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Functional Effects of Adult Human Olfactory Stem Cells on Early-Onset Sensorineural Hearing Loss

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Key Words. Auditory brainstem response • Auditory threshold • Cell therapy • Hearing loss • Inner ear • Olfactory stem cell

ABSTRACT

Transplantation of exogenous stem cells has been proposed as a treatment to prevent or reverse sensorineural hearing loss. Here, we investigate the effects of transplantation of adult human olfactory mucosa-derived stem cells on auditory function in A/J mice, a strain exhibiting early-onset progressive sensorineural hearing loss. Recent evidence indicates that these stem cells exhibit multipotency in transplantation settings and may represent a subtype of mesenchymal stem cell. Olfactory stem cells were injected into the cochleae of A/J mice via a lateral wall cochleostomy during the time period in which hearing loss first becomes apparent. Changes in auditory function were assessed 1 month after transplantation and compared against animals that received sham injections. Hearing threshold levels in stem cell-transplanted mice were found to be significantly lower than those of sham-injected mice ($p < .05$) for both click and pure tone stimuli. Transplanted cells survived within the perilymphatic compartments but did not integrate into cochlear tissues. These results indicate that transplantation of adult human olfactory mucosa-derived stem cells can help preserve auditory function during early-onset progressive sensorineural hearing loss. STEM CELLS *2011;29:670–677*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Hearing impairments that arise during infancy and childhood can lead to marked deficits in speech and language acquisition, as well as hinder cognitive and psychosocial development. In industrialized nations, genetic factors are the major causes of childhood hearing loss [1, 2]. For example, approximately 50% of prelingual hearing loss cases in humans are attributable to genetic causes [1, 3]. Although the etiologies of early-onset hereditary hearing loss are heterogeneous [1, 4], the majority of cases are nonsyndromic and sensorineural (i.e., arise through the loss of auditory hair cells and/or neurons; [2, 5]). As the regenerative capacity of the mammalian cochlea is severely limited, one approach currently being explored to treat hearing loss is the transplantation of endogenous stem cells [6–8]. Transplanted stem cells have the potential to repair tissues by replacing damaged cells and/or by secreting factors that enhance the survival or repair of endogenous cells.

A number of animal models have been developed for the examination of physiological, cellular, and molecular bases of hereditary sensorineural hearing loss. A/J mice represent a valuable model for examining early-onset sensorineural hearing loss, as these mice exhibit a severe decline in auditory function that commences immediately following weaning, the genetic basis of which has been well-characterized [9–13]. Although elevated hearing thresholds are first apparent in A/J mice at postnatal day 25 (P25), hair cell loss is evident in the cochlea as early as P14 [9]. This impairment in auditory function subsequently progresses to near complete hearing loss by 3 months of age as the number of hair cells progressively declines [11]. No evidence of progressive loss of spiral ganglion neurons or cells of the cochlear lateral wall (stria vascularis and spiral ligament) has been observed [9]. Three loci contributing to early-onset hearing loss in A/J mice have been identified, including the gene variants *Cdh23*^{ahl} and *ahl4* and a single nucleotide insertion in the mitochondrial DNA gene *tRNA-Arg* [9, 12, 14]. The *Cdh23* gene encodes cadherin 23, a membrane-bound glycoprotein present in hair cell stereocilia tip links, which plays important role in mechanoelectrical transduction [15]. Mutations in this gene have been linked to a form of early-onset nonsyndromic autosomal recessive deafness in humans (DFNB12 disorder) [16]. A/J mice are homozygous for the *Cdh23*^{ahl} allele [9]. Single nucleotide polymorphism in the mitochondrial *tRNA-Arg* gene has been shown in other mice strains to increase the production of reactive oxygen species [17] and to modify the phenotypic expression of hearing loss associated with 12S rRNA A1555G

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mutation in humans [18]. Molecular characterization of *ahl4* has not yet been completed.

Two important factors influencing the success of stem cell transplantation are the stem cell type and the site(s) of transplantation. Adult stem cells have a number of advantages for transplantation, as they can be used for autologous transplantation (to resist host rejection) and are less tumorigenic than embryonic stem cells [19]. Adult stem cells from the olfactory mucosa are readily accessible by biopsy and exhibit a broad differentiation potential both in vitro and in transplantation settings [20–22]. Transplantation of adult olfactory mucosa-derived stem cells (hereafter referred to as olfactory stem cells) has been shown to have beneficial effects in animal models of human disorders relating to a variety of tissues, including Parkinson's disease (transplantation into brain: [23]) and cardiac infarction (transplantation into heart: [22]). Recent studies suggest that adult olfactory stem cells represent a subtype of mesenchymal stem cell of neural crest origin [24].

Transplantation of stem cells into the cochlea requires an accessible route of entry to enable the delivery of stem cells in close proximity to sites of degeneration. Previous studies have described multiple delivery routes for stem cell transplantation into animal models of hearing loss, including via cochlear, intravenous, and vestibular injection sites (reviewed in [25]). The round window provides a natural access route to the cochlea (scala tympani) with minimal surgical damage but requires extensive cellular migration to influence affected tissues. In contrast, lateral wall or basal turn cochleostomies are invasive yet provide a wide distribution of transplanted cells within the cochlea throughout multiple compartments.

