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Survival and neural differentiation of adult neural stem cells transplanted into the mature inner ear☆

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Abstract

The cochlear sensory epithelium and spiral ganglion neurons (SGNs) in the adult mammalian inner ear do not regenerate following severe injury. To replace the degenerated SGNs, neural stem cell (NSC) is an attractive alternative for substitution cell therapy. In this study, adult mouse NSCs were transplanted into normal and deafened inner ears of guinea pigs. To more efficiently drive the implanted cells into a neuronal fate, NSCs were also transduced with neurogenin 2 (*ngn2*) before transplantation. In deafened inner ears and in animals transplanted with *ngn2*-transduced NSCs, surviving cells expressed the neuronal marker neural class III β-tubulin. Transplanted cells were found close to the sensory epithelium and adjacent to the SGNs and their peripheral processes. The results illustrate that adult NSCs can survive and differentiate in the injured inner ear. It also demonstrates the feasibility of gene transfer to generate specific progeny for cell replacement therapy in the inner ear.

Keywords: Gene transfer; Neural stem cell; Neurogenin; Organ of Corti; Spiral ganglion; Transplantation

Introduction

Hearing impairment is a common disability. The most frequent cause is a degeneration of the sensory epithelium (the organ of Corti) within the auditory part of the inner ear, the cochlea. There is evidence that the cochlea in lower vertebrates and avian possesses a self-repair mechanism that can be activated following damage to the sensory epithelium [1–3]. However, in mammals, the cochlear sensory epithelium and neural components do not regenerate, and there is currently no effective intervention for their repair. Moreover, in the auditory system, as in other afferent systems, degeneration of the spiral ganglion neurons (SGNs) and auditory nerve occurs secondary to the loss of the cochlear sensory epithelium, thus aggravating the functional impairment and reducing the possibilities for rehabilitation [4–7]. To replace missing or severely impaired neurons or sensory cells, novel approaches are required.

Theoretically, there are several possible methods to regenerate or replace the cells within the mammalian cochlea. Generation of new sensory cells or neurons either by activating cochlear progenitor cells or stem cells or by conversion of supporting cells would be an exciting approach [8–11]. However, in cases where hair cells and spiral ganglion neurons are severely degenerated or absent, a cell replacement therapy based on tissue implantation may offer an interesting and more immediate alternative. The transplanted cells would be expected not only to take the position of missing cochlear cells, but also to become fully integrated with the auditory system both structurally and functionally. The choice of cells for transplantation is a key issue, and there are several candidates for cell therapy. One

☆ Adult neural stem cell in the mature inner ear.

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option is using embryonic neuronal tissue. Transplantation of embryonic dorsal root ganglion cells into the adult inner ear has demonstrated that neurons survive quite well in the cochlea and, more importantly, also migrate into the spiral ganglion region and along the peripheral processes projecting to the organ of Corti [12]. Another exciting alternative for cell therapy is to use stem cells. Embryonic stem (ES) cells are pluripotent and can differentiate into a variety of cell types [13,14]. One challenge of stem cell transplantation into the inner ear will be to promote the implanted cells to a cochlear or sensorineural lineage, that is, cochlear sensory cells and spiral ganglion neurons. Using neural stem cells (NSCs) rather than ES cells would possibly facilitate differentiation into cells of functional interest for the auditory system. NSCs have been reported to possess the capacity to self-renew and differentiate into the major cell types of the neural tissue, that is, neurons, astrocytes, and oligodendrocytes [15,16]. It has been documented that adult NSCs can generate olfactory bulb neurons and that, following injury, their proliferation rate increases and their progeny migrates to the lesion site where they differentiate into astrocytes [17]. Furthermore, NSCs have been tested in the treatment of several severe nervous system disorders, that is, Parkinson disease, Huntington disease, Alzheimer disease, and spinal cord injuries in animal models [18–22]. Following transplantation into the damaged retina, neuronal differentiation and morphological integration of adult neural progenitor cells have also been demonstrated [23–25]. These results suggest the potential of adult NSCs for differentiation into neural cell types within the injured mature neural system. Neural stem cells preferentially differentiate to astrocytes both in vitro and upon transplantation, and few neurons are made. However, several molecular pathways instructing neuronal differentiation during development have been characterized, raising the possibility to affect neurogenesis from NSC. Consequently, transducing the stem cells before implantation with a gene promoting differentiation into a neuronal fate could prove beneficial for neuronal replacement. For example, it has been reported that neurogenin 2 (*ngn2*) is required for the generation of progenitors of cranial sensory ganglia [26,27]. Other observations provide evidence for a complex crosstalk between pathways of neurogenesis and dorsoventral patterning in the nervous system and place *ngn2* in a central position as a regulator of genetic pathways that identity and specify neuronal fates [28]. It has previously been shown that transducing NSCs with *ngn2* results in around 95% of the transduced cells differentiating into a neuronal fate in vitro [29], lending support to this strategy.

