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Potential of Pluripotent Stem Cells for the Replacement of Inner Ears

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1. Introduction

The inner ear, which manages our senses of hearing and balance, has mechanosensitive hair cells, which convert vibration into electronic signal to depolarize auditory or vestibular neurons. Inner ear functions depend largely on hair cells, their associated neurons and cochlear lateral wall, and defects in these cells result in hearing loss and deafness. Although some investigations indicated hair cell regeneration in mammalian vestibular sensory epithelia, loss of mammalian auditory hair cells is currently irreversible, which is the reason why hundreds of millions of people worldwide with hearing impairment have no way of restoring their auditory function. To date, the cochlear implant, which is designed to electrically stimulate the auditory neurons, is the only available prosthesis for severe to profoundly deaf individuals. However, it depends on remaining auditory neurons, named as spiral ganglion neurons, and their loss severely compromises its efficacy. In this context, several research strategies are directed toward replacing the degenerating spiral ganglion neurons following hearing loss. Here we review recent advances in the field of inner ear regeneration using pluripotent stem cells.

2. Inner ear anatomy

The inner ear consists of the vestibule, three semicircular canals, and cochlea. The vestibular sensory epithelia are located on the maculae of the saccule and utricle, and the cristae of the three semicircular canals. The vestibular sense organs contain two types of hair cells: The type I hair cells with round bottoms and thin necks, and type II hair cells shaped like cylinders with a flat upper surface covered by a cuticle. A tuft of cilia, or the stereocilia protrudes from the apical surface of each hair cell. Most afferent fibers terminate on type I hair cells, whereas the small efferent fibers terminate on type II hair cells. The cochlea is divided into three chambers: the scala tympani and vestibuli, which are filled with perilymph, and the scala media, which is filled with endolymph, containing potassium ions at higher concentrations than perilymph. The organ of Corti, the excitatory structure of the cochlea, contains hair cells and supporting cells, including pillar cells, Deiters' cells, Hensen's cells, inner phalangeal cells, and inner and outer sulcus cells. The afferent innervation of the organ of Corti consists of the dendritic terminals of neurons whose cell bodies comprise the spiral ganglion in Rosenthal's canal in the modiolus. The major projection of the afferent input is to the ventral cochlear nucleus. When the organ of Corti vibrates in response to incoming sound waves, the stereocilia of each hair cells bend,

opening the mechanoelectrical trasduction channels that are in the wall of the stereocilia. The entry of potassium and calcium ions into the hair cells through these channels causes the hair cells to depolarize, releasing neurotransmitters to stimulate the afferent terminal of spiral ganglion neurons.

3. Stem cells in the inner ear

In mammals, some hair cell generation has been observed in vestibular sensory epithelia [8,49], however, lost hair cells were replaced by transdifferentiation of supporting cells, not by cell proliferation of hair cells or supporting cells [55]. The belief that no tissue stem cells might exist in the inner ear was overturned by the finding that stem cells were still present in the vestibular organs of adult mice [19]. Several laboratories adopted a sphere-forming assay to isolate stem/progenitor cells from complex cell mixtures [6,19,20,21,30,33, 39,37,48,51,53,54] derived from inner ear tissues. Sphere-forming cells from the utricle of adult mice are pluripotent and can give rise to a variety of cell types, including cells representative of ectodermal, mesodermal and endodermal lineages [19]. Unfortunately, the lack of regenerative capacity in the adult mammalian cochlea is explained by the findings that the adult cochlea loses the ability for sphere formation by the third week of age [30]. Although attempts to establish stem cells from embryonic rat otocysts [18, 53] have been made, it is not clarified that these established stem cells correspond to which developmental stage. Identification of stem cells in the human fetal cochlea [3] contributes to study stem cell biology of the auditory organ in humans, while advances in identification of stem cells heve been made in rodents.

4. Hair cell regeneration

The inner ear sensory epithelia contains less than 20,000 sensory cells, or hair cells, although there are about a million photoreceptors in the eye. Hair cells are damaged by various causes, including acoustic trauma, ototoxic drugs, and aging. Most non-mammalian vertebrates are able to regenerate sensory hair cells after injury. However, mammalian cochlear hair cells do not regenerate spontaneously, although vestibular hair cells in adult mammals regenerate at levels so low as to rule out any significant functional recovery [8, 49].

