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Effect of epithelial stem cell transplantation on noise-induced hearing loss in adult mice

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ABSTRACT

Noise trauma in mammals can result in damage to multiple epithelial cochlear cell types, producing permanent hearing loss. Here we investigate whether epithelial stem cell transplantation can ameliorate noise-induced hearing loss in mice. Epithelial stem/progenitor cells isolated from adult mouse tongue displayed extensive proliferation in vitro as well as positive immunolabelling for the epithelial stem cell marker p63. To examine the functional effects of cochlear transplantation of these cells, mice were exposed to noise trauma and the cells were transplanted via a lateral wall cochleostomy 2 days post-trauma. Changes in auditory function were assessed by determining auditory brainstem response (ABR) threshold shifts 4 weeks after stem cell transplantation or sham surgery. Stem/progenitor cell transplantation resulted in a significantly reduced permanent ABR threshold shift for click stimuli compared to sham-injected mice, as corroborated using two distinct analyses. Cell fate analyses revealed stem/progenitor cell survival and integration into suprastrial regions of the spiral ligament. These results suggest that transplantation of adult epithelial stem/progenitor cells can attenuate the ototoxic effects of noise trauma in a mammalian model of noise-induced hearing loss.

Introduction

Hearing loss due to noise overexposure is one of the most common sensory disabilities in humans, particularly in industrialised countries. This debilitating disease significantly reduces quality of life by negatively impacting upon communication in social and professional settings. The ætiology of noise-induced hearing loss (NIHL) is multifactorial involving a complex interplay between environmental and genetic factors (Ohlemiller, 2008; Konings et al., 2009). Acute noise trauma results in mechanical damage, enhanced mitochondrial free radical formation, and reduced cochlear blood flow (Henderson et al., 2006; Le Prell et al., 2007). The sequelae of these changes are extensive and include necrosis, apoptosis and sublethal pathologies in tissues throughout the cochlea (McFadden et al., 2005; Ohlemiller, 2008). Stem cell transplantation is rapidly gaining interest as a potential therapy to prevent or reverse this cell loss and thereby provide a treatment for NIHL (Martinez-Monedero and Edge, 2007; Parker et al., 2007; Revoltella et al., 2008; Brigande and Heller, 2009).

The effects of noise trauma include both transient and persistent increases in hearing threshold levels (temporary and permanent threshold shifts, respectively) (Nordmann et al., 2000; White et al., 2009). Susceptibility to the permanent effects of noise exposure differs markedly between individuals in humans and animal models of NIHL with respect to both the extent of hearing loss and the cochlear tissues affected (human: Taylor et al., 1965; Pawelczyk et al., 2009; animal: Erway et al., 1996; Davis et al., 2001; Ohlemiller and Gagnon, 2007). The CBA/Ca inbred mouse strain family has proven to be an invaluable model for the study of the pathology and treatment of NIHL as their hearing levels remain stable with age (Hunter and Willott, 1987; Schone et al., 1991; Jimenez et al., 1999), thus eliminating conflicting contributions of presbycusis.

Several studies have identified the cellular targets of noise trauma in CBA/Ca mice. Depending upon the degree of trauma, these can include the cochlear lateral wall (fibrocytes of the spiral ligament, and marginal, intermediate and basal cells of the stria vascularis), the organ of Corti (hair cells and supporting cells), and the spiral limbus (Wang et al., 2002; Hirose and Liberman, 2003; Ohlemiller and Gagnon, 2007). As several of these cochlear cell types are epithelial in origin (e.g., hair cells, supporting cells, marginal cell layer of the stria vascularis), we postulated that epithelial stem/progenitor cell transplantation could possess the potential to ameliorate NIHL. Transplanted stem cells can repair tissues by replacing damaged

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cells or by secreting factors that enhance the survival and/or proliferation of endogenous cells (Bernardo et al., 2009; da Silva Meirelles et al., 2009; Lai et al., 2010).

We and others have demonstrated that the epithelium of the tongue represents an accessible and abundant source of adult stem and progenitor cells (Ookura et al., 2002; Luo et al., 2009; Okubo et al., 2009; Sullivan et al., 2010). 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 and Williams, 2005). Tissue homeostasis in adult epithelia is maintained by stem cells residing in the basal-most cell layer that give rise to progenitor cells which proliferate for a finite number of times generating several epithelial and taste bud cell types (Jones et al., 1995; Lavker and Sun, 2000; Fuchs and Horsley, 2008; Blanpain and Fuchs, 2009; Haegebarth and Clevers, 2009).

In the present study, we isolated adult stem/progenitor cells from CBA/CaH mouse tongue epithelium and characterised their proliferative capacity and phenotypes in vitro. Subsequently, we examined the efficacy of cochlear transplantation of these cells in reducing noise ototoxicity. Epithelial stem/progenitor cells were transplanted into the cochleae of CBA/CaH mice shortly after noise trauma (48 h) and hearing levels were then measured after 4 weeks. Survival and incorporation of the transplanted cells were also investigated by cell fate analyses. Together, the results of these studies provide evidence that epithelial stem/progenitor cell transplantation can engender a functional rescue of hearing in an animal model of NIHL.