In this study, we examined the effects of transplantation of adult human olfactory stem cells on auditory function in A/J mice at 4 weeks of age. Olfactory stem cells were injected into the cochleae of these mice via a lateral wall cochleostomy site that provided robust access to the scala tympani and scala vestibuli and limited access to the scala media. Examination of auditory brainstem responses (ABRs) to sound stimuli showed that transplanted olfactory stem cells contribute to a reduction in hearing loss when compared with sham-injected animals. Systematic anatomical analyses revealed that transplanted olfactory stem cells survived within the cochlea for at least 2 weeks. The transplanted cells did not integrate into cochlear tissues, however, suggesting that paracrine effects on endogenous cells may mediate the observed functional preservation of hearing levels.

Materials and Methods

Adult Human Olfactory Stem Cell Culture and Characterization

Adult human olfactory stem cells were kindly provided by Prof. Alan Mackay-Sim (National Centre for Adult Stem Cell Research, Griffith University, Griffith, Australia) [20]. Previous reports differ in their characterization of olfactory stem cells with suggestions that olfactory stem cells are a subtype of mesenchymal stem cell [24] or alternatively a subtype of neural stem cell [22, 23]. Therefore, we characterized the cell line used in the present experiments for their expression of mesenchymal stem cell markers and capacity to differentiate into mesodermal cell types, which are two defining criteria of mesenchymal stem cells [26]. Mesenchymal stem cell marker expression was examined with immunocytochemistry; whereas, mesodermal differentiation potential was investigated with adipogenesis and osteogenesis assays.

To investigate mesenchymal stem cell marker expression, olfactory stem cells were plated onto glass coverslips at a density of 6,500 cells per cm^2 and grown to 70%–80% confluency. Cells were fixed for 15 minutes at room temperature in 4% paraformaldehyde (PFA) in 0.1 M phosphate buffered saline (PBS), pH 7.4, and then blocked for 1 hour in 10% normal goat serum in PBS containing 0.1% Triton X-100 (NS-PBSTx). Primary antibodies were diluted in NS-PBSTx and applied overnight at 4°C. The following primary antibodies were used: monoclonal rat anti-5-bromo-2′-deoxyuridine (BrdU) (1:50; AbD Serotec, East Brisbane, Australia, www.abdserotec.com; MCA2060), monoclonal mouse anti-β-tubulin III (1:500; Promega, Sydney, Australia, www.promega.com; G712A), monoclonal mouse anti-CD44 (1:10; Developmental Studies Hybridoma Bank; H4C4), monoclonal mouse anti-Stro-1 (1:1; Developmental Studies Hybridoma Bank; STRO-1), and monoclonal mouse anti-vimentin (1:10; Developmental Studies Hybridoma Bank; AMF-17b). Cells were then rinsed for 4 hours in several changes of PBS and incubated overnight at 4°C in the appropriate secondary antibodies diluted in PBS. Following rinsing for 4 hours in PBS, cells were mounted in Gelmount (Biømeda, Kirwan, Australia). In experiments examining mitotic activity, BrdU (Sigma, Castle Hill, Australia, www.sigmaaldrich.com/australia.html) was added to the culture medium 2 hours before fixation. The fixed cells were then incubated in 1 N HCl at 4°C for 10 minutes, 2 N HCl at room temperature for 10 minutes, and then 2 N HCl at 37°C for 20 minutes. Following rinsing in NS-PBSTx for 20 minutes, cells were processed for BrdU immunolabeling as described above.

To examine the capacity of the olfactory stem cells to differentiate into adipocytes, cells were plated into 24-well plates (Corning, Sigma) at 5,000–6,000 cells per cm^2 and grown to 70%–80% confluency. The culture medium was then replaced with adipocyte induction medium that comprised Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Mulgrave, Australia, www.invitrogen.com) supplemented with 10% fetal calf serum (Gibco), 0.5 mM 3-isobutyl-1-methyl-xanthine (Sigma), 1 μM dexamethasone (Sigma), 60 μM indomethacine (Sigma), and 10 μg/ml insulin (Novo Nordisk, Baulkham Hills, Australia, www.novonordisk.com.au). Two different adipogenic induction protocols ("short" and "long") were employed. In the first (short) protocol, cells were cultured in adipocyte induction medium for 3 days and then incubated in maintenance medium containing DMEM, 10% fetal calf serum, and 10 μg/ml insulin for 6 days, with medium changing every 2 days. The second (long) protocol involved exposing the cells to three differentiation cycles, each comprised 3 days in adipocyte induction medium followed by 1 day in maintenance medium. The differentiated cells from both protocols were then fixed in 4% PFA for 15 minutes at room temperature and stained with oil red O (Sigma).

