally active HCs [53]. Several authors investigated the possibility of obtaining new HCs and SGNs from endogenous sources and from putative cochlea-resident SCs. New functional sensory epithelia were obtained from endogenous avian inner ear cells by mesenchymal-to-epithelial transition after several culture freezing and expansion cycles, without co-culture with other tissues [54]. Isolated SGNs have been shown to able to survive *in vitro*, forming synapses with other neurons and HC in co-culture [48]. In *in vitro* co-cultures, also the neural progenitors derived from embryonic SCs were able to regenerate the complex neural network of the inner ear by producing neurites (positive to synaptic markers) elongating toward HCs [43,47,55,56]. These *in vitro*