Recently, Oshima et al., reported on stepwise protocols to induce hair cell-like cells from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [31]. The early development of the inner ear occurs in three phases. The first phase is the formation of the otic placode, followed by the second phase, the transformation of the otic placode into the otocyst, and finally, regional patterning of the otocyst occurs. Taking advantage of the knowledge of early inner ear development, they reproduced the developmental events in vitro starting with undifferentiated ESCs and iPSCs of mice, which are directed toward the ectodermal lineage. ESC/iPSC-derived ectodermal cells respond to otic inducing growth factors, for example, basic fibroblast growth factor (bFGF). Finally, induced otic progenitors are subjected to chicken utricular stromal feeders, which promoted the differentiation of otic progenitors into epithelial clusters displaying hair cell-like cells with stereociliary bundles. These hair-cell like cells respond to mechanical stimulation with currents that are reminiscent of nascent hair cell transduction currents. Further studies are needed to elucidate the signals to specify hair cell subtypes such as auditory or vestibular, inner or outer hair cell, or type I or type II hair cell, so one possible use of hair cell-like cells from ES/iPS cells is to study the steps

leading to hair cell maturation. Another use of hair cell-like cells from ESCs/iPSCs is to evaluate effectiveness and toxicity of various drugs to hair cells in vitro. Finally, the generation of human iPSCs with mutations for genes required for hair cell development and function could elucidate the pathogenesis that causes hearing impairment.

Another approach for inner ear regeneration is stem cell transplantation. Ito et al., performed the first animal experiments in the auditory systems to examine the potential for repairing the central auditory pathway and reported that embryonic brain tissue transplanted into a lesion in the ventral cochlear tract resulted in tissue regeneration and associated functional recovery [14]. Tateya et al., examined the potential of neural stem cell (NSC) transplantation to restore inner ear hair cells in mice [47]. Although the majority of grafted cells differentiated into glial or neural cells in the inner ear, a few transplanted NSCs integrate in vestibular sensory epithelia and expressed specific markers for hair cells in vivo. However, a small number of hair cell-marker positive grafted cells and no evidence of synaptic connections between transplants and host spiral ganglion neurons hampered well-established methods for functional recovery.

5. Regeneration of spiral ganglion neurons

Spiral ganglion neurons (SGNs) are the neurons which relay auditory signals from hair cells to the central systems. Cochlear implants, which bypass the damaged hair cells, directly stimulate the SGNs in profoundly deaf patients. Some animal studies suggest that degeneration of SGNs may compromise cochlear implant function [9, 41], although some conflicting reports demonstrated no correlation between clinical performance and the number of surviving auditory neurons [2,7,25]. Many attempts have been made to regenerate SGNs by transplanting pluripotent stem cells into the inner ear. We review previous reports and discuss obstacles to overcome for successful functional recovery. Several kinds of pluripotent stem cells have been delivered into the cochlea for the regeneration of SGNs, including NSCs [10,13,46], ESCs [4,5,11,12,29,34,36,38], bone marrow stem cells (BMSCs) [26,28,40], and iPSCs [27].

Tamura et al., evaluated the ability of NSCs to achieve neural differentiation in the modiolus of the cochlea and demonstrated that some grafted NSCs expressed β-III tubulin, a neuronal marker, although the majority of them differentiated into glial cells [46]. However, NSC transplantation can be utilized for protection of SGNs, because transplantation of neurospheres can reportedly be utilized for local application of neurotrophins into the brain [32,42], and several neuroprophins are known to have protective effects for SGNs [24,43,50].