In this study, adult mouse NSCs were transplanted into the mature guinea pig inner ears to investigate their survival and differentiation, with an emphasis on the potential replacement of auditory neurons. Both normal-hearing animals and animals chemically deafened before implantation were used. To efficiently derive a homogenous population of a specific neuronal type from the NSCs, the cells were transduced with *ngn2* before inner ear transplantation.

Materials and methods

Implantation of adult NSCs expressing *Lac*Z or enhanced green fluorescence protein (GFP) was made in a total of 60 adult pigmented guinea pigs (body weight 300–400 g). The care and use of animals and all experimental procedures were approved by the regional ethical committee (approval nos. 283a–d/02 and 464/03).

Three different strategies for exploring the potential of NSC implantation were tested in the present study. First, the survival of adult NSC was investigated in the mature and intact cochlea. Thirty-six normal-hearing animals were implanted with NSCs and sacrificed after 1 week (GFP NCSs, $n = 4$), 2 weeks (GFP NCSs, $n = 16$; *Lac*Z NCSs, $n = 4$), 3 weeks (GFP NCSs, $n = 4$), and 4 weeks (GFP NCSs, $n = 5$; *Lac*Z NCSs, $n = 3$). Subsequently, to test whether survival and differentiation of NSC would be greater in the injured cochlea, implantation was also made in 12 chemically deafened guinea pigs, sacrificed after 2 weeks (GFP NCSs, $n = 6$) and 4 weeks (GFP NSCs, $n = 6$). Finally, with the aim of driving the NSCs to a neuronal fate in the mature inner ear, NSCs were transduced with *ngn2* and GFP before the transplantation into 12 normal-hearing guinea pigs (2 weeks' survival, $n = 6$; 4 weeks' survival, $n = 6$).

Adult NSCs preparation

Neurospheres were generated as previously described [17]. Briefly, following anesthesia (CO_2) and decapitation of the adult donor mice (2 months old; ROSA26 mice for *Lac*Z; [30]; GFP mice; [31]), the lateral wall of lateral ventricle was taken out and enzymatically dissociated in 0.7 mg/ml hyaluronic acid, 1× trypsin-EDTA (Life Technology), and 200 U/ml DNaseI (Roche). Following the first triturating, the thin brain tissue layer was incubated for 10 min at 37°C before a second triturating. The dissociated cells were kept at 37°C for another 10 min and mixed with the same volume of ice-cold 4% BSA solution (Sigma). The cells were further triturated, filtered with a 40-μm strainer (Falcon) and centrifuged at 1000 rpm for 5 min. The supernatant was removed; the cell pellet was resuspended in a culture dish containing neurosphere medium (DMEM/F12, B27) supplemented with EGF (20 ng/μl) and bFGF (20 ng/ml). Finally, the culture dish was kept in incubator at 37°C with 5% CO_2 and 3% O_2 in a humid environment. At day 6, neurospheres could be identified, harvested, and kept at 4°C for transplantation at a cell density of 10^4/μl.