To examine osteogenic differentiation, cells were plated into 24-well plates at 5,000 cells per cm^2 and grown to 70%–80% confluency. The culture medium was then replaced for 21 days with osteogenic differentiation medium containing DMEM supplemented with 10% fetal calf serum, 0.1 μM dexamethasone, 0.15 mM L-ascorbic acid (Sigma), and 1 mM sodium dihydrogen phosphate (Sigma), with medium changing every 2–3 days. Cells were then fixed in 4% PFA for 15 minutes at room temperature and stained using the von Kossa procedure, in which cells were incubated in 2% silver nitrate for 30 minutes, exposed to ultraviolet light for 30 minutes, and then incubated in 2.5% sodium thiosulfate for 5 minutes.

Olfactory Stem Cell Transplantation and Hearing Threshold Determination

To investigate the functional effects of stem cell transplantation, male and female A/J mice (P28-P37; $n = 21$) with equivalent hearing levels were divided into two cohorts.

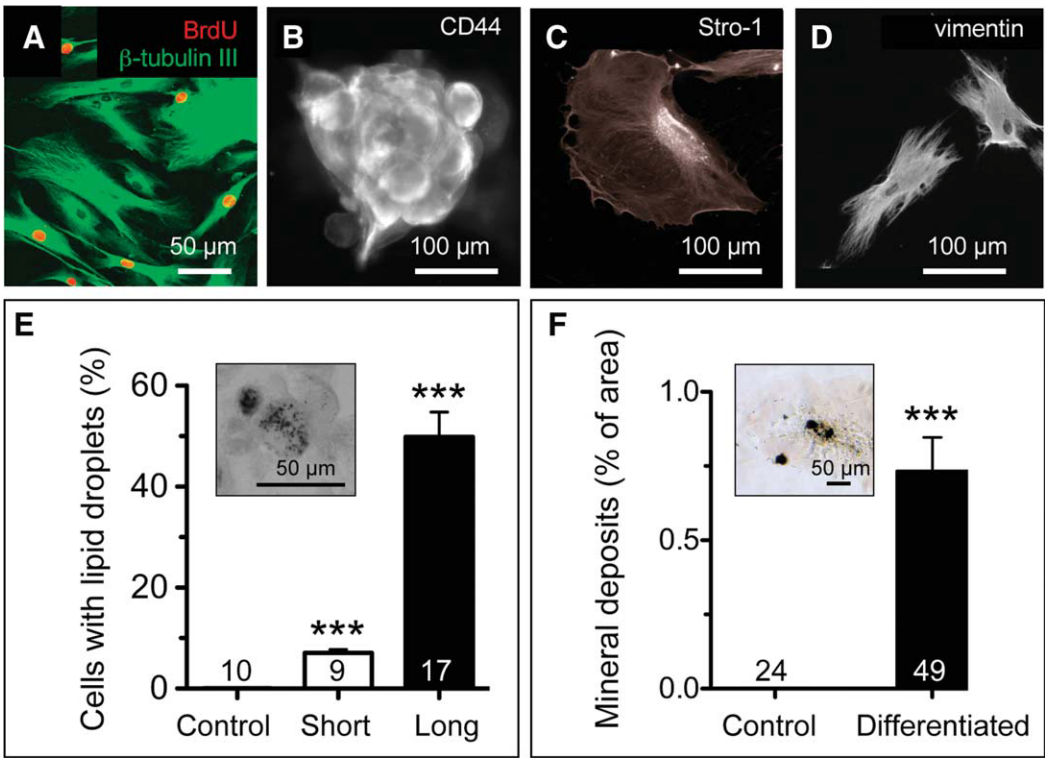

Figure 1. Transplanted adult human olfactory stem cells are mesenchymal stem cell-like. (**A**): Immunolabeling for 5-bromo-2′-deoxyuridine incorporation by adult human olfactory stem cells 2 hours after addition of the substitute nucleoside to the culture media. The cells are also immunoreactive for the neuronal marker β-tubulin III in the undifferentiated state, a characteristic of certain types of mesenchymal stem cells. (**B–D**): Human olfactory stem cells are immunoreactive for the mesenchymal stem cell markers CD44 (**B**), Stro-1 (**C**), and vimentin (**D**). (**E**): Short- and long-exposure to adipogenic induction media resulted in significant formation of oil red O-stained lipid droplets compared with control media (n = fields of view across two wells; ***, $p < .0001$). The proportion of cells containing stained lipid droplets was influenced by the length of exposure to the adipogenic induction media, with a significantly greater percentage of cells exhibiting staining following a long period of induction than after a shorter induction ($p < .0001$). Inset: two cells containing stained lipid droplets. (**F**): Osteogenic induction resulted in a significant increase in the formation of von Kossa-stained mineral deposits (n = fields of view across two wells; ***, $p < .0001$). Inset: darkly stained mineral deposits in the osteogenesis assay. Abbreviation: BrdU, 5-bromo-2′-deoxyuridine.