ESCs are promising candidates for restoration of SGNs, because they have the potential to replace the lost auditory nerve due to their pluripotency. Sakamoto et al., first examined the fate of ESCs transplanted into the inner ears of adult mice and demonstrated that damaged inner ear has some activity inducing ESCs to develop into ectodermal cells, but the effect was insufficient to induce inner ear specific cells, including SGNs and hair cells [36]. The methods for generation of neurons from ESCs, including retinoic acid treatment of embryoid bodies [1], and co-culture of ESCs with PA6 cells, stromal cells derived from skull bone marrow [17] have been utilized for neural induction of ESC to regenerate SGNs. In this context, the regenerative potential of ESC-derived neural progenitors transplanted into the modiolus of the gerbil cochlea was examined and extensive migration of transplants along the auditory nerve was demonstrated [5]. Furthermore, transplantation of neural progenitors recovered the function of auditory neurons [29]. The evidence that ESC-derived

neurons have the potential to make synapse formation with auditory hair cells justifies the strategies of stem cell transplantation for the regeneration of auditory neurons [22,23]. Toward successful replacement of damaged SGNs by ESCs, establishment of SGN-specific cell types from ESCs is important. Transient expression of Neurog1, which is expressed in developing otocysts and is required for SGN differentiation, migration and survival, and treatment with glial cell line-derived neurotrophic factor (GDNF) turned undifferentiated ESCs into auditory nerve-like glutamatergic neurons [35].

Although previous studies identified ESCs as the promising candidates as transplants, ESC-based therapy is complicated by immune rejection and ethical problems. In this context, iPSC-based regenerative medicine has been developed recently [44,45,52]. iPSC-derived neural progenitors survived and expressed glutamatergic neuronal marker, VGLUT1, one week after transplantation into the cochlea, which indicated iPSCs can be used as transplants for the regeneration of SGNs as well as ESCs [27].

BMSCs, which can be readily obtained from an individual's own bone marrow, are also good candidates as transplants, because recent studies have shown that BMSCs can produce not only osteoblasts, chondrocytes, adipocytes, or myoblasts, but also neurons [15,16]. The survival of autologous BMSCs grafted in the cochlea was proven [26,28,40]. The enhanced survival of BMSCs was confirmed in deafened cochleae [26]. Autologous BMSC-derived neurospheres transplanted into the cochlear modiolus of the deafened guinea pigs settled predominantly in the internal acoustic meatus [28]. Combined with those findings, BMSCs can be a source for replacement of SGNs.