Viral transduction of NSCs

Production of vesicular stomatitis virus G-protein (VSV-G) pseudotyped retroviral particles was performed as

previously described [29] with the exception that virus packaging cells were grown in serum-free neurospheres media supplemented with only B27, and ultracentrifugation of retroviral particles was omitted. Two previously described retroviral constructs were used [29]. Briefly, one construct encoded *myc*-tagged *ngn2* followed by an internal ribosomal entry site and GFP. The control construct was identical, except that it did not include the *ngn2* gene. By delivering retroviral particles to cell media, the retroviral genome was transduced into the cells and inserted in the cell genome, encoding both *ngn2* and GFP (*ngn2*-transduced cells). Primary NSCs were cultured as described above using C57/BL 6J mice (B&K, Sweden). Primary neurospheres were passaged after 1 week, and secondary neurospheres were infected 24 h after passage at a multiplicity of infection of 3.5. The efficiency of the transduction protocol is approximately 60% [29]. Cells were harvested after another 48 h and resuspended in DMEM-F12 to the same cell density as above for transplantation.

Procedure to damage inner ear cells

To destroy the sensory epithelium, anesthetized (ketamine, 4 mg/100 g body weight, and xylazine, 1 mg/100 g body weight, i.m.) guinea pigs were deafened by an injection of 10% neomycin through the tympanic membrane [12]. The middle ear cavity was filled with the neomycin solution, and the head of the animal was kept for 15 min in the appropriate position for the drug to diffuse via the round window into the cochlea. This procedure generally causes severe loss of the sensory cells and a subsequent and progressive loss of spiral ganglion neurons. Neomycin treatment was performed 2 days before implantation.

Adult NSC transplantation

The transplantation procedures, illustrated in Fig. 1, have been described before [12]. Briefly, under deep anesthesia (ketamine, 4 mg/100 g body weight, and xylazine, 1 mg/100 g body weight, i.m.), the left postauricular region of the animal was shaved and sterilized with 70% ethanol. The animal was then placed on a heating pad (37°C), and using a postauricular approach, the left bulla was exposed and opened to visualize the basal cochlea. A small hole was made into the scala tympani at the basal cochlear turn. A volume of 10 μl culture medium containing NSCs (10^4/μl) was infused into the scala tympani using a microsyringe (Exmire microsyringe, Ito corporation) at the speed of 5 μl/min. The needle of the microsynringe was kept in the inner ear for several minutes to prevent the NSCs and the culture medium from flowing out. A small piece of fascia was then placed over the cochleostomy, and the incision was approximated with sutures.

Histology

Following an overdose of pentobarbital (i.p.), the animals were transcardially perfused with 0.9% saline at 37°C followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C. The cochleas were excised, trimmed, and kept in the fixative before being transferred to 0.1 M phosphate buffer, in which they were stored until further processing.

X-gal staining

An X-gal-based histochemical method was used to detect β-galactosidase activity in the cochleas transplanted with adult NSCs expressing *Lac*Z as described before [12]. Briefly, following preincubation for 2 h at room temperature in 2 mM $MgCl_2$, 0.02% Nonidet P40 substitute (Fluka), 0.01% sodium deoxycholate, and 0.1 M PBS, the cochleas were incubated at 37°C for 8 h in a reaction mixture solution containing 1 mg/ml X-gal (5-bromo-4-chloro-3-indolyl beta-D-galactopyranoside, Sigma), 2 mM $MgCl_2$, 20 mM potassium ferrocyanide [$K_4Fe(CN)_6$, Sigma], 20 mM potassium ferricyanide [$K_3Fe(CN)_6$, Sigma], 150 mM NaCl, and 0.1 M PBS, pH 7.4. X-gal was dissolved in N, *N*-dimethylformamide (Sigma) as a stock solution of 100 mg/ml. Following staining, the cochleas were decalcified in 0.1 M EDTA for approximately 2 weeks, embedded in JB4 resin, and sectioned at 5 μm.

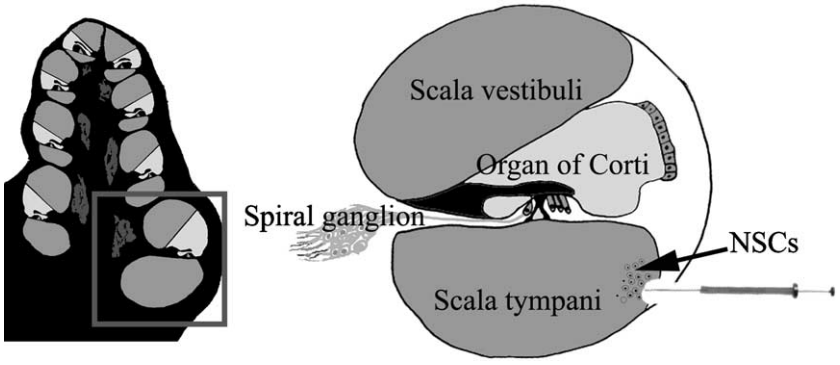

Fig. 1. Schematic illustrations of a midmodiolar section of the guinea pig cochlea and the location of implantation.