Auditory function was assessed by measuring ABR thresholds to click and pure tone stimuli, as described previously [27, 28]. One cohort received a unilateral cochlear injection of olfactory stem cells (Transplant; $n = 8$) and the other received a unilateral injection of the vehicle solution alone (Sham; $n = 13$). Hearing levels in the injected ears were assessed 4 weeks after surgery and compared cohorts with presurgery levels.

Preparation of Olfactory Stem Cells for Injection into the Cochlea

Olfactory stem cells were grown in flasks (Corning, Sigma) to 70%–80% confluency and then harvested using TrypLE Express (Invitrogen). The collected cells were rinsed in DMEM/F12 (Invitrogen), centrifuged for 5 minutes at 300g, and resuspended in PBS at 4,000–15,000 cells per μl.

Microsurgery for Stem Cell Injection into the Cochlea

A/J mice were immunosuppressed by subcutaneous injection of cyclosporin A (Sigma; 15 mg/kg body weight) dissolved in olive oil, 1 day before surgery and 2 days following the procedure. Cochleostomies were performed in the lateral wall of the left cochlea at the basal turn, as described previously [28]. Stem cell transplantations were performed using glass capillary needles (tip diameter 100 μm) attached to a Hamilton syringe. The capillary needles were inserted into the cochlea via the cochleostomy and 1–1.5 μl of stem cell suspension is injected over 1–2 minutes. The cochleostomy was then sealed with bone wax, with all surgeries completed in 30–40 minutes.

ABR Threshold Determination

ABRs were recorded immediately before stem cell transplantation or sham injection, and 4 weeks after these procedures. Acoustic stimuli were delivered to anesthetized mice via an electrostatic insert speaker (Tucker Davis Technologies, FL) fitted into the external ear canal. Clicks (1 millisecond duration) and pure tone pips (16 kHz; 1 millisecond rise/fall; 3 milliseconds duration) were delivered, and ABRs were recorded while sound intensity was reduced in 5 dB sound pressure level (SPL) steps beginning at 90 dB SPL. The recorded signal was obtained with a minimum of 512 averages. ABR (hearing) thresholds were determined by identifying the lowest sound intensity level at which the peak amplitude of the evoked ABR signal exceeded four times the standard deviation of the baseline noise (Fig. 2A; AxoGraph X, Sydney, Australia, www.axographx.com [27]).

Survival of Adult Human Olfactory Stem Cells Transplanted into the Cochlea

To investigate the survival of adult human olfactory stem cells following transplantation into the mouse cochlea, stem cells were labeled with the lipophilic dye Vybrant CM-DiI (Invitrogen) and injected into the cochleae of immunosuppressed A/J mice (P28–P37; $n = 10$) using the microsurgical approach described above. Mice were sacrificed either

immediately to determine the site(s) of transplantation, or 1–4 weeks after surgery to examine stem cell fate. Preparation of cells for transplantation included growth of adult human olfactory stem cells in flasks to 70%–80% confluency and harvesting using TrypLE Express. The collected cells were then rinsed in DMEM/F12, centrifuged (5 minutes at 300*g*), and incubated for 20 minutes at 37°C in Vybrant CM-DiI (5 μl/ml) diluted in PBS. Following rinses in cold PBS, cells were centrifuged (5 minutes at 300*g*) and resuspended in PBS at 10,000–12,000 cells per μl. To confirm that the dye labeling had no effect on cell survival, unlabeled stem cells were transplanted and tracked with Nissl staining.

Tissue Processing

Mice were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (20 mg/ml) and perfused through the left ventricle with 4% PFA. Transplanted cochleae were removed and a small hole was made at the cochlear apex. Tissues were then postfixed in 4% PFA for 24 hours at 4°C. For decalcification, cochleae were exposed to 10% EDTA (Sigma) for 48 hours at 4°C. Tissues were rinsed in PBS for 1 hour, cryoprotected in graded sucrose/PBS solutions to 30% sucrose (w/v), frozen in optimal cutting temperature (Sakura Finetek, Tokyo, Japan, www.sakura-finetek.com), sectioned along the modiolar axis at 7 μm on a cryostat, and mounted in Gelmount.

Microscopy and Image Processing

Specimens were viewed using a Zeiss Axioplan epifluorescence microscope equipped with Plan-Neofluar 10 × 0.30 numerical aperture (NA) and Plan-Neofluar 20 × 0.50 NA dry objective lenses and an AxioCam MRm digital camera (Zeiss, Göttingen, Germany, www.zeiss.de). Images were processed to adjust brightness and contrast using Adobe Photoshop 8.0 (Adobe Systems).