6. Tables and figures

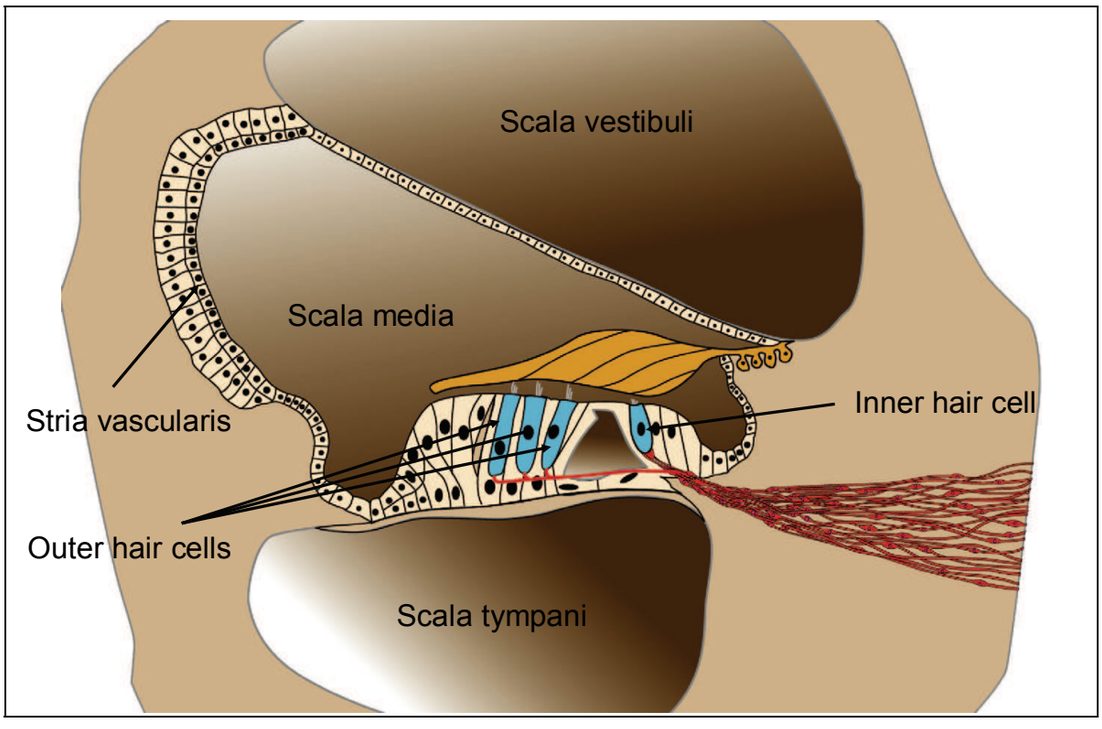

Fig. 1. The cochlea

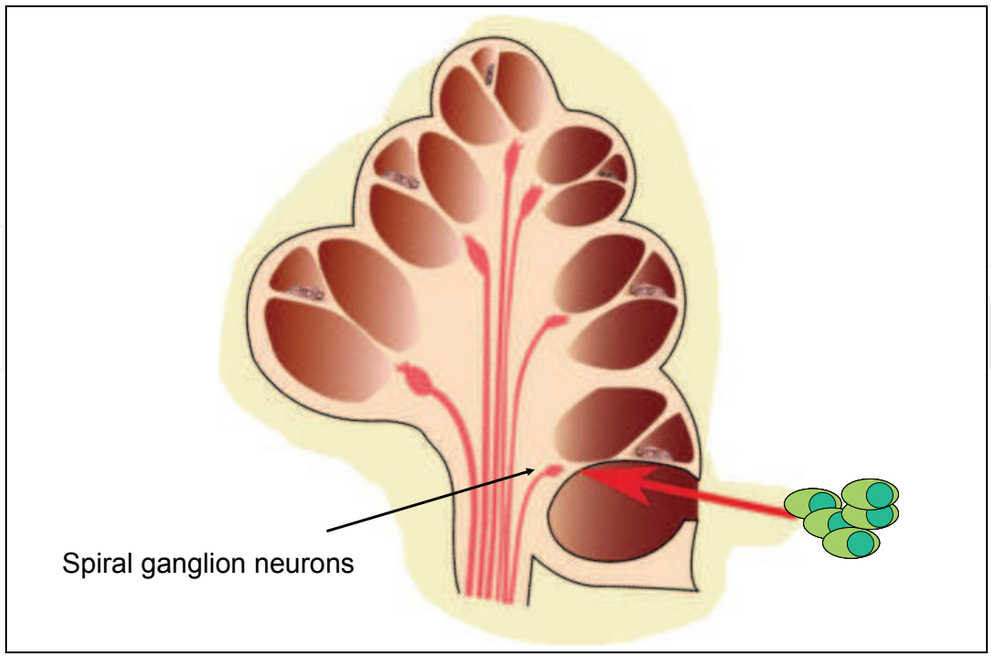

Fig. 2. Cell transplantation into the cochlear modiolus

7. Conclusions

Here we report the present status of development of stem cell-based therapy aiming for inner ear regeneration. Several experimental studies have demonstrated that pluripotent stem cells including ESCs and iPSCs are useful tools to examine detailed mechanisms of inner ear development, leading to reveal strategies for inner ear regeneration, and have the potential as a source of transplants for cell-based therapy for inner ear regeneration. However, many problems to be resolved still remain before realization of cell-based therapy for treatment of inner ears. More detailed analyses should be done to reveal key molecules that play critical roles in inducing differentiation of pluripotent stem cells into inner ear cells.

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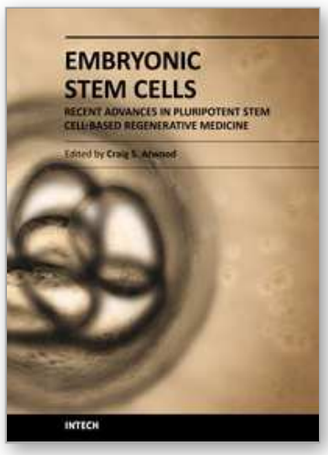

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Pluripotent stem cells have the potential to revolutionise medicine, providing treatment options for a wide range of diseases and conditions that currently lack therapies or cures. This book describes recent advances in the generation of tissue specific cell types for regenerative applications, as well as the obstacles that need to be overcome in order to recognize the potential of these cells.

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