Materials and methods

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 minimise discomfort to the animals.

Epithelial stem/progenitor cell isolation and culture

CBA/CaH mice (6 weeks; $n=5$) were anaesthetised with CO_2 and decapitated. The tongue was dissected free and injected with a solution of 2 mg/ml collagenase D (Roche). Following 90 min, the dorsal epithelium at the rear of the tongue (surrounding and including the circumvallate papilla) was peeled off the underlying muscle with fine forceps. This region was selected as the circumvallate papilla represents a readily identifiable landmark, thus enabling the same tissue to be isolated across animals. Tissues were minced with fine scissors and incubated in TrypLE Express (Invitrogen) containing 1 mg/ml collagenase D and 1 mg/ml hyaluronidase (Sigma) at 37 °C for 1 h. Dissociated cells were cultured in Advanced DMEM/F12 medium containing 20 mM glutamine, 10% fetal bovine serum, B-27 supplement minus vitamin A, 20 ng/ml EGF, 20 ng/ml bFGF, 100 U/ml penicillin G and 100 μg/ml streptomycin on plastic tissue culture dishes coated with rat-tail collagen (5 μg/cm^2; Roche) at 37 °C with 5% CO_2. Cells at passage 6 were used for transplantation experiments.

Immunocytochemistry

Cells were grown on glass coverslips coated with rat-tail collagen (5 μg/cm^2; Roche) and fixed at confluency for 10 min in methanol at −20 °C (for cytokeratin 8 and cytokeratin 14 immunolabelling) or 4% paraformaldehyde in 0.1 M phosphate-buffered saline pH 7.4 (PBS; for p63 immunolabelling) at 4 °C. Cells were then blocked for 1 h in 10% normal goat serum in PBS containing 0.3% Triton X-100 (NS-PBSTx). Primary antibodies were diluted in NS-PBSTx and applied for 2 h at room temperature. The following primary antibodies were used: monoclonal mouse anti-p63 (1:50; Santa Cruz Biotechnology; sc-8431), monoclonal rat anti-cytokeratin 8 (1:20; Developmental Studies Hybridoma Bank; TROMA1), monoclonal mouse anti-cytokeratin 14 (1:50; Chemicon; CBL197), monoclonal rat anti-5′-bromo-2′-deoxyuridine (BrdU; 1:250; AbD Serotec; MCA2060) and monoclonal mouse anti-BrdU (1:20; Developmental Studies Hybridoma Bank; G3G4). Cells were then rinsed for 4 h in several changes of PBS and incubated for 1 h at room temperature in the appropriate secondary antibodies diluted in PBS. Fluorescent secondary antibodies used were as follows: Alexa 488-conjugated goat anti-mouse IgG (1:100; Invitrogen; A-11029) and DyLight 649-conjugated goat anti-rat IgG (1:100; Jackson ImmunoResearch; 112-495-167). Sections were counterstained by incubation overnight at 4 °C in rhodamine-conjugated *Phaseolus vulgaris* leucoagglutinin (PHAL; 1:100; Vector Laboratories; RL-1112), fluorescein-conjugated Jacalin (1:100; Vector Laboratories; FL-1151), or the nuclear stain DAPI (0.3 μM; Invitrogen). In experiments examining mitotic activity, BrdU (Sigma) was added to the culture medium at a final concentration of 10 μM 2 h prior to fixation. The fixed cells were then incubated in 2 N HCl at room temperature for 20 min. Following rinsing in 0.1% Triton X-100 in PBS for 20 min, cells were processed for BrdU immunolabelling as described above.

Noise trauma and hearing threshold detection

CBA/CaH mice (male and female; 4–6 weeks; $n=11$) were deafened in both ears by noise overexposure (120 dB SPL, 1–80 kHz broadband noise, 2–2.5 h under general anaesthesia) in a foam-padded, shielded acoustic chamber. This strain of mice was selected to match the stem/progenitor cell donors and minimise immunorejection. Animals of this age were selected as vulnerability to noise exposure declines after 8 weeks of age in CBA/CaJ mice (Kujawa and Liberman, 2006).

Auditory function was assessed by measuring auditory brainstem response (ABR) thresholds to click and pure tone stimuli, as described previously (Bogaerts et al., 2008). Briefly, acoustic stimuli were delivered to anaesthetised mice via an electrostatic insert speaker (Tucker Davis Technologies) fitted into the external ear canal. Clicks and pure tone bursts (20 kHz) were delivered and ABRs were recorded while sound intensity was reduced in 5 dB SPL steps beginning at 90 dB SPL. ABR 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 (Bogaerts et al., 2009).

To assess the extent of NIHL, permanent ABR threshold shifts were determined by comparing the pre-trauma threshold levels in the operated (left) ear to threshold levels in the non-operated (right) ear 30 days post-trauma (Miller et al., 1963). Animals that did not display a permanent threshold shift (i.e., shift<10 dB SPL) were excluded from study.