Table 1. Hearing threshold levels (dB SPL) to click stimuli presurgery and postsurgery

Cohort	Presurgery	Postsurgery	Difference
Transplant	60	75	15
	50	70	20
	85	80	−5
	75	85	10
	85	90	5
	60	70	10
	55	75	20
Mean ± SEM		79 ± 3**	13 ± 4**
Sham	55	90	35
	45	90	45
	70	90	20
	35	85	45
	65	85	20
	60	75	15
	50	75	25
	60	80	20
	50	85	35
	70	90	20
	55	90	35
	80	90	10
	80	85	5
Mean ± SEM		85 ± 2**	25 ± 4**

**, $p < .01$; Transplant vs. Sham.

Table 2. Hearing threshold levels (dB SPL) to tone stimuli presurgery and postsurgery

Cohort	Presurgery	Postsurgery	Difference
Transplant	80	80	0
	65	80	15
	75	90	15
	80	90	10
	70	70	0
	75	70	−5
	70	75	5
	65	60	−5
Mean ± SEM		78 ± 3*	5 ± 2
Sham	75	85	10
	75	85	10
	80	85	5
	70	85	15
	70	75	5
	75	90	15
	75	75	0
	70	90	20
	80	85	5
	80	90	10
	80	85	5
Mean ± SEM		85 ± 2*	9 ± 2

*, $p < .05$; Transplant vs. Sham.

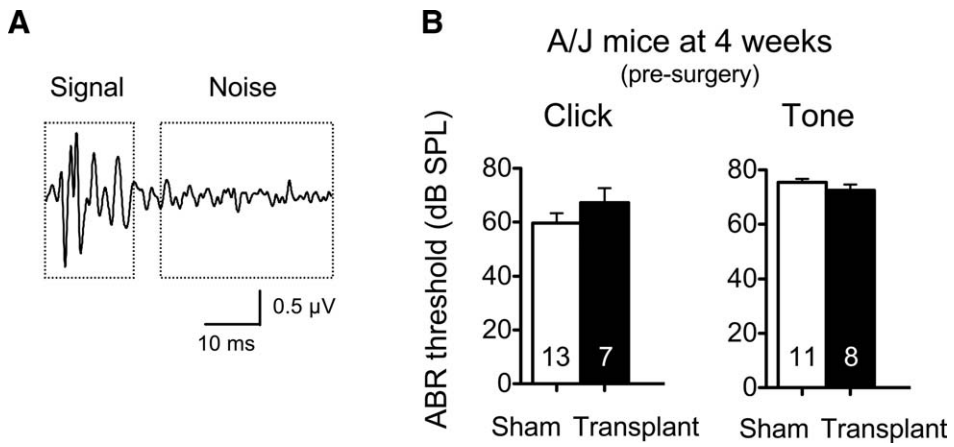

Figure 2. Hearing loss is similar for Sham and Transplant cohorts. (**A**): Hearing thresholds were calculated using the automated threshold detection method, which calculates the sound level at which the mean evoked auditory brainstem response (ABR) signal exceeds four times the standard deviation of the baseline noise (boxed areas). (**B**): Mean ABR threshold levels to click and pure tone (16 kHz) stimuli did not differ between the Sham and Transplant cohorts before surgery, indicating comparable levels of progressive hearing loss in the two groups. Number of animals is indicated in the bar. Mean ± SEM. Abbreviation: ABR, auditory brainstem response.

Statistics

Statistics are quoted as mean ± SEM. Significant differences in mean threshold values were determined using the D'Agostino and Pearson omnibus normality test with a one sample *t* test against a hypothetical value of 0 for Gaussian distributed data (comparison of short and long adipogenesis protocols vs. Control; Fig. 1E), an unpaired two-tailed Student's *t* test (comparison of short vs. long adipogenesis protocols; Fig. 1E), a Wilcoxon signed-rank test against a hypothetical value of 0 for non-Gaussian distributed data (comparison of osteogenesis assay vs. Control; Fig. 1F), or unpaired one-tailed and two-tailed Student's *t* tests (comparison of ABR thresholds for Transplant vs. Sham cohorts; Fig. 3B, 3C; Tables 1, 2). All statistical analyses were performed using Prism 5.0a (GraphPad, La Jolla, CA, www.graphpad.com).

All procedures were approved by the Garvan Institute of Medical Research/St Vincent's Hospital Animal Ethics Committee and conducted according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004) of the National Health and Medical Research Council of Australia. Every effort was taken to minimize discomfort to the animals.

Results

Transplanted Adult Human Olfactory Stem Cells Are Mesenchymal Stem Cell–Like

Stem cells derived from the adult human olfactory mucosa are multipotent [20] and exhibit extensive proliferation in vitro (Fig. 1A). Consistent with recent findings [24, 29], cells of the stem cell line used in this study were found to express mesenchymal stem cell markers, CD44, Stro-1, and vimentin (Fig. 1B-1D; [30, 31]) and to possess the capacity to differentiate into mesodermal cell types (Fig. 1E, 1F). Undifferentiated olfactory stem cells also expressed immunoreactivity to the neuronal marker β-tubulin III (Fig. 1A), a characteristic of certain types of mesenchymal stem cells [32–34]. Together, these results are consistent with the recent classification of human olfactory stem cells as a subtype of mesenchymal stem cell [24] rather than previous classifications as a subtype of neural stem cell [22, 23].