GFP detection

Following trimming the bone around the inner ear, the cochleas transplanted with adult NSCs expressing GFP were cryosectioned at a thickness of 12 μm (embedded in tissue-freezing medium, OCT compound tissue Tek Sakura).

Cell counting

Surviving NSCs, identified by positive X-gal staining and GFP fluorescence, were counted in the animals showing cell survival 2 weeks following implantation. Counting was made on sections (12-μm thick) collected every 24–25 μm throughout the entire cochlea. Student *t* test was used for the statistic analysis.

Immunohistochemistry

Differentiation of implanted NSCs was explored using immunohistochemical detection of tissue type-specific protein markers. The TUJ1 antibody (Berkeley Antibody Company) was used to detect neural class III β-tubulin. Following preincubation in goat serum (Santa Cruz Biotechnology, Inc.), the sections were incubated with TUJ1 antibody overnight at 4°C. After rinsing, cy3-conjugated affiniPure $F(ab')_2$ fragment goat antimouse IgG (Jackson Immunoresearch Laboratories, Inc.) was applied at room temperature for 2 h. To identify glial cells, an antibody against the glial fibrillary acidic protein (GFAP; DAKO) was used. Following preincubation in goat serum, the sections were further incubated with polyclonal rabbit anti-GFAP overnight at 4°C. After rinsing, amca affinity-purified goat antirabbit IgG (H+L) (Vector Laboratories) was applied at room temperature for 2 h. To detect a possible differentiation of the implanted cells into a hair cell fate, a hair cell marker was used. An affinity-purified rabbit antimyosin-VIIa antibody (Dr. Tama W. Hasson, Division of Biological Sciences, University of California, San Diego, USA) and amca affinity-purified antirabbit IgG (H+L) (Vector Laboratories) were applied to the cochlear section using the same procedures as above.

Microscopy

Tissue sections were observed using either a Zeiss fluorescent microscope equipped with a digital camera (Spot RT, Diagnostic Instrument or Polaroid DMC le) or a confocal microscope (Zeiss LSM 510).

Results

Survival of adult NSCs in the mature inner ear

The implanted cells were identified using either X-gal histochemistry (*Lac*Z-expressing cells; Fig. 2) or GFP fluorescence (GFP-expressing cells; Figs 3A, 4B and 5B). The survival rate differed with the experimental manipulations, normal-hearing and deafened host animals, and transduced cells (Table 1).

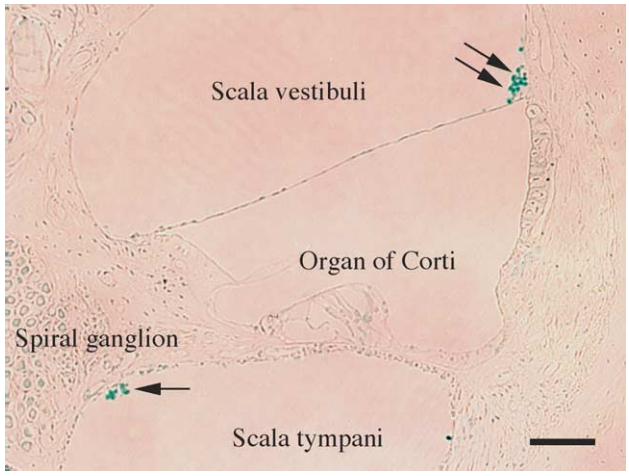

Fig. 2. Image illustrating the survival of adult mouse NSCs in the normal guinea pig inner ear 2 weeks following transplantation. Blue-appearing *Lac*Z-expressing implanted cells were found in the scala tympani and scala vestibuli of the inner ear. The implanted cells (arrow) were attached close to the spiral ganglion and the organ of Corti. Surviving transplanted cells (double arrows) were also observed in the scala vestibuli, attached to the lateral bony wall of the scala vestibuli, close to the Reissner membrane. Scale bar: 100 μm.

