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Sensory Cell Regeneration and Stem Cells: What We Have Already Achieved in the Management of Deafness

*Petros V. Vlastarakos, †Thomas P. Nikolopoulos, *Evangelia Tavoulari, *George Papacharalambous, †Antonios Tzagaroulakis, and ‡Stefan Dazert

**ENT Department, Hippokrateion General Hospital of Athens; †ENT Department, Atticon University Hospital, Athens, Greece; and ‡ENT Department, Ruhr University of Bochum, Bochum, Germany*

Background/Objective: Genetic manipulation of the cell-cycle exit, induction of new hair cells (HCs) through gene modification therapy, and introduction of stem cells (SCs) into damaged cochleas potentially offer exciting new strategies in treating sensorineural hearing loss.
Materials and Methods: Literature review from Medline and database sources.
Study Selection: Ex vivo models, animal studies, in vitro studies, and review articles.
Data Synthesis: Embryonic SCs, neural SCs, or bone marrow SCs survive in the mammalian inner ear after transplantation. The scala media and the modiolus seem more functionally appropriate injection sites. The clear evidence that transplanted neural SCs can adopt the morphologic phenotypes of HCs was the most significant milestone achieved in the related research. The normal cytoarchitecture in the organ of Corti may also be restored through mouse atonal homologue 1 transgene expression and transduction of the nonsensory cells, producing clinically measured improvement in hearing thresholds. Embryonic SC–derived neurons have the potential for synapse formation with auditory HCs and reinnervation of the auditory epithelia. However, fluctuations in survival rates, functional recovery of the spiral-ganglion neurons, integration to the host tissue, and potential immune barriers are also areas of utmost importance.
Conclusion: There is an already exciting progress in the fields of sensory cell regeneration and SC research in an attempt to restore hearing or prevent deafness. However, further understanding of the underlying mechanisms of auditory genetics, continuing investigation of the human genome, refinement of the delivering techniques, and specification of the therapeutic strategies have to be developed before functional regeneration of the cochlea can be achieved in clinical practice. **Key Words:** Bone marrow—Embryonic—Inner ear—Mouse atonal homologue 1—Neural—Neurotrophic—Stem cells.

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The increase in life expectancy in modern societies has not only increased the burden of chronic diseases but has also raised issues with regard to quality of life. In this context, the treatment of sensorineural hearing loss (SNHL), which appears as one of the most prevalent chronic diseases (1), and the restoration of normal hearing have been intensively pursued.

The developments in the field of digital hearing aids, although continuous and remarkable, will always be subjected to limitations because they need a certain number of surviving hair cells (HCs). Cochlear implants, on the other hand, represent an important milestone in hearing rehabilitation because they bypass the HCs and stimulate directly the spiral ganglion cells. However, they are also subjected to device- and/or recipient-related limitations (2–4).

Hence, restoration of the normal HC function seems to be the objective, and thus, attempts have been made to regenerate the damaged inner ear. Such efforts involve either renewed cell proliferation of the mitotically quiescent auditory epithelium or transplantation of cells that can differentiate into highly specialized sensory cells. In view of this perspective, gene manipulation and stem cell (SC) therapy represent new and exciting approaches for the treatment of SNHL.

The aim of the present article is to review the accomplished milestones on these treatment modalities and address critical issues that should be taken into account before they can be considered as successful therapeutic options for the management of deafness.

Address correspondence and reprint requests to Petros V. Vlastarakos, M.D., M.Sc., ENT Department, Hippokrateion General Hospital of Athens, 114 Vas. Sofias Ave, Athens 11527, Greece; E-mail: pevlast@hotmail.com; pevlast@yahoo.gr

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MATERIALS AND METHODS

An extensive search of the literature was performed in Medline and other available database sources using the keywords "ENT," "inner ear," "stem cells," "precursor," "embryonic," "neural," "growth factor," "transcription factor," and "treatment." The keyword stem cells was considered primary and was either combined to each of the other keywords individually or used in groups of 3. In addition, reference lists from the retrieved articles were manually searched. Language restrictions limited the included literature into English-speaking articles. The number of studies initially selected was 232.

Three main categories of outcomes were chosen for further analysis: 1) assessment of the efficacy of different treatment modalities in regenerating the damaged inner ear, 2) already established methods for delivering treatment directly into the inner ear, and 3) identification of the critical issues that should be considered and resolved before these modalities can be applied in clinical practice.