Stem/progenitor cell transplantation

Prior to transplantation, isolated adult epithelial stem/progenitor cells were grown in flasks (Corning) to 70–80% confluency and then harvested using TrypLE Express. The collected cells were rinsed in DMEM/F12 (Invitrogen), centrifuged for 5 min at 300 × *g*, resuspended in PBS at 2000–4000 cells/μl, and stored on ice until transplanted.

To investigate the functional effects of stem/progenitor cell transplantation, mice with equivalent hearing levels at 2 days post-trauma were divided into two cohorts: Transplant and Sham. The Transplant cohort received a unilateral cochlear injection of epithelial stem/progenitor cells ($n=7$) and the Sham cohort received a unilateral injection of the vehicle solution alone ($n=4$). Cochleostomies were performed in the lateral wall of the left cochlea at the basal turn, posterior to the stapedial artery and in line with the round window as described previously (Bogaerts et al., 2008). This cochleostomy site corresponds to the 51.4 ± 2.8 kHz ($n=3$) region

of the mouse cochlea according to the place-frequency map of Müller et al. (2005). It is important to note, however, that the place-frequency map of the mouse cochlea can shift by up to one octave following noise damage (Müller and Smolders, 2005). This cochleostomy site has been shown to deliver transplanted cells primarily to the two perilymphatic compartments, scala vestibuli and scala tympani (Bogaerts et al., 2008). For stem/progenitor cell transplantations, 1 µl of cells suspended in PBS was injected over 1 min to transplant 2000–4000 cells. The cochleostomy was then sealed with bone wax, with all surgeries completed in 30–40 min.

Cell fate analyses of transplanted adult epithelial stem/progenitor cells

To investigate the fate of transplanted cells in the mouse cochlea, stem/progenitor cells (prepared as above) were labelled with the lipophilic dye Vybrant CM-DiI (5 µl/ml; Invitrogen) and injected into the cochleae of CBA/CaH mice 4–5 weeks old (10,000 cells/µl; $n=6$) using the microsurgical approach described above. Mice were sacrificed 1–4 weeks after surgery by perfusion through the left ventricle with 4% paraformaldehyde. Transplanted cochleae were removed and postfixed in the same fixative for 24 h at 4 º C. For decalcification, cochleae were exposed to 10% EDTA (Sigma) for 48 h at 4 °C. Tissues were rinsed in PBS for 1 h, cryoprotected in graded sucrose/PBS solutions to 30% sucrose (w/v), frozen in OCT (Sakura Finetek), sectioned along the modiolar axis at 7 µm on a cryostat, counterstained with NeuroTrace 500/525 green fluorescent Nissl stain (1:50; Invitrogen), and mounted in Gelmount. Sections containing transplanted cells were then immunolabelled for Na^+/K^+-ATPase expression using a rabbit monoclonal anti-Na^+/K^+-ATPase α antibody (1:100; Epitomics; 2047-1) and standard immunohistochemical methods (Sullivan et al., 2010).

To examine the distribution of transplanted cells along the cochlear axis, Vybrant CM-DiI-labelled cells were injected into the cochleae of CBA/CaH mice (4 weeks of age; 4000 cells; $n=5$) 2 days post-noise trauma. Mice were sacrificed 5 days after surgery by perfusion through the left ventricle with 4% paraformaldehyde. Transplanted cochleae were postfixed in the same fixative for 24 h at 4 º C and decalcified by exposure to 10% EDTA for 48 h at 4 °C. Surface preparations of the cochlear spiral were prepared from the apex to the base and the frequency locations of transplanted cells were determined using the place-frequency map of Müller et al. (2005).

Microscopy and image processing

Specimens were viewed using a Zeiss Axioplan epifluorescence microscope equipped with Plan-Neofluar 10 × 0.30 NA and Plan-Neofluar 20 × 0.50 NA dry objective lenses and an AxioCam MRm