The first step of the study was to explore the potential of adult NSC to survive in the mature inner ear. NSCs were thus transplanted into normal-hearing animals (i.e., animals not subjected to chemical deafening). Implanted cells were found surviving in the host cochleas for up to 3 weeks following implantation (cf. Table 1). In about 50% of the animals, NSCs were found at 1 and 2 weeks following transplantation (2 of 4 and 11 of 20, respectively). However, the survival went down dramatically at later stages: one of four animals had remaining NSCs at 3 weeks, and none out of eight animals at 4 weeks following transplantation.

In an attempt to enhance the survival of NSC in the inner ear and to test the hypothesis that survival and differentiation of NSC would be greater when transplanted into an injured system, guinea pigs were deafended before the NSC implantation. Indeed, the survival rate was better in animals that had been neomycin-treated (deafened) before transplantation. Here, surviving cells were found in five of six animals at 2 weeks and in two of six hosts at 4 weeks (Fig. 4). Similar survival rates were seen in normal host animals transplanted with NSCs which had been genetically modified before implantation. In experiments with *ngn2*-transduced NSCs, implanted cells were found in three of six animals at 2 weeks and in two of six hosts at 4 weeks (Figs 5 and 6).

Cell counting, however, revealed a relatively poor survival of the implanted adult NSCs. At 2 weeks following implantation, the number of surviving cells ranged from 17 to 107: 0.4‰ in the normal-hearing group, 0.7‰ in the deafened group, and 0.6‰ in the neurogenin

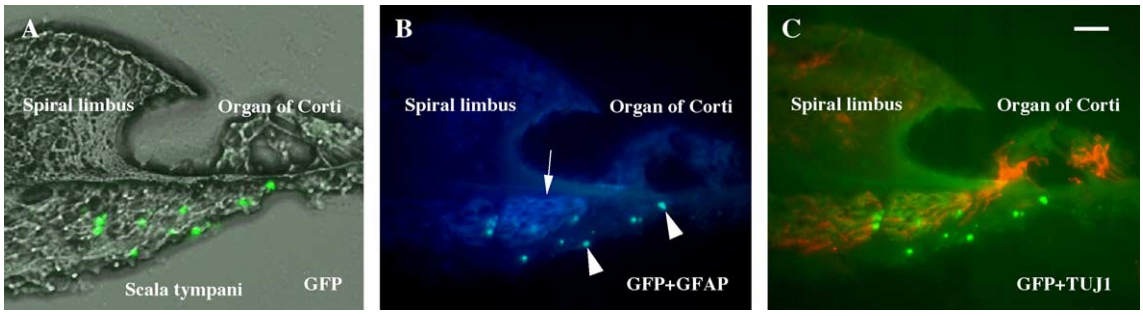

Fig. 3. Two weeks following transplantation, GFP-expressing adult NSCs had migrated and differentiated in the normal-hearing inner ear. The GFP-expressing implanted cells (green fluorescence) were located along the auditory nerve fibers close to the organ of Corti (A, confocal image). The GFP-expressing implanted cells (arrow heads, green + blue fluorescence) and the auditory nerve fibers of host animals (arrow, blue fluorescence) were labeled with GFAP antibody (B, GFP+GFAP, fluorescence microscope image). The implanted cells (green fluorescence) were not labeled with TUJ1 antibody, while the hair cells and auditory nerve fibers of the host animals (red fluorescence) were labeled with TUJ1 antibody (C, GFP+TUJ1, fluorescence microscope image). Scale bar: 20 μm shown in C.

group. There was no significant difference in cell survival between these groups ($P > 0.05$, unpaired t test).