Using this framework of results, the retrieved studies were critically appraised according to evidence-based guidelines for the categorization of medical studies (Tables 1–4) (20). As a result of the previously mentioned methodology, the number of studies that were finally included in data synthesis was curtailed by 172.

A great difficulty in the area of research explored in this article was that most of the related surveys investigated only a small fraction of auditory genetics and/or SC transplantation potentials per se, and that several fields still remain to be investigated. Therefore, the results obtained could not be readily extrapolated with regard to the greater picture of hearing restoration. However, the synthesis of nonvertebrate, avian, mammalian, and human evidence, despite the well-acknowledged differences betweenspecies, managed to provide a fair estimation of the latter.

RESULTS

Two ex vivo models, 29 animal studies, 19 in vitro studies, and 9 review articles met the defined criteria and were included into the study selection.

DISCUSSION

Analysis of Evidence

Sensorineural hearing loss is usually nonreversible (in mammals) because the human auditory epithelium is composed of terminally differentiated cells. Therefore, SC transplantation and/or sensory cell regeneration represent unique treatment modalities for the severely impaired human inner ear. The origination of SCs and the basic auditory genetics with regard to HC commitment and survival have been discussed elsewhere (21). However, another key element for the successful management of deafness is the analysis of the evidence that derive from animal and in vitro studies from the standpoint of either restoring the damaged organ of Corti or preventing the initialization and/or advancement of damages.

TABLE 1. *Evidence-based categorization of medical studies*

Category of evidence	Origin of evidence
Ia	Evidence from meta-analysis of randomized controlled trials
Ib	Evidence from at least 1 randomized controlled trial
IIa	Evidence from at least 1 controlled study without randomization
IIb	Evidence from at least 1 other type of quasiexperimental study
III	Evidence from nonexperimental descriptive studies such as comparative studies, correlation studies, and case-control studies
IV	Evidence from expert committee reports or opinions or clinical experience of respected authorities, or both

STEM CELL TRANSPLANTATION

Basic Methodology

An important milestone in the fields of sensory cell regeneration and SC research is the development of special techniques for delivering treatment directly into the inner ear.

Indeed, the efficiency of the approaches for local drug administration to the inner ear, which were used in the past for electrophysiologic studies, was restricted by the fact that only low molecular weight particles (less than 400 Da) could passively surpass the round window barrier (22). Drug introduction through the round window (23), on the other hand, and perilymphatic perfusion through the cochlear scalae (24) had also been used for such studies with promising results, taking into account the numerous technical difficulties that they were facing.

However, efficient transplantation of SCs into the murine cochlea largely depends on a system that can allow multiple infusions of very small amounts of solutions directly into the inner ear (i.e., the scala tympani), the volume of which can vary among injections, and the time of administration would be under the control of the investigator. Such a system may consist of 2 major parts (25): the microinjector system, which basically harbors and regulates the volume of the infused solution; and the catheter system with infusion tip, which is implanted into the scala tympani. The microinjector can be operated either manually, through a fine screwdriver, accomplishing a minimum volume for injection at a 15-degree adjustment (25), or via an osmotic pump, allowing an infusion rate of 0.5 μl/h (26). A small silicone cushion located 0.5 mm from the end of the infusion tip can limit the extent of the tip introduction into the scala tympani, minimizing the risk of mechanical trauma to the neighboring structures (25–27).

The surgical approach includes a midline incision on the dorsal surface of the head just posterior to the bregma, which is continued postauricularly until it becomes approximately even with the base of the pinna. Through a 1.0- to 2.0-mm hole in the auricular bulla, the round window and the basal turn of the cochlea are visualized, and a small hole is drilled into the basal turn (~0.1 mm), just 2.0 mm anterior to the round window, allowing access to the scala tympani. With fine forceps holding the silicone guard, the infusion catheter is inserted into the hole in the lateral wall until the silicone drop is seated against the otic

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TABLE 2. *Study characteristics regarding ESC transplantation*

