Current Status of Stem Cell Therapy for Cochlear Hair Regeneration

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Abstract

Technologies such as hearing aids and cochlear implants do provide useful benefit to a large number of individuals with hearing loss, but they are incapable of truly "correcting" a hearing loss. In contrast to these, replacement of hair cells via stem cell therapies holds promise for a cure. Despite the discovery of stem/progenitor cells in the mammalian cochlea, no regeneration of either damaged hair cells or auditory neurons has been observed in mammals, in contrast to what is seen in birds and other vertebrates. Transplantation of exogenous stem cells has been proposed as a treatment to prevent or reverse sensorineural hearing loss. Although there is no clinical trial of stem cell therapies for hair cell regeneration, the research on animal models such as rats and mice is promising and is likely to lead to the development of novel therapeutic approaches for inducing hair cell regeneration in the mammalian cochlea. We review here current status of adult stem cells that are being used for seeding the cochlea for new hair cell formation. Overall, the data are encouraging and indicate that the technical problem of how to implant properly primed precursors into the cochlea or modiolus for hair cell and sensory neuron replacement is solvable. The main obstacles seem to be the identification of a source that provides enough stem cells to allow such therapies to have a good chance of success.

Keywords: Gene therapy, hair cell, adult stem cell.

The 21st century is witnessing an uprising in cellular therapy. Stem cell technology is proving to be a valuable tool not only for the development and regeneration of various tissue and organ systems, but also as a unit in evolution by natural selection. In the future, medical research anticipates treating many diseases including cancers, genetic and infectious diseases, diabetes, HIV, hearing loss, and heart diseases with the use of gene therapy and stem cell technology (Sheridan, 2011; Singec, Jandial, Crain, Nikkhah, & Snyder, 2007). Gene therapy was first conceptualized in 1972, with the authors urging caution before commencing gene therapy studies in humans (Friedmann & Roblin, 1972). The first FDA-approved gene therapy experiment in the United States occurred in 1990, when Ashanti DeSilva was treated for X-linked severe combined immunodeficiency (Sheridan, 2011). Since then, over 1,700 clinical trials have been conducted using a number of techniques for gene therapy.

A number of adult stem cell therapy techniques already exist; particularly bone marrow transplants that are used to treat leukemia (Gahrton & Björkstrand, 2000) and related bone/blood cancers (Bone Marrow Transplant). Stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies (Totey, Totey, Pal, & Pal, 2009; Tuch, 2006). Thousands of patients around the world have already benefited from bio-technologies using stem cells, delivered safely by skilled physicians. Diseases once considered incurable are responding well to stem cell therapies and are restoring a quality of life to patients they thought they had lost forever.

Here, we will highlight the strengths and weakness of various approaches starting with the different cellular material with respect to hearing loss. The ultimate goal of stem cell therapy for cochlear hair regeneration is to achieve restoration of the hearing loss. Before embarking on the stem cell therapy it is worth spending some time in understanding the role of gene therapy in generating hair cell replacement.

Gene Therapy

Gene therapy has been focused on generating replacement hair cells by re-expression of the atonal homolog 1 (Atoh1, also known as Math1) gene. This gene is essential for hair cell development as its targeted disruption in mice results in the absence of auditory and vestibular hair cells (Bermingham, 1999). Virus-mediated gene transfer of Atoh1 into the deafened cochlea of adult Guinea pigs resulted in improved auditory brainstem responses (Izumikawa, 2005). Although the extent of hearing improvement that is possible with Atoh1 gene transfer in adult laboratory animals requires further investigation, it is obvious from a number of studies that the Atoh1 gene is able to convert some types of cochlear cells into hair cells (Woods, Montcouquiol, & Kelley, 2004; Zheng & Gao, 2000). These Atoh1-induced hair cells are functional, at least when the Atoh1 gene is