Location of the implanted NSCs

As illustrated in Fig. 1, the NSCs were transplanted into the fluid of scala tympani in the most basal turn of the cochlea. In the 26 host animals where surviving NSCs were found, implanted cells were observed to attach both to the osseous Rosenthal canal (where the spiral ganglion neurons are located) and close to the organ of Corti in the scala tympani. Implanted cells were also found in the scala tympani of more apical cochlear turns. Moreover, NSCs were found in the adjacent scala vestibuli (cf. double arrows in Fig. 2) in eight host animals, suggesting that the cells distribute throughout the entire cochlea. Interestingly, implanted NSCs were also observed outside of fluid-filled compartments (scala tympani and scala vestibuli). As seen in Fig. 3A, NSCs were found located along the auditory nerve fibers projecting to the hearing organ. This was observed in four host guinea pigs at 2 weeks following transplantation. In two host animals transplanted with *ngn2*-transduced NSCs, implanted cells were found close to spiral ganglion neurons (Fig. 6).

Neuronal differentiation of implanted cells

The fate of the transplanted cells was further characterized using tissue type-specific markers. Cells located along the auditory nerve fiber tract projecting to the hearing organ (cf. Fig. 3A) expressed glial fibrillary acidic protein (GFAP) suggesting a glial fate (Fig. 3B) in 2 of 20 normal-hearing animals. None of these cells, implanted into normal-hearing animals, expressed the neuronal marker βIII-tubulin. However, in animals that had been chemically deafened before implantation, βIII-tubulin-positive implanted cells were found in 2 of 12 host animals, and GFAP-positive implanted cells were observed in 1 of 12 animals. Interestingly, in the *ngn2*-transduced group, relatively more host animals showed neuronal differentiation of the NSCs. In animals (normal) transplanted with *ngn2*-transduced NSCs, TUJ1-positive cells were found in 5 of 12 inner ears (cf. Table 1; Figs 5 and 6). None of the surviving cells were labeled with the myosin VIIa antibody.

Discussion

The cochlear sensory epithelium and neural components do not, at least in mammals, regenerate or repair naturally

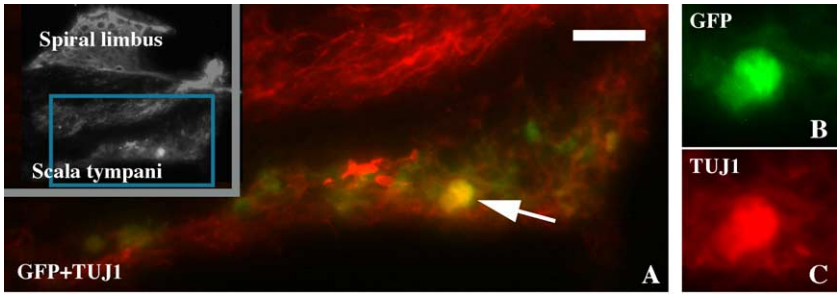

Fig. 4. Images showing surviving and differentiated adult NSCs in the chemically deafened inner ear at 4 weeks following transplantation. The insert in A (black–white image in gray window) shows a structural overview of the inner ear. The implanted cells (in blue window and in color image with a higher magnification) were found surviving in scala tympani. The differentiated cell (arrow) was double-labeled with GFP (green, enlarged in B) and the neuron marker, TUJ1 antibody (red, enlarged in C), thus appearing yellow. Scale bar: 20 μm.

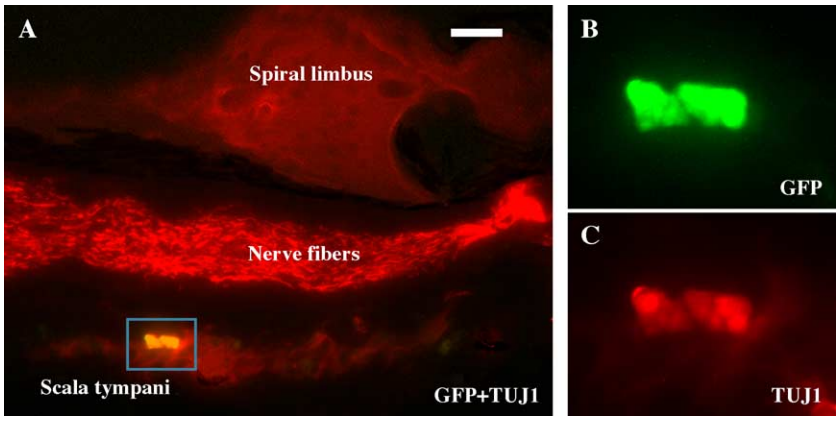

Fig. 5. Neurogenin 2 transduced NSCs in the normal inner ear at 4 weeks following implantation. The implanted cells were double-labeled with GFP (B, green) and neuronal marker, TUJ1 (C, red), thus appearing yellow (A). Scale bar: 20 μm shown in A.

