# Can neurosphere production help restore inner ear transduction?

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The exquisite sensitivity of the human ear to sound depends on the proper function of mechanosensory hair cells that transduce mechanical stimuli into electrical signals. Sound-induced deflections of bundles of actin-rich microvilli that protrude from the apical surface of hair cells evoke changes in the open probability of nonselective ion channels that modulate cellular excitability and synaptic transmission. The signals are transmitted to auditory spiral ganglion neurons (SGNs) which, in turn, convey the sensory information to the brain. A multitude of factors including excessive noise, trauma, ototoxic drugs, aging, and genetic factors can cause degeneration and death of mechanosensory hair cells and SGNs. Unlike the primary sensory cells for touch, taste, and smell, sensory hair cells do not regenerate in humans; thus, their loss leads to permanent hearing deficits, which collectively are projected to affect 69 million Americans by the year 2030. In a recent issue of PNAS, Wei *et al.* (1) describe a novel source of precursor cells that could be used to replace lost hair cells and SGNs. Here, we highlight the significance of these findings, the prospects that they may be developed into viable treatment strategies and several challenges that remain.

The current state-of-the-art therapy for hearing loss, the cochlear implant, offers only partial restoration of hearing function and is indicated in only a limited number of cases. Deaf patients who have suffered significant hair cell loss but retain auditory SGNs can be considered as candidates for a cochlear implant. The device attempts to reproduce the sensory function of hair cells and stimulates the auditory SGNs electrically. Although a great success story for the field, cochlear implants do not restore the full spectrum of sound frequencies that the human ear can perceive and survival of SGNs is a prerequisite. As such, other therapeutic approaches for restoration of hearing including cellular replacement via stem cell therapy, introduction of exogenous genetic material through gene therapy (2, 3), and pharmacological induction of intrinsic tissue regeneration are being investigated and hold the promise for complete recovery of auditory function.

**A Novel Source for Hair Cell Replacement**

In pursuit of a cellular replacement strategy, Wei *et al.* (1) identified proliferative cells from the ependyma of the lateral ventricles of the adult mouse brain that share similar molecular, morphological, and physiological characteristics with inner ear hair cells and therefore may serve as a potential source for replacement of lost hair cells. In previous work, stem cells have been isolated from various sources and examined for their potential to differentiate into cells with a hair cell-like phenotype. For example, stem cells isolated from mammalian inner ear tissues such as the stria vascularis, the auditory organ (4, 5), the greater epithelia ridge of the cochlea (6), and the inner ear organs of balance (4, 7) appear to possess the potential to proliferate and generate hair cell-like cells under certain conditions or after transplantation. Cells with bona fide hair bundles have been generated in vitro from cell lines derived from embryonic chick ears (8). Stem cells derived from sources outside the inner ear such as embryonic stem cells (9), bone marrow mesenchymal stem cells (10), and adult mouse olfactory precursor cells (11) are also able to differentiate into cells that express hair cell markers. Moreover, neural stem cells have been transplanted into the inner ear and their survival and differentiation into sensory cells and/or neurons has been encouraging (12–15), but practical application remains a challenge because yield is low and obstacles such as robust immune reactions and proper tissue integration have impeded progress. Some obstacles, such as strong immune reactions, may be overcome by isolation of donor adult stem cells for reintroduction into the same host. A novel source of autologous cells for hair cell replacement, the ependymal cells from the germinal zone of the adult brain, has emerged in the new work from Wei *et al.* and may overcome these obstacles.

