

COMMENTARY

Human stem cells ameliorate auditory evoked responses in a model of neuropathy

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Abstract

Stem cells have been touted as a potential source of replacement cells for the treatment of severe-to-profoundly deaf individuals, including possible combined therapy with a cochlear implant. The success of such a therapy is dependent on a number of factors, but of critical importance is the functional incorporation of transplanted cells into the peripheral and central auditory systems. In a major breakthrough, Chen and colleagues recently reported the restoration of hearing thresholds by up to 46% following the transplantation of human pluripotent stem cells in a rodent auditory neuropathy model. Improved function was matched with new synapse formation in the peripheral and central aspects of the auditory system. The findings have promising clinical implications for patients with auditory neuropathy. Still to be elucidated are the long-term survival and function of transplanted cells, the precise mechanism by which hearing is restored, and whether further improvement is possible when combined with electrical stimulation from a cochlear implant.

Over the last decade, more than 40 *in vivo* studies have been conducted to investigate the viability of various stem cell types for the replacement of auditory neurons (ANs) in the deaf cochlea (recently reviewed in [1]). Although the majority of these studies have shown survival and differentiation of transplanted cells for various lengths of time, none has been able to demonstrate the combined integration of human stem cells into the cochlea with functional improvement in hearing thresholds. In addition, the directed differentiation of human embryonic stem cells (hESCs) toward an auditory

neural lineage has been limited, and just one other study has reported differentiation of cells into auditory-like neurons expressing β III tubulin, Brn3a, peripherin, and GATA3 [2]. The recent *Nature* paper by Chen and colleagues represents a major breakthrough in the field by illustrating the directed differentiation of human pluripotent stem cells into defined otic progenitors and, subsequently, into functional auditory neurosensory lineages. Impressively, the auditory-like neurons derived from these protocols improved hearing function when delivered into the denervated cochlea [3].

The directed differentiation of hESCs toward an auditory neural lineage has been challenging, owing partially to the limited knowledge of the origin of placodal progenitors during normal development. Studies have elucidated the important role of fibroblast growth factors in inner ear development [4] and the specification of the otic placode [5,6], and these findings were applied by Chen and colleagues to sequentially produce otic progenitors (from three hESC lines). These otic progenitors were further differentiated under defined conditions to produce both sensory hair cell-like cells and AN-like cells which biochemically and physiologically resembled their *in vivo* counterparts. Although the AN-like cells expressed both K^+ and Na^+ currents, these stem cell-derived neurons were observed to fire single action potentials, which more closely resemble ANs during embryonic development [7] rather than the electrical profile of ANs *in vitro* [8]. While electrical activity is encouraging, these neurons presumably would need to recapitulate their *in vivo* physiology in order to faithfully transmit precise pitch and timing cues to the brain. This would include the expression of a specific subset of ion channels [1] and the capability to follow normal acoustic stimulation, which is in the order of 200 spikes per second for ANs [9]. Moreover, for potential future therapies combining stem cells with cochlear implantation, these stem cell-derived neurons would need to be capable of following higher rates of stimulation from the implant (>800 spikes per second [9,10]).

The transplantation of human stem cell-derived otic neural progenitors described by Chen and colleagues also represents several major advances, the combination of

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which has eluded other investigators in the field to date. These advances include the following: a significant improvement of neuronal density within Rosenthal's canal after hESC transplantation into the cochlear modiolus, expression of the afferent type I auditory neural marker NKA α 3 in transplanted hESCs, extension of stem cell-derived processes peripherally to the hair cells (including synaptic marker expression within hESC-derived fibers contacting the basal surface of the hair cells), and growth of hESC-derived processes in a central direction (with a small number of central synapses detected in the cochlear nucleus by immunochemistry). In further support of these observations, functional recovery of varying degrees was reported in all stem cell-transplanted animals (n = 18) by using auditory evoked responses. These evoked responses improved significantly from 4 until 10 weeks post-transplantation (the end-point for the study).

Although one study has reported the recovery of auditory evoked thresholds following transplantation of human-derived olfactory stem cells into the deaf cochlea [11], the transplanted cells did not integrate into the cochlear tissues and the mechanisms for repair were attributed to paracrine signaling by the cells (which remained in the perilymphatic compartments). Conversely, a different study reporting integration of hESCs into the cochlear modiolus following hearing loss [2] did not report functional improvement following transplantation. The article by Chen and colleagues combines both hESC integration and recovery of auditory evoked thresholds, and 18 transplanted animals showed significant functional improvement post-transplantation. Interestingly, the authors noted that there were considerably fewer stem cell-derived central projections growing toward the cochlear nucleus (in comparison with numbers of transplanted somata), and evidence of central synapses was detected in just three of the transplanted animals. Moreover, the authors report functional improvement across all frequencies, despite the observation that hESCs are detected only in the base of the cochlea. This suggests that further work is still required to fully elucidate the mechanism(s) by which function is restored in this model. While the detection of hESC-derived synapses in the cochlear nucleus poses several technical challenges, this difficulty will ultimately be an important aspect to investigate given the synaptic complexity and cochleotopic connectivity required for the correct transmission of sound information to the brain [12].

Future investigations should therefore examine the role of electrical stimulation in promoting the outgrowth of stem cell-derived neurons toward their central targets, which may facilitate improved synaptic and cochleotopic connectivity [1]. This idea is supported by evidence that patterned electrical stimulation delivered to the deaf

mammalian cochlea can restore synapses in the cochlear nucleus [13]. In addition, the long-term survival of donor stem cells in the cochlea warrants further investigation since the side effects of chronic immunosuppression can include cancer and infection [14]. This challenge may be overcome with the use of patient-matched induced pluripotent stem cells if genomic and functional instabilities can be controlled [15] along with the directed differentiation into an appropriate neural phenotype. The promising results reported by Chen and colleagues in *Nature* mean that we are a step closer to the clinical application of stem cells for the treatment of hearing loss from auditory neuropathy, and perhaps these findings can be extended to treat other types of hearing loss in the future.

Abbreviations

AN, auditory neuron; hESC, human embryonic stem cell.

Competing interests

The author declares that she has no competing interests.

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BAN is a Senior Research Fellow in the Department of Otolaryngology of the University of Melbourne. She holds a PhD and leads the investigations relating to stem cell therapy for auditory nerve regeneration in the Department.

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