Olfactory Stem Cell Transplantation Protects Against Loss of Auditory Function

To examine the effects of olfactory stem cell transplantation on early-onset hearing loss, A/J mice at P28–P37 (the age at which elevations in hearing thresholds are first apparent) were separated into two cohorts: Transplant and Sham. Comparable levels of hearing loss were present in the animals assigned to the two cohorts ($p > .05$; Fig. 2B), as assessed by ABR (hearing) thresholds to click and pure tone (16 kHz) stimuli. ABR threshold levels were comparable with those reported previously [9]. All mice were immunosuppressed before surgery. Mice within the Transplant cohort received a unilateral cochlear injection of olfactory stem cells, whereas those of the Sham cohort received a unilateral cochlear injection of the vehicle solution (PBS). Hearing threshold levels for the injected ears of the two cohorts were examined 4 weeks following transplantation/sham surgery.

Two separate comparisons were completed to assess the functional effects of olfactory stem cell transplantation. In the first comparison, hearing threshold levels in the injected ears of the Transplant and Sham cohorts at 4 weeks after surgery were compared (Fig. 3A, 3B; Tables 1, 2). This analysis revealed that for both click and 16 kHz pure tone stimuli, ABR threshold levels were significantly lower in mice of the Transplant cohort (click: 77.9 ± 2.9 dB SPL; 16 kHz pure tone: 76.9 ± 3.7 dB SPL) than those of Sham cohort (click: 85.4 ± 1.6 dB SPL, $p < .01$; 16 kHz pure tone: 84.6 ± 1.6 dB SPL, $p < .05$). Strikingly, four cases were observed in the Transplant cohort in which hearing thresholds to 16 kHz pure tone stimuli remained at or improved to below presurgery levels (Table 2). This

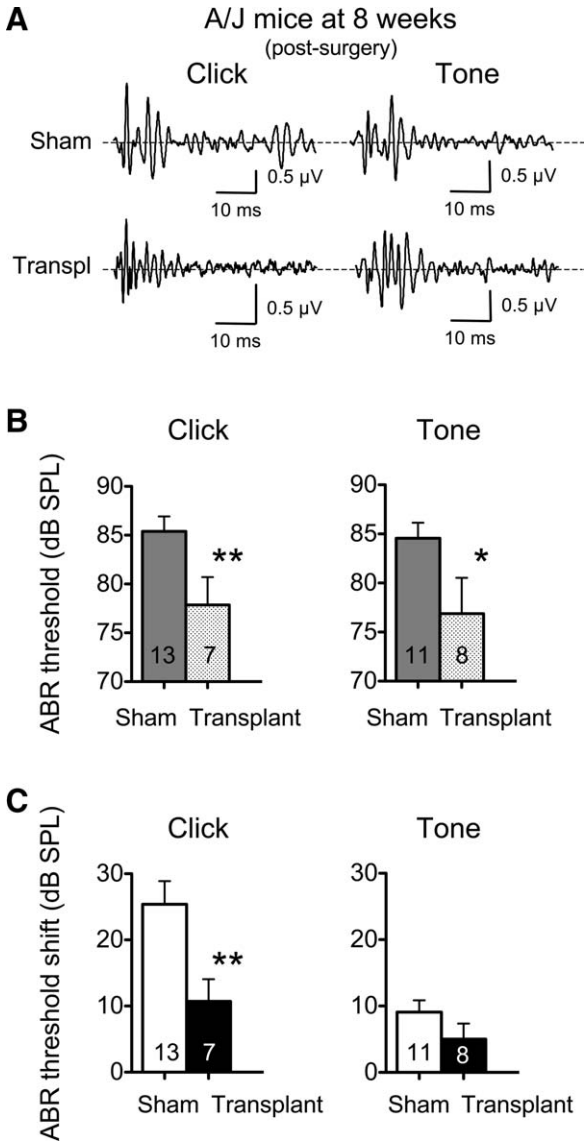

Figure 3. Transplanted olfactory stem cells reduce hearing loss. (**A**): Representative averaged auditory brainstem response (ABR) signals 4 weeks after surgery for Sham and Transplant cohorts in response to click and pure tone stimuli. (**B**) Postsurgery ABR threshold levels were significantly lower in mice of the Transplant cohort than those of Sham cohort for both click and pure tone stimuli. (**C**) The mean difference between predeafening and postsurgery ABR threshold levels (ABR threshold shift) to click stimuli was significantly less for the Transplant versus Sham cohort. No difference was observed between the two cohorts in the threshold shifts to pure tone stimuli. Number of animals is indicated in the bar. Mean ± SEM; *, $p < .05$, **, $p < .01$. Abbreviations: ABR, auditory brainstem response; SPL, sound pressure level.