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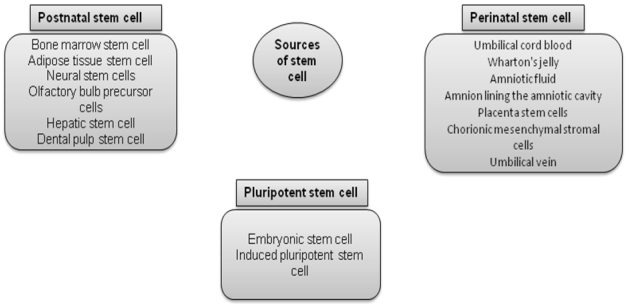
over expressed in a developing cochlea (Gubbels, Woessner, Mitchell, Ricci, & Brigande, 2008). The major limitation of virus-mediated gene therapy is, beside safety concerns, the problem of delivering the virus into all regions of the cochlear spiral. An injection at the base of the cochlea will very likely have effect only on the high frequencies. Treatment of middle and lower frequencies will require multiple injections at different sites. Opening of the cochlea always bears a high risk of doing additional damage, and it is inconceivable that utilization of multiple sites along the cochlear spiral will be a surgically feasible approach in the future, particularly in humans. Beside virus delivery obstacles, there is another limitation of the approach, which is that the therapeutic agent is able to induce so-called ectopic or supernumerary hair cells. These additional hair cells that are not located at the correct location within the organ of Corti do not contribute to proper hearing; instead, in all experimental cases investigated thus far, their presence is accompanied with profound hearing loss (Chen & Segil, 1999; Chen et al., 2003; Lowenheim et al., 1999).

Stem cell therapy

Stem cells are "generic" cells characterized by undifferentiated potency (totipotent, pluripotent, multipotent) and self-renewing cells that have the capacity to differentiate into any cell type of the body given appropriate intracellular gene regulation, intercellular communication, and environmental cues. The prospect of using specific cell types derived from stem cells or from reprogrammed adult somatic cells provides a unique opportunity in cell therapy and drug discovery for developing novel strategies for cochlear repair. Cell-based therapeutic approaches for treating hearing loss caused by disease or injury aim to promote structural repair of the injured or diseased tissue, an outcome currently not achieved by drug therapy. Specific cell types have been derived from human embryonic stem cells, induced pluripotent stem cells and directly transdifferentiated from adult somatic cells, such as skin cells. It is yet to be determined if the latter approach will evolve into a paradigm shift in the fields of stem cell research and regenerative medicine. These multiple sources of stem cells cover a wide spectrum of safety that needs to be balanced with efficacy to determine the viability of the cellular product (Daadi, 2011).

Transplanted stem cells can repair damaged tissues either through triggering direct differentiation of resident stem cells or indirectly by paracrine secretion. The latter increases the survival and/or proliferation of endogeneous cells (Bernardo, Locatelli, & Fibbe, 2009; Lai et al., 2010; Meirelles, Fontes, Covas, & Caplan, 2009). Stem cell therapy is budding as a prospective therapy for auditory nerve rehabilitation. It has been postulated that in the mammalian ear, the supporting cell layer may contain progenitor cells that could lead to hair cell regeneration. If such cells exist, they need to be identified. Furthermore it has already been shown that the mammalian ear contains cells that have the capacity for self-renewal and multilineage differentiation, both in vitro and after xenograft transplantation into the ears of chick embryos developing in ovo (Li, Liu, & Heller, 2003). On the other hand, the remnant of the once powerful regenerative ability can be detected in the mammalian adult vestibular sensory epithelia as well as in the neonatal cochlea. Particularly, it has been possible to isolate self-renewing progenitor cells from these organs and to use the progeny of these cells to generate hair cell-like cells in vitro and in vivo (Li et al., 2003; Oshima et al., 2007; Savary et al., 2007; Savary et al., 2008; Senn, Oshima, Teo, Grimm, & Heller, 2007; White, Doetzlhofer, Lee, Groves, & Segil, 2006; Zhai et al., 2005; Zhang et al., 2007). These inner ear derived stem/progenitor cells are probably well-suited for proof-of-principle experiments aimed to replace lost hair cells in the organ of Corti, if the hurdles of cell delivery and proper cell homing could be overcome. It has been postulated that a population of cells localized in the supporting cell layer in mammalian ears may contain progenitor cells that could lead to hair cell regeneration. Adult stem cells were recently found in the mouse utricle, a part of the inner ear involved in balance and motion.

Several stem cell types have now been delivered into the inner ear for the replacement of auditory neurons, including bone marrow stem cells (Matsuoka, Kondo, Miyamoto, & Hashino, 2007; Naito et al. 2004; Sharif et al., 2007), neural stem cells (Fu et al., 2009; Hu et al., 2005; Iguchi et al., 2003; Regala, Duan, Zou, Salminen, & Olivius, 2005; Tamura et al., 2004; Tateya et al., 2003) and embryonic stem cells (Ahn et al., 2008; Altschuler, O'Shea, & Miller, 2008; Coleman et al., 2006; Hu, Ulfendahl, & Olivius, 2004a; Lang et al., 2008; Okano et al., 2005; Praetorius, Vicario, & Schimmang, 2008; Regala et al., 2005; Reyes et al., 2008; Sekiya et al., 2006; Shi, Corrales, Liberman, & Edge, 2007). In addition, stem or stem-like cells including neural stem cells (Iguchi et al., 2003; Tamura et al., 2004; Tateya et al., 2003), olfactory bulb precursor cells (Liu et al., 2010; Pandit, Sullivan, Egger, Borecki, & Oleskevich, 2011) and dorsal root ganglia (Hu, Ulfendahl, & Olivius, 2004b; Olivius et.al 2003; Olivius et al., 2004), have been used to enhance the survival of endogenous auditory neurons, by means of their secretion of trophic factors and/or their expression of a supportive protein matrix (Figure 1).