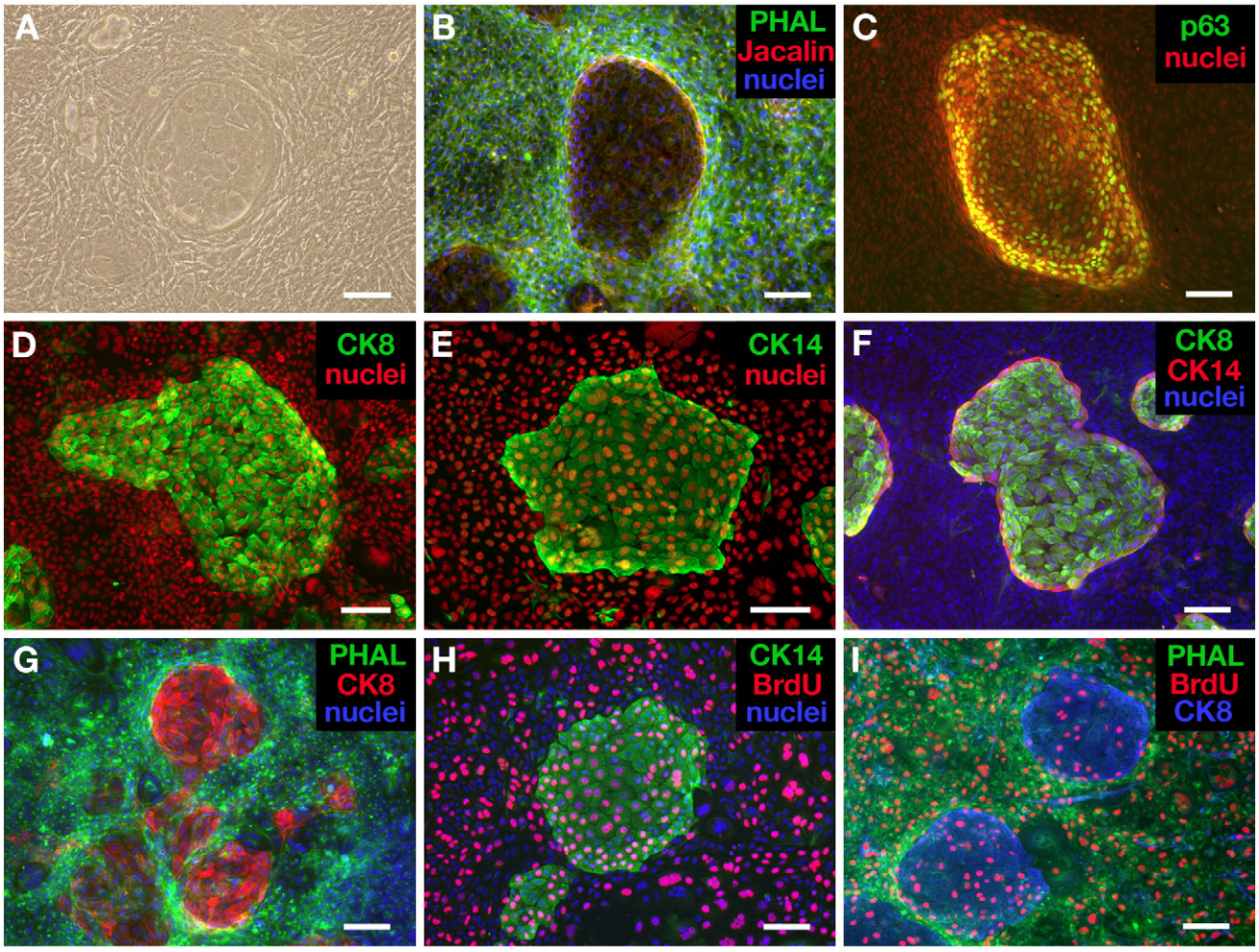

Fig. 1. Characterisation of tongue epithelial stem/progenitor cells in vitro. A. Light micrograph showing detail of a colony generated by cells isolated from the dorsal tongue epithelium. These colonies are comprised of small, densely packed cells surrounding islands of squamous cells. A representative island can be seen in the centre of the image. B. The small, densely packed cells within the colonies bind the lectins PHAL and Jacalin. C–G. Cells comprising the squamous islands express the epithelial stem cell marker p63 (C), and the epithelial markers CK8 (D, F, and G) and CK14 (E and F). H and I. Immunolabelling for BrdU incorporation showing that mitotic activity is distributed throughout the colonies, including both the populations of lectin-binding cells and the islands of squamous cells. Abbreviations: CK8, cytokeratin 8; CK14, cytokeratin 14; PHAL, *Phaseolus vulgaris* leucoagglutinin. Scale bars = 100 µm.

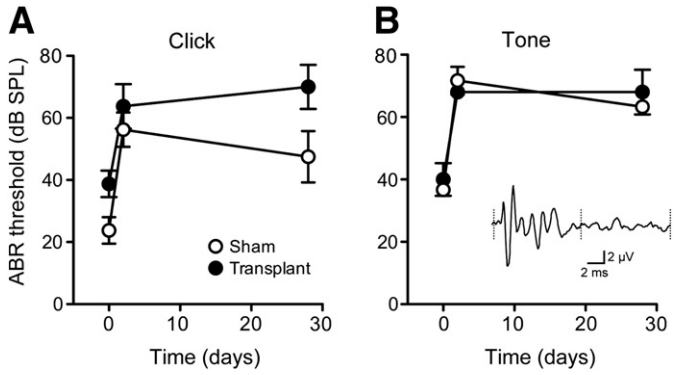

Fig. 2. Transplant and Sham cohorts exhibit similar levels of NIHL. A and B. Noise trauma caused significant increases in mean ABR threshold levels ($P<0.05$) in the Transplant and Sham cohorts for click (A) and pure tone (B; 20 kHz) stimuli 2 days and 30 days (permanent threshold shift) post-trauma. Mean ABR threshold shifts did not differ between the Transplant and Sham cohorts, indicating comparable levels of deafening in the two groups. Inset: Representative averaged ABR signal prior to noise trauma in response to click stimuli. Mean ± SEM.

digital camera (Zeiss). Images were processed to adjust brightness and contrast using Adobe Photoshop 8.0 (Adobe Systems).

Statistical analysis

Statistics are quoted as mean ± standard error of the mean (SEM). Significant differences in mean threshold values were determined using the non-parametric Mann–Whitney one-tailed test (due to sample size<12) for comparison of ABR thresholds before and after noise trauma (Fig. 2), and for comparison of ABR thresholds in Transplant versus Sham cohorts (Figs. 3 and 4). All statistical analyses were performed using Prism 5.0a (GraphPad).