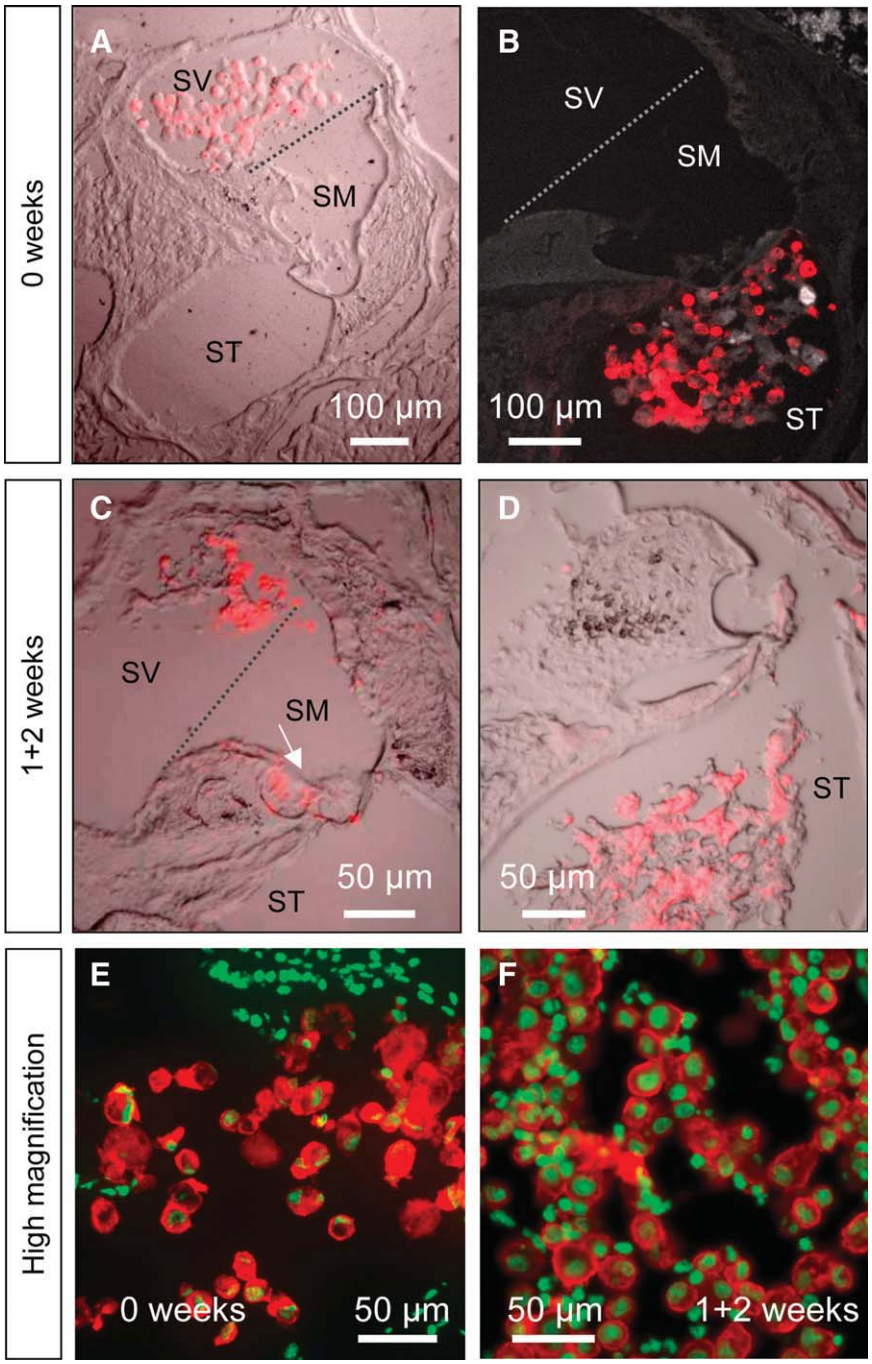

Figure 4. Transplanted olfactory stem cells survive for up to 2 weeks in cochlear compartments. (**A-F**): Human olfactory stem cells labeled with Vybrant CM-DiI are distributed primarily in clusters in the scala vestibuli and scala tympani immediately (**A, B, E**) and 1–2 weeks following transplantation (**C, D, F**). Images show low (**A–D**) and high (**E, F**) magnification photomicrographs of DiI-labeled transplanted cells (red) with 4′,6-diamidino-2-phenylindole–labeled nuclei (green; **E, F**). Abbreviations: SM, scala media; ST, scala tympani; SV, scala vestibule.

was observed only in one animal of the Sham cohort. No correlation was observed in the Transplant cohort between postsurgery hearing levels and either sex or the number of transplanted stem cells (data not shown).

In the second comparison, hearing threshold shifts in the injected ear were determined by calculating the difference between presurgery levels in this ear and the levels observed 4 weeks postsurgery (Tables 1, 2). Threshold shifts in the Transplant and Sham cohorts were then compared (Fig. 3C). This analysis revealed that the ABR threshold shift between presurgery and postsurgery levels in the injected ear of mice in the Transplant cohort (10.7 ± 3.4 dB SPL) was significantly less in mice of the Sham cohort (25.4 ± 3.5 dB SPL) in response to click stimuli ($p < .01$). For the 16 kHz pure tone stimuli, the ABR threshold shift between presurgery and postsurgery levels was similar for mice in the two cohorts (Transplant: 4.4 ± 2.9 dB SPL; Sham: 9.1 ± 1.8 dB SPL).