Embryonic stem cells

Human embryonic stem (hES) cells are pluripotent cells derived from the inner cell mass of blastocysts. These hESCs are capable of growing indefinitely and retaining their potential to differentiate into almost all cell types of the adult body.

ESCs and induced pluripotent (iPS) cells are considered to be the most appropriate source of stem cells for the development of sensory hair cells (Beisel, Hansen, Soukup, & Fritzsch 2008; Brigande & Heller, 2009). Embryonic stem cell-derived inner ear progenitors have the capability to differentiate into hair cell-like cells in any case, either in cell culture or when transplanted into embryonic ears. Thus they emerge to be highly appropriate for transplantation studies. (Li, Roblin, Liu, & Heller, 2003). ES cells have the potential for self-renewal or regeneration that can be accredited to the expression of specific genes such as OCT4, NANOG, and SOX2 (Boyer et al., 2005).. Deregulation of any or all of these genes causes ES cells to lose their pluripotent nature and differentiation ability. Prominently, ES cells are more prone to immune rejection by the host although immunosuppressive therapy can counteract ES cell rejection. This decreases the potential to fight against opportunistic infections and also leads to several side effects such as kidney failure, osteoporosis, diabetes and hypertension (Grinnemo, Sylven, Hovatta, Dellgren, & Corbascio, 2008). One of the possibilities for preventing rejection is by creating embryonic stem cells that are genetically identical to the patient via therapeutic cloning. The major apprehension with ESC transplantation into patients as therapy is their ability to form teratoma. (Knoepfler, 2009).

Apart from ESCs, another source of stem cell gaze is induced pluripotent stem cell, which can be generated from a patient's skin. However there are several roadblocks which need to be overcome while using these stem cell based therapies for curing hearing loss. The obstacles which need to be addressed for includes finding a suitable surgical access to cochlea (as already discussed for gene therapy); establishing that the stem cell-derived cells survive, integrate and mature at the correct locations (and not at ectopic places); and finally, it needs to be ensured that the stem cell-derived grafts do not develop into tumors.

Adult stem cells

Adult stem cells, also known as somatic stem cells are found throughout the body. They are undifferentiated cells which can be coaxed to become different cells in the body (heart tissue, neural matter, skin cells etc). They can be isolated from different organs of the body such as fat, bone marrow, umbilical cord blood, placentas, neuronal sources, and olfactory tissue which resides in the upper nasal cavity (Banerjee & Bhonde, 2007; Jiang et al., 2002; Thiese & Krause, 2002). This simple fact has significant implications for medicine as the infusion of such stem cell can make diseased or damaged tissue healthy and robust.

Adult stem cells from the olfactory mucosa are readily accessible by biopsy and exhibit a broad differentiation both in vitro and in transpanatation settings (McDonald, Mackay-Sim, Crane, & Murrell, 2010; Murrell et al., 2005; Murrell, Sanford, Anderberg, Cavanagh, & Mackay-Sim, 2009). Several researchers have demonstrated that the epithelium of the tongue represents an accessible and abundant source of adult stem and progenitor cells (Luo, Okubo, Randell & & Hogan, 2009; Ookura et al., 2002; Okubo, Clark, & Hogan, 2009; Sullivan, Borecki, & Oleskevich, 2010). Transplantation of epithelial stem/progenitor cell into mice with noise induced hearing loss resulted in a significantly reduced ABR threshold shift to click stimuli. These findings provide evidence that epithelial stem/progenitor cell transplantation can lessen permanent threshold shifts resulting from noise trauma (Sullivan et al., 2010). Previous studies have tested for a functional rescue of hearing via adult stem cell transplantation in animal models of cochlear ischaemia (Hakuba et al., 2005; Yoshida et al., 2007).