Results

Tongue epithelium as a source of adult stem/progenitor cells

Isolated cells from the posterior tongue epithelium gave rise to a rapidly growing colony after 7 days in vitro (Fig. 1), capable of propagating into additional colonies upon serial passage ($n=12$ passages). These colonies were comprised of small, densely packed cells whose plasma membranes bound the lectins Jacalin and PHAL (Fig. 1B), which label rodent tongue epithelial cells of the basal and granular layers in vivo (Wakisaka, 2005). Squamous cells expressing the epithelial stem cell marker p63 (Fig. 1C; Senoo et al., 2007), and the epithelial markers cytokeratin 8 and 14 (Hudson, 2002) were observed in islands within the colonies (Fig. 1D–G). Mitotic activity was extensive throughout the colonies, including both the lectin-binding cells and the islands of squamous epithelial cells, as shown by cell proliferation assays using the thymidine analogue BrdU (Fig. 1H and I). The extensive proliferative potential of these colonies is characteristic of holoclones, stem cell derived keratinocyte colonies (Barrandon and Green, 1987; Pellegrini et al., 2001). Similarly, previous studies have reported the isolation of holoclone-forming cells from the anterior portion of the adult mouse tongue epithelium (Luo et al., 2009).

Transplant and sham cohorts exhibit similar levels of NIHL

Animals were exposed to noise trauma and separated into two cohorts: Transplant and Sham. To assess the extent of NIHL, ABR threshold levels were tested 30 days post-trauma and compared to pre-trauma levels. Previous studies indicate that noise-induced threshold shifts reach permanent levels 2–4 weeks after exposure (Miller et al., 1963). For both Transplant and Sham cohorts, ABR threshold levels for click and pure tone stimuli were significantly increased at 2 days post-trauma and at 30 days post-trauma in the non-operated ear (permanent threshold shift) compared to pre-trauma levels (Fig. 2). Mean ABR threshold shifts did not differ between the Transplant and Sham cohorts at either time point, indicating comparable levels of NIHL were present in animals assigned to the two cohorts ($P>0.05$; Fig. 2). Permanent threshold shifts of 31 ± 8 and 28 ± 6 dB SPL were observed in the Transplant cohort to click and pure tone (20 kHz) stimuli, respectively, while threshold shifts of 24 ± 5 and 27 ± 3 dB SPL were present in the Sham cohort. Consistent with previous studies examining noise-induced threshold shifts of less than 40 dB SPL (Liberman and Beil, 1979; Kujawa and Liberman, 2006), significant hair cell loss was not observed at 30 days post-trauma (data not shown).

Transplantation of epithelial stem/progenitor cells attenuates NIHL

To examine the effects of epithelial stem/progenitor cell transplantation on NIHL, mice within the Transplant cohort received a unilateral cochlear injection of epithelial stem/progenitor cells, while those of the Sham cohort received a unilateral injection of the vehicle solution. Surgeries were performed 2 days post-trauma, the time point providing maximal integration of transplanted stem/progenitor cells following noise trauma (Parker et al., 2007). Transplanted cells occurred primarily within the region of the cochlea spanning 8.5 ± 1.8 to 14.0 ± 1.2 kHz ($n=5$), according to the place-frequency map of

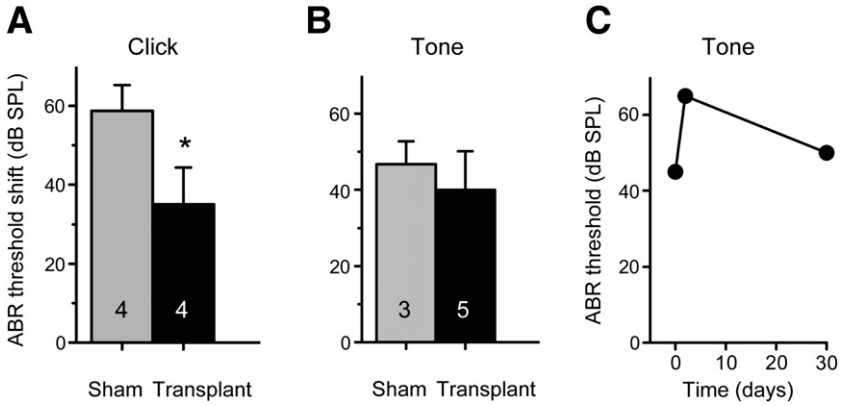

Fig. 3. Transplanted epithelial stem/progenitor cells attenuate NIHL (Analysis 1). A and B. The mean difference between pre-trauma and 28 day post-surgery ABR threshold levels (ABR threshold shift) to click stimuli (A) in the operated (left) ear was significantly less for mice transplanted with stem/progenitor cells (Transplant) than for sham-injected mice (Sham). No difference was observed between the two cohorts in the threshold shifts to pure tone (20 kHz) stimuli (B). Number of animals indicated in each bar. Mean ± SEM; * $P<0.05$. C. Return to approximate pre-trauma levels after stem cell transplantation in an animal's response to pure tone stimuli.