following severe injury [32,33]. The development of therapeutic techniques designed to replace damaged cells and to restore inner ear function thus remains a clinical challenge. A substitution approach using cell therapy has been used as treatment in severe neurological disorders such as Parkinson disease [34]. Applying a similar strategy to the impaired inner ear raises many practical questions. Survival of the implanted exogenous tissue or cells in the mature inner ear would be the first critical issue to demonstrate. In this study, surviving adult NSCs were found in about half of the normal-hearing host animals at 1–2 weeks following implantation. However, the survival of implanted NSCs decreased significantly at later stages, and no NSCs were found at 4 weeks. The reason is obscure but probably due to the host local environment, for example, lack of nutrition and/or essential growth factors in the scala tympani. Also, an immunological response cannot be excluded, although no signs of a tissue reaction were observed in the present study. Interestingly, better survival was seen in the host animals that had been chemically deafened before implantation (NSCs found survival in five of six hosts at 2-week survival and in two of six hosts at 4-week survival). In these animals, the sensory epithelium is severely damaged, and as a secondary effect, the spiral ganglion neurons start to degenerate. The finding may suggest that, in the host, tissue in damaged inner ears releases growth factors (e.g., neurotrophic factors) enhancing NSCs survival in the early stage following implantation. It is likely that a release of growth factors is a normal response of the inner ear to injury, an attempt to stimulate endogenous repair mechanisms [35–37]. However, the absolute number of surviving NSCs in the inner ear is still very low. It has previously been reported that around 0.5‰ of implanted embryonic mouse NSCs survived following transplantation into mice (i.e., allograft transplantation, mice to mice) [38]. In the present study, where mouse adult neural stem cells were transplanted into guinea pigs (i.e., xenografted transplantation), we had similar results, a survival rate of 0.4–0.7‰. Although survival rate is low, the results prove the principle that adult stem cells can survive in the mature inner ear. To have a future clinical application, much more works are needed to enhance the stem cell survival, that is, using embryonic stem cell as a donor or providing a more suitable extracellular matrix in the inner ear.

If a transplantation approach is to be successful in treating inner ear injuries, it is of course essential that the cells not only survive, but also migrate to functionally relevant regions and differentiate into an appropriate cell fate, that is, a neuronal fate when attempting to replace auditory neurons. In all experiments, the adult NSCs were

Table 1
Survival and differentiation of adult neural stem cells in the mature guinea pig inner ear

Experimental group	Normal-hearing				Neurogenin		Deafened	
Survival period	1 week	2 weeks	3 weeks	4 weeks	2 weeks	4 weeks	2 weeks	4 weeks
Number of implanted animals	4	20	4	8	6	6	6	6
Number of animals showing								
NSC survival	2	11	1	–	3	2	5	2
TUJ1 (+) NSC	–	–	–	–	3	2	1	1

Adult neural stem cell (NSC) survived for up to 3 to 4 weeks postoperatively when transplanted into normal-hearing animals transplanted with adult NSCs (normal-hearing group), when the cells were transduced with neurogenin 2 (neurogenin group), and when the cells were transplanted into neomycin-treated animals (deafened group). Implanted cells were labeled with the neuronal marker TUJ1 antibody in the neurogenin and deafened groups but not in the normal-hearing group.