Olfactory Stem Cells Survive but Do Not Integrate into the Cochlea

To examine the fate of adult human olfactory stem cells on cochlear transplantation, cells were injected into the cochleae of immunosuppressed mice and tracked using Vybrant CM-DiI labeling. Examination of the distribution of olfactory stem cells within the cochlea immediately after transplantation showed that they occurred in clusters within both the scala vestibuli and scala tympani (Fig. 4). A comparable distribution of stem cells within the cochlea was also observed 1–2 weeks after the transplantation surgery (Fig. 4C, 4D). In addition, small numbers of cells were observed within the scala media of a limited number of animals (data not shown). Integration into metabolic (lateral wall) or sensory (organ of Corti and spiral ganglia) cochlear tissues was not observed. Evidence of tumor or teratoma formation was also not observed, consistent with previous transplantation studies with adult olfactory stem cells [23]. Together, these results indicate that the cochleostomy site used in this study delivers human olfactory stem cells primarily to the scala vestibuli and scala tympani of the mouse cochlea, and that these stem cells survive within the cochlea for at least 2 weeks following transplantation.

Discussion

A recent study has provided evidence that drug therapy can preserve auditory function in animal models of early-onset sensorineural hearing loss [35]. Here, we examine the effects of adult human olfactory stem cell transplantation on hearing levels in A/J mice exhibiting well-characterized early-onset sensorineural hearing loss. Multipotent olfactory stem cells were injected into the cochleae of A/J mice at P28–P37, the time point at which elevations in hearing thresholds in these mice are first apparent [9]. Cell tracking studies indicated that transplanted stem cells are distributed primarily within the scala vestibuli and scala tympani, and survive for at least 2 weeks following transplantation. Examination of ABR (hearing) thresholds 4 weeks after surgery demonstrated that hearing threshold levels in transplanted cochlea were significantly lower than in the cochleae of mice that received a sham injection of vehicle solution. Together, these results provide evidence of a beneficial effect of olfactory stem cell transplantation on hearing levels in A/J mice.

Recent studies of adult olfactory and oral mucosae reveal that the lamina propria within these tissues are a source of mesenchymal stem cells with extensive differentiation potential [24, 36–38]. The olfactory mucosa also contains neural stem cells, situated within the olfactory epithelium, maintaining a lifelong turnover of olfactory receptor neurons [39]. Our in vitro characterization of the olfactory stem cells used in this study suggests that these cells are mesenchymal stem cells. The cells are immunopositive for the mesenchymal stem cell markers CD44, Stro-1, and vimentin [30, 31] and are capable of differentiation into mesenchymal cell types (adipocytes and osteocytes). As Stro-1 is expressed exclusively in the lamina propria of the human olfactory mucosa [36], these cells appear to be similar to previously described populations of olfactory lamina propria-derived mesenchymal stem cells [24].

Transplanted mesenchymal stem cells have been shown to produce functional benefits in a wide variety of animal models of human disorders. These effects can be mediated by the functional integration of transplanted stem cells into host tissues and/or by the secretion of factors (e.g., growth factors and cytokines) influencing endogenous cells [40–42]. Our cell tracking studies indicated that transplanted olfactory stem cells survive for at least 2 weeks within the perilymphatic compartments of the A/J mouse cochlea, but did not appear to integrate into cochlear tissues. The extent of integration of mesenchymal stem cells into host tissues after cochlear transplantation has differed between studies. Although Sharif et al. [43] described integration of bone marrow-derived stromal cells into mouse cochlear tissues following transplantation via a lateral wall cochleostomy, Matsuoka et al. [44] reported no integration of mesenchymal stem cells following transplantation via a lateral wall cochleostomy but extensive integration after injection directly into cochlear tissues. Direct transplantation of stem cells into cochlea tissues has not yet been examined in mice because of technical difficulties relating to the size of the murine cochlea.

Conclusion

We examined the effects of transplantation of adult human olfactory stem cells on auditory function in a mouse model of early-onset sensorineural hearing loss. Examination of hearing thresholds showed that transplanted olfactory stem cells contribute to a reduction in hearing loss when compared with sham-treated animals. The transplanted stem cells survived for 2 weeks and did not integrate into cochlear tissues. The absence of integration of olfactory stem cells into cochlear tissues in this study suggests that paracrine signaling could mediate the observed amelioration of hearing impairment. This interpretation is consistent with the proposed paracrine effects of transplanted hematopoietic stem cells mediating preservation of hearing levels after transient ischemia via promotion of hair cell survival in gerbils [45]. As hair cell loss is a significant pathology accompanying progressive hearing loss in A/J mice [9], future studies will examine whether paracrine effects on hair cell survival may mediate the preservation of auditory function observed in this study.

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Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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