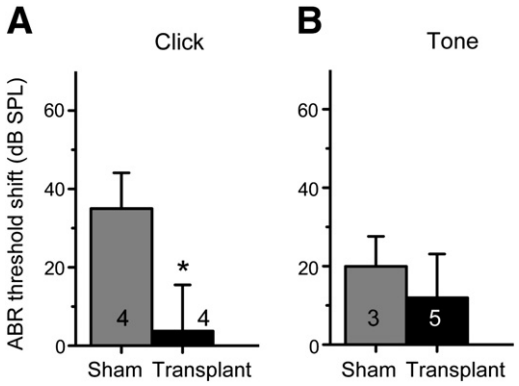

Fig. 4. Transplanted epithelial stem/progenitor cells attenuate NIHL (Analysis 2). A and B. The mean difference between the post-surgery ABR threshold levels of the operated (left) and non-operated (right) ears in the Transplant cohort was significantly less than in the Sham cohort for click stimuli (A). Differences were not observed in the threshold shifts to pure tone (20 kHz) stimuli (B). Number of animals indicated in each bar. Mean ± SEM; * $P<0.05$.

Müller et al. (2005), though small numbers of cells were distributed sparsely along the remainder of the cochlea (data not shown).

Two distinct analyses were performed to assess the effects of epithelial stem/progenitor cell transplantation following noise trauma (Figs. 3 and 4). In Analysis 1, hearing threshold shifts were determined for the operated ear by comparing pre-trauma levels in this ear with the levels observed at 28 days post-surgery (30 days post-trauma; Fig. 3A). Threshold shifts in the Transplant and Sham cohorts were then compared. This analysis revealed that the ABR threshold shift between pre-trauma and post-surgery levels in the operated (left) ear of mice in the Transplant cohort was significantly less than in mice of the Sham cohort in response to click stimuli ($P<0.05$). For pure tone stimuli, the ABR threshold shift was similar in the two cohorts ($P>0.05$; Fig. 3B). Interestingly, one animal showed a return to approximate pre-trauma levels for pure tone stimuli following cell transplantation (Fig. 3C), an outcome not observed in the Sham cohort.

In Analysis 2, the non-operated (right) ear of each animal was used as an internal control and compared against the operated (left) ear. Differences between the hearing threshold levels of the two ears at 28 days post-surgery were compared between the Transplant and Sham cohorts (Fig. 4). This second analysis was consistent with Analysis 1 in that mice in the Transplant cohort again showed a significantly smaller ABR threshold shift for click stimuli ($P<0.05$) than those of the Sham cohort (Fig. 4A). For pure tone stimuli, the ABR threshold shift did not differ significantly between the two cohorts (Fig. 4B). No correlation was observed in the Transplant cohort between improvements in hearing levels and either sex or the number of transplanted stem/progenitor cells (data not shown).

Stem/progenitor cells survive and integrate into the cochlea

To examine the fate of adult epithelial stem/progenitor cells upon cochlear transplantation, cells were injected into the cochleae of mice and tracked using Vybrant CM-DiI labelling (Fig. 5). Incorporation of stem/progenitor cells was observed primarily into the suprastrial regions of the spiral ligament (Fig. 5A and B), Reissner's membrane (Fig. 5A), and the simple squamous epithelial lining of scala tympani 1–4 weeks after transplantation (Fig. 5A–C). These locations are consistent with the epithelial origins of the transplanted cells. Transplanted cells integrating into suprastrial regions expressed the enzyme Na^+/K^+-ATPase (Fig. 6), a protein abundantly expressed by superficial fibrocytes of the suprastrial region (Schulte and Adams, 1989; Spicer and Schulte, 1991; Furukawa et al., 1996; Xia et al., 1999). Immunolabelling for Na^+/K^+-ATPase was not observed in transplanted cells present in other regions of the cochlea (Fig. 6). Examination of the epithelial stem/progenitor cells in vitro indicated that cells of the squamous islands express Na^+/K^+-ATPase (data not shown). As only those transplanted cells integrating into suprastrial regions showed expression of this enzyme in vivo, the observed immunolabelling suggests either that cells of the squamous islands specifically integrate into the cochlear lateral wall or that expression was induced in lectin-binding cells following their integration.