**Wei *et al.* describe a novel source of precursor cells that could be used to replace lost hair cells and SGNs.**

Ependymal cells, which form a multiciliated single cell layer lining the lateral ventricles, play an important role in transport of cerebrospinal fluid. Although ependymal cells were once suggested to have stem cell characteristics (16), their proliferative or stem cell capacity is still somewhat controversial (17, 18). However, Wei and colleagues (1) identified BrdU-positive and MyosinVIIA-positive cells in the ependymal layer of the lateral ventricle in vivo as well as in neurospheres derived from the walls of the lateral ventricles. The proliferative cells corresponded to ependymal cells and, interestingly, expressed several markers that are also expressed by hair cells such as myosin VIIa, myosin VI, and CtBP2/RIBEYE. Furthermore, although they had some morphological differences from hair cells, Wei *et al.* (1) showed that, similar to vestibular hair cells of the inner ear, the ependymal cells had both cilia and actin-based microvilli on their apical surfaces. Also in a fashion similar to hair cells, ependymal cells rapidly took up the styryl dye FM1–43 that permeates hair cell transduction channels, suggesting that the ependymal cells express similar large-conductance, nonselective ion channels. When cocultured with SGNs, the myosin VIIA-positive ependymal cells appeared to make synapse-like contacts with SGNs and, when depolarized, they evoked glutamate-dependent synaptic currents in SGNs. Although the authors did not demonstrate the ability of ependymal cells to respond to mechanical stimulation and the morphology of their microvillar bundles were not identical to authentic hair bundles, the ependymal cells shared a number of similarities with hair cells. A distinct advantage is that, unlike hair cells, ependymal cells appear to retain the

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ability to proliferate, which raises their prospects for use in cellular replacement strategies. Further examination of the differences between the proliferative capacity of ependymal cells and the quiescence of hair cells may make ependymal cells a valuable comparative model for understanding some basic biological questions.

### Generation of Auditory Neurons from Neural Stem Cells

The results of Wei and colleagues (1) are also significant because they demonstrate that cells harvested from the same brain region can be driven toward an auditory SGN phenotype. After hair cell loss, many auditory SGNs retract their synaptic processes and degenerate. Neurotrophic factors have been used to prevent afferent degeneration after hair cell damage (reviewed in ref. 19); however, in many cases the damage to SGNs may be quite advanced and may require cell replacement therapies. Because cochlear implants are ineffective without intact SGNs, combined implant/cell replacement strategies could be envisioned.

Previously, transplanted embryonic stem cell-derived neural progenitor cells were shown to extend processes and make contacts with hair cells when transplanted into denervated auditory organs (20, 21) in vivo. Progenitor cells transplanted into organ cultures also expressed synaptic markers, suggesting the formation of functional synapses. In the Wei *et al.* (1) study, neural stem cells (NSCs) isolated from the subventricular zone (SVZ) of the lateral ventricles were shown to differentiate into neurons with characteristics similar to SGNs. In vivo, the NSCs found in the germinal region of the brain, the SVZ, are responsible for the continuous generation of new neurons destined for the olfactory bulb in adult mice. The authors showed that these NSCs isolated from the SVZ established synapse-like synapsin 1-positive contacts with inner ear hair cells in cocultures or in SGN-depleted auditory organ cultures in vitro. Moreover, depolarization of the contacted hair cells evoked action potentials in the NSCs. In addition, the NSCs were shown to make contacts with SGNs when cocultured with auditory organs; 60% of the NSCs behaved as presynaptic neurons and 30% as postsynaptic neurons. Thus, NSCs from the SVZ could serve both functions required for replacement for auditory spiral ganglion neurons. Together with the previous observations that adult neural stem cells transplanted into normal and deafened ears can survive and be driven to differentiate into neurons (12), the identification of NSCs from the SVZ that can be driven toward a SGN phenotype is particularly exciting.

In summary, new sources of cells that may have the potential to develop into hair cell and afferent SGNs for replacement after hair cell loss have been identified in the germinal zone of the adult mammalian brain. Follow-up work will be required to assess the ability of the transplanted ependymal cells to respond to mechanical stimulation in a manner similar to hair cells. If forthcoming, such novel sources could potentially provide substitutes for lost hair cells. In addition, because of their ability to differentiate into neurons and functionally connect with hair cells, adult neural stem cells could potentially be transplanted to replace lost spiral ganglion neurons and thereby reconstitute the neurosensory circuitry in the inner ear.

A significant challenge remains that may limit the clinical utility of the approach. Because the cells that line the lateral ventricles are buried deep within the brain, harvest of the cells for use in inner ear cell replacement strategies may not be practical in humans. Nonetheless, as a tool for understanding the basic science of cell replacement in the inner ear, the work of Wei *et al.* (1) represents an important step forward. Although the finish line in the race to develop effective treatments for hearing loss may be distant, it is clear the starting bell has sounded.


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