Adult stem/progenitor cells have a number of advantages for cochlear transplantation in that they can be used for autologous transplantation (to resist host rejection) and are less tumourigenic than embryonic stem cells (Bithell & Williams, 2005). Overall, the data are encouraging as they indicate that the technical problem of how to implant properly primed precursors into the cochlea or modiolus for hair cell and sensory neuron replacement is solvable. The main obstacles seem to be the identification of a source that provides enough stem cells to allow such therapies to have a good chance of success.

Mesenchymal stem cells

Mesenchymal stem cells, or MSCs, which are found in almost all the postnatal organs are glass adherent population of multipotent stem cells having

immunomodulatory property.

The scarcity of stem cells for transplantation calls for other alternative sources. MSCs can be derived from the human umbilical cord matrix, human placenta, bone marrow, human amnion, or human breast milk (Kadam & Bhonde, 2010; Kadam, Muthyala, Nair, & Bhonde, 2010; Kadam, Sudhakar, Nair, & Bhonde, 2010; Patki, Kadam, Chandra, & Bhonde, 2010; Phadnis et al., 2011) which are good sources of abundant stem cells for treatment. MSCs have been identified and characterized by: 1) their ability to adhere to plastic culture flasks; 2) the positive expression of CD105, CD73, CD90 membrane antigens, and the lack of expression of others (e.g. CD45 and CD34) and 3) the ability of differentiation under adequate conditions along the osteogenic, chondrogenic and adipogenic lineages. In recent years, cells with these characteristics have been isolated from the Wharton jelly (WJ) of the Umbilical Cord (UC). Similar to bone marrow MSCs, they have shown multilineage differentiation potential and ability to provide trophic support to neighboring cells. According to the literature, there are two main populations of cells with a mesenchymal character within the human UC: Wharton's Jelly Mesenchymal Stem Cells (WJ-MSCs) and Human Umbilical Cord Perivascular Cells (HUCPVCs). In the present work our aim is to make a comprehensive review on MSC populations of the WJ and how these cell populations may be used for future applications in central nervous system (CNS) regenerative medicine. Following a brief insight on the general characteristics of MSC like cells, we will discuss the possible sources of stem cells within the WJ and the cord itself (apart UC blood), as well as their phenotypic character. As it has already been shown that these cells hold a strong trophic support to neighboring cell populations, we will then focus on their secretome, namely the molecules that have already been identified within it, and their role in phenomena such as immunomodulation (Carvalho, Teixeira, Reis, Sousa, & Salgado, 2011). The possible application of these cell populations to CNS regenerative medicine will be addressed by critically reviewing the work that has been performed so far in this field. Finally, a brief insight will be made on what, in the author's opinion, are the major challenges in the field for the future application of these cell populations in CNS regenerative medicine.

Past study has shown the active regeneration of cochlear fibrocytes after severe focal apoptosis, without any changes in the organ of Corti by the transplantation of the MSC derived from bone marrow, and with a significant hearing recovery ratio (Kamiya et al., 2007). Recent studies suggest that adult olfactory stem cells represent a subtype of MSCs (Delorme et al., 2010; Pandit et al., 2011). Numerous studies have demonstrated that human MSCs avoid allorecognition, interfere with dendritic cell and T-cell function, and generate a local immunosuppressive microenvironment by secreting cytokines (Ryan, Barry, Murphy, & Mahon, 2005). It has also been shown that the immunomodulatory function of the human MSCs is enhanced when the cells are exposed to an inflammatory environment characterized by the presence of elevated local interferon-gamma levels (Ryan, Barry, Murphy, & Mahon, 2007).

Future Directions

Identification of a suitable source of MSCs either from perinatal tissues like the umbilical cord, amnion, placenta or postnatal tissues like dental pulp, adipose tissue is of prime importance for targeted delivery of cells into the cochlea (Figure 1). It is worth exploring the potential of dental pulp stem cells (DPSCs) for this purpose as these cells are derived from the neural crest. Recently we demonstrated the potential of DPSCs to differentiate into insulin producing islet-like cells (Govinadasamy et al., 2010). As the cochlea originates from an embryonic ectoderm similar to that of dental pulp, DPSCs appear to be a bonafide candidate of choice for cochlear hair cell repair. A team work of ENT surgeons and stem cell biologists would pave the way for translational research from bench to bedside in the area of cochlear hair regeneration. Much has been accomplished since the discovery of postembryonic hair cell production and hair cell regeneration in lower vertebrates. No therapies for hair cell regeneration are under clinical trials, but research is yielding potentially important discoveries that are likely to lead to the development of therapeutic methods for inducing hair cell regeneration in the mammalian inner ear.

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