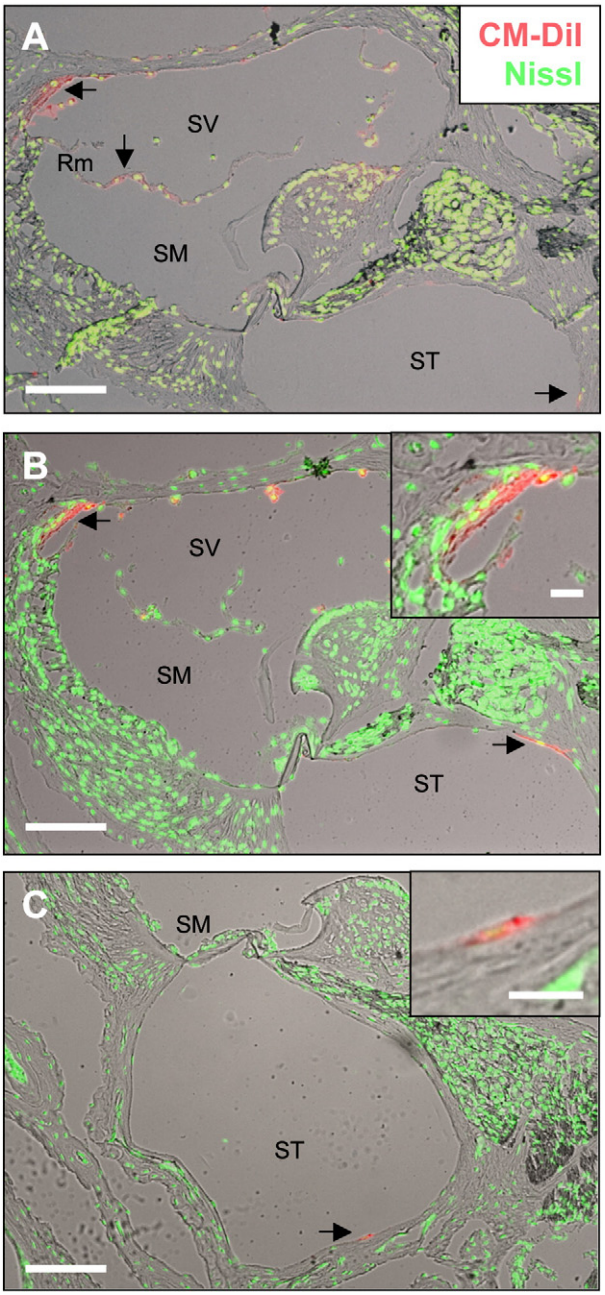

Fig. 5. Stem/progenitor cells survive and integrate into the cochlea. A and B. Overlaid fluorescence and light micrographs showing the locations of transplanted stem/progenitor cells (arrows) labelled with Vybrant CM-DiI (red) in the spiral ligament of scala vestibuli (SV), Reissner's membrane (Rm), and the squamous epithelial lining of scala tympani (ST) 1 week after transplantation. C. Transplanted cells are present 4 weeks post-surgery and incorporated into the epithelial lining of scala tympani. Preparations were counterstained with the nuclear dye NeuroTrace 500/525 (green). Insets: High magnification views showing incorporation of stem/progenitor cells into the spiral ligament (B) and into the simple squamous epithelial lining of the scala tympani (C). Abbreviation: SM, scala media. Scale bars = 10 μm (insets) and 50 μm.

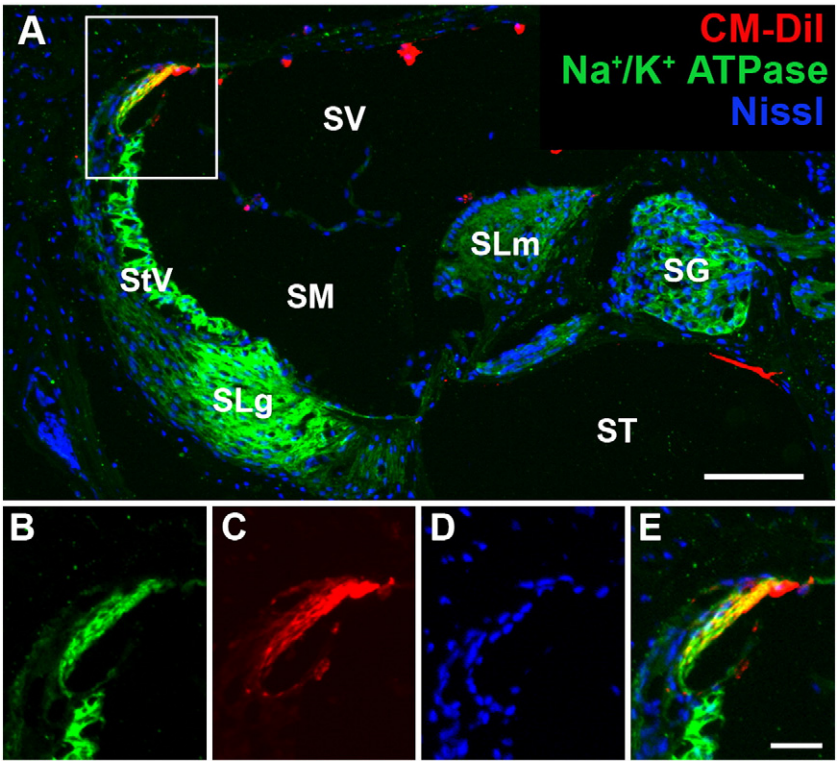

Fig. 6. Transplanted stem/progenitor cells express markers of endogenous cochlear cells. A. Transplanted stem/progenitor cells labelled with Vybrant CM-DiI (red) integrate into the lateral wall of the cochlea (square) and express the ion transport-mediating enzyme Na^+/K^+-ATPase (green), strongly expressed in cells of the spiral ligament (SLg) and stria vascularis (StV). Note that immunolabelling for Na^+/K^+-ATPase is not observed in transplanted cells present in other regions of the cochlea. Cell nuclei are labelled with a fluorescent Nissl stain (blue). B–E Higher magnification views of the region highlighted by the square in (A) showing labelling for Na^+/K^+-ATPase (B), Vybrant CM-DiI (C), cell nuclei (D), and a merged image of the three labels (E). Abbreviations: SG, spiral ganglion; SLg, spiral ligament; SLm, spiral limbus; SM, scala media; ST, scala tympani; StV, stria vascularis; SV, scala vestibuli. Scale bars = 100 μm in A; 25 μm in B–E.