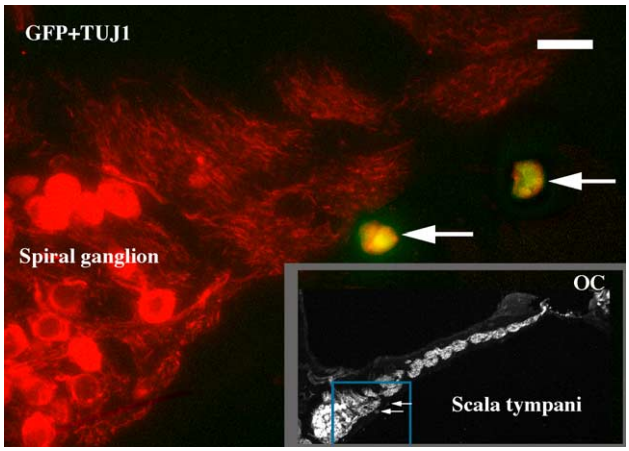

Fig. 6. Neurogenin 2 transduced NSCs survived within modiolus and close to the spiral ganglion neurons at 2 weeks following transplantation. The GFP-positive cells (arrows) were also double-labeled with a neuronal marker, TUJ1, and appeared yellow. OC, organ of Corti. Scale bar: 20 μm.

transplanted into scala tympani, a procedure that involves minimal surgical trauma to the inner ear. The location, however, is essentially functionally irrelevant and most likely also lacks some of the essential growth factors. It is thus very interesting to note that the surviving cells had ended up at, or had migrated to, sites closer to growth factors or nutritional support, for example, bone or adjacent to the sensory epithelium and neurons (cf. Figs. 2–6) [39,33]. More importantly, it was shown that NSCs also migrated from the implantation site within the fluid-filled scala tympani, through the bony modiolus, towards locations along the auditory nerve tract close to the sensory epithelium (cf. Fig. 3A), and spiral ganglion neurons. This result suggests that adult NSCs indeed possess the capacity to migrate to functionally important structures following implantation into mature inner ear. It has recently been reported that dorsal root ganglion neurons implanted into the inner ear migrated to the spiral ganglion region and along the nerve fibers projecting to the hearing organ [12]. It was suggested that the implanted cells were directed by the release of growth factors from the host neural tissues and migrating by means of the minute holes that have been shown to pass from scala tympani through the bone towards the Rosenthal canal (Schuknecht canaliculae perforantes) [40,41]. In the present study, implanted NSCs were also observed in the scala vestibuli (cf. Fig. 2), suggesting that the implanted cells may move significant distances along the cochlea, possibly following the flow of perilymph from the transplantation site in scala tympani through helicotrema to scala vestibuli [42].

When transplanting embryonic dorsal root ganglion neurons [12], the cell fate is obviously already established. However, for stem cells, the final outcome is much more dependent on local factors. In normal-hearing hosts, no neuronal differentiation was detected, whereas in the deafened animals, some surviving implanted cells were labeled with a neuronal marker. To ascertain a higher degree of neuronal differentiation, it is, however, feasible to genetically engineer the cells before implantation. It has previously been shown that transducing NSCs with *ngn2* results in around 95% of the transduced cells differentiating into a neuronal fate [29]. It was thus attempted to genetically modify the NSCs before implantation using *ngn2*. Indeed, this resulted in cells positively labeled with a neuronal marker (TUJ1) in all five host animals where surviving implanted cells were found. It has, however, been reported that *ngn1* is the main factor to define the precursors of cochlear ganglia [43]. Indeed, several neural-specific basic helix–loop–helix genes, for example, *ngn1* and *ngn2*, are expressed in the epibrancial placodes, suggesting that a similar regulatory cascade may underlie the development of sensory neurons in cranial ganglia. It has also been demonstrated that *ngn1* is not activated in *ngn2* mutant placodes, which suggests *ngn2* as the first regulator to operate in this cascade and *ngn1* as a potential transcriptional target of *ngn2* in the placodes [26].

In conclusion, the present findings demonstrate that adult NSCs may survive for up to at least 4 weeks following transplantation into the mature inner ear and that they seem to migrate to locations near functionally important structures such as the auditory nerve tract, the organ of Corti, and the spiral ganglion. The results were especially encouraging in deafened animals where the NSCs were also observed to differentiate to cells positively stained with the neuronal marker TUJ1. Neural differentiation was also enhanced by transducing the NSCs with *ngn2* before transplantation. The study lends support to the notion that a cell therapy strategy, in combination with genetic engineering, may replace degenerated or absent host cochlear neurons or sensory epithelia and possibly act to restore the auditory function in deafened subjects. However, more work is required to enhance the survival and differentiation of implanted cells.

Acknowledgments

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