Together, these results indicate that the cochleostomy site used in this study delivers epithelial stem/progenitor cells primarily to scala vestibuli and scala tympani of the mouse cochlea, and that these stem/progenitor cells survive within the cochlea for at least 4 weeks (the time period of the present study) following transplantation.

Discussion

Previous studies have tested for a functional rescue of hearing via stem cell transplantation in animal models of cochlear ischaemia and aminoglycoside exposure (Hakuba et al., 2005; Hildebrand et al., 2005; Yoshida et al., 2007). The present study is the first to examine the functional effect of stem cell transplantation on NIHL. As several of the cellular targets damaged by NIHL are of epithelial origin, we focussed our attention on the effects of epithelial stem cell transplantation. Our findings demonstrate, via two distinct analyses, that allotransplantation of epithelial stem/progenitor cells into adult mice following noise trauma 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.

The exposure of animals to noise levels capable of producing permanent cochlear damage leads to large thresholds shifts that recover exponentially to smaller stable shifts at 2–4 weeks after exposure (Miller et al., 1963). The permanent threshold shifts arise from mechanical, metabolic, and vascular changes that result in apoptotic and sublethal pathologies in the organ of Corti and lateral wall (McFadden et al., 2005; Ohlemiller, 2008). The nature and extent of these pathologies differ across frequencies in the cochlear tonotopic map (Hamernik et al., 1989; Ou et al., 2000; Lim et al., 2008). Our finding of an improvement in the ABR threshold shift for click but not pure tone stimuli (20 kHz) suggests that the observed effects of epithelial stem/progenitor cell transplantation occurred primarily outside the 20 kHz cochlear location (the mid-point of the cochlear axis in normal hearing mice; Viberg and Canlon, 2004). As the transplanted cells were located primarily within the portion of the cochlea corresponding to 8.5–14.0 kHz, effects in this region could mediate the observed improvement to click stimuli. Determination of the specific region/s involved, however, will be complicated by the shifts that occur in the place-frequency map of the mouse cochlea following noise damage (Müller and Smolders, 2005).

Transplanted stem cells can repair tissues by replacing damaged cells or by secreting bioactive factors that enhance the survival and/or proliferation of endogenous cells (Bernardo et al., 2009; da Silva Meirelles et al., 2009; Lai et al., 2010). Cell fate analyses in the present study show incorporation of transplanted cells into the spiral ligament, one of the principal sites of cochlear damage in NIHL. Constitutive fibrocyte turnover in the spiral ligament within the cochlear lateral wall is thought to be essential in maintaining normal cochlear function by regulating potassium recycling, and enhancement of this turnover may act as an endogenous cochlear repair mechanism (Lang et al., 2003; Yamasoba et al., 2003). Consistent with the importance of potassium recycling for cochlear function, several NIHL susceptibility genes in humans are linked to potassium homeostasis (Pawelczyk et al., 2009). As upregulation of local proliferation via paracrine signalling is commonly observed following stem cell transplantation (Mahmood et al., 2004; Munoz et al., 2005; Einstein et al., 2009; Madhavan et al., 2009), enhancement of fibrocyte turnover may represent one avenue by which the transplanted epithelial stem/progenitor cells can influence hearing levels. Additional mechanisms of intervention could include enhancement of cochlear blood flow or normalisation of mitochondrial free radical levels, which peak at 7–10 days following noise trauma (Ohlemiller et al., 1999; Yamashita et al., 2004), as transplanted adult stem cells

can upregulate angiogenesis (Kim et al., 2009; Zou et al., 2010) and efficiently scavenge reactive oxygen and nitrogen species (Valle-Prieto and Conget, 2010). As the transplanted cells integrating into suprastrial regions of the spiral ligament expressed the ion transport-mediating enzyme Na^+/K^+-ATPase, these cells could potentially also contribute directly to the maintenance of cochlear fluid homeostasis (Kikuchi et al., 2000).

Our cell fate studies indicated that transplanted epithelial stem/progenitor cells survive for at least 4 weeks within the cochlea and incorporate within tissues lining the perilymphatic compartments. Interestingly, integration of neural stem cells injected into noise-deafened mice was not observed in these tissues, but instead within the spiral ganglion, spiral limbus, and organ of Corti (Parker et al., 2007). Together these results suggest that cochlear integration sites may be stem cell type specific. Stem cell therapies combining diverse stem cell types may therefore enable intervention at multiple sites within the cochlea and provide additive beneficial effects on NIHL. These studies are currently underway.

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