Type of study	Level of evidence	Type of SCs	Injection site	Technical details	Type of mammals	Reported advantages	Reported disadvantages	Remarks
Prospective control (5)	IIa	ESC	Scala media	1) Polyimide canula; 2) microsyringe pump	Deafened experimental/ normal hearing controls	1) Minimal mechanical trauma; 2) high survival rate; 3) NEM cells in aggregations	No integration into endogenous tissue	1) Highly successful results; 2) xenotransplantation
Prospective control (6)	IIa	ESC	Perilymphatic space	ND	Damaged experimental IEs/normal hearing controls	1) Survival in the IE; 2) differentiation into SCs; 3) integration with SCs	1) Endocochlear duct could not be approached; 2) aggregation of cells on bony walls	IEs provide important cues for survival and differentiation
Prospective (7)	IIb	ESC	PSCC	Hamilton syringe	Damaged IEs	Differentiation of transplanted cells into ECT cells	Characteristics of undifferentiated cells remained	Nonsuccessful attempt
Prospective control (8)	IIa	ESC	Scala tympani	1) Micro-syringe; 2) Electronic micropump	Deafened	1) Minimal mechanical trauma; 2) no inflammatory response; 3) survival of implanted cells	1) Cells decrease after 4 wk; 2) dispersal to CSF; 3) nonsignificant cell count into the Rosenthal canal	The deafened cochlea environment can support the survival of exogenous tissue
Prospective control (9)	IIa	Embryonic DRG	Scala tympani	1) Forceps; 2) microcannula; 3) osmotic micropump	Deafened NT-treated/ NT-deafened and normal-hearing non–NT treated	1) Survival of healthy DRG neurons; 2) DRG migration into the modiolus; 3) no signs of apoptosis/ inflammation	Similar numbers of animals with surviving grafts irrespective of NT treatment	Larger number of surviving cells than in other studies with DRG
Prospective control (10)	IIa	Embryonic DRG	Scala tympani	Forceps	Damaged IEs experimental/ normal-hearing controls	1) Implant survival up to 10 wk; 2) survival close to the OC/modiolus; 3) migration toward functionally relevant sites	1) No difference in survival rate of deafened versus control animals; 2) no structural/functional integration of transplanted tissue	1) The adult auditory system can accept foreign nervous tissue; 2) xenotransplantation
Prospective control (11)	IIa	Embryonic DRG	Scala tympani	1) Forceps; 2) fine catheter; 3) mini osmotic pump	NGF experimental/ non-NGF controls	1) NGF promotes DRG survival; 2) NGF stimulates neurite outgrowth; 3) DRG migrate toward SGN	None reported	Xenotransplantation

CSF indicates cerebrospinal fluid; DRG, dorsal root ganglion; ECT, ectodermal; ESC, embryonic SC; IE, inner ear; ND, not defined; NEM, neuroectodermal; NGF, nerve growth factor; NT, neurotrophin; OC, organ of Corti; PSCC, posterior semicircular canal; SC, stem cell; SGN, spiral ganglion neuron.

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TABLE 3. *Study characteristics regarding NSC and BMSC transplantation*

Type of study	Level of evidence	Type of SCs	Injection site	Technical details	Type of mammals	Reported advantages	Reported disadvantages	Remarks
Prospective (12)	IIb	NSC	Perilymphatic space	1) Hamilton syringe; 2) micropipette	Normal hearing	1) Development of HC; 2) integration into the OC; 3) migrational capacity; 4) wide adaptation	1) Not high HC count; 2) new HCs do not express all HC features	NSCs migrate/differentiate over a wider area than expected
Prospective control (13)	IIa	NSC	Scala tympani	Microsyringe	Deafened and NGN-treated experimental/ normal-hearing controls	1) Better survival in deafened animals; 2) better neuronal differentiation in NGN animals; 3) migration toward functionally relevant sites; 4) minimal mechanical trauma	1) Poor survival after 2 wk; 2) Dramatic decrease in surviving cells in 4 wk	1) Injection site is functionally irrelevant and lacks growth factors; 2) xenotransplantation
Prospective (14)	IIb	NSC	Modiolus	1) Hamilton syringe; 2) Infusion pump	Deafened	1) Robust survival in all experimental animals; 2) migrational activity into the modiolus	Differentiation predominantly into glial cells	Relatively poor neuronal differentiation
Prospective (15)	IIb	BMSC	Modiolus	30-G needle	Damaged IEs	1) Robust survival in multiple regions of the cochlea in all animals; 2) neuronal differentiation; 3) migrational capacity	Paucity of cells in the scala media	Perhaps more appropriate for SGN regeneration
Prospective (16)	IIb	BMSC	Scala tympani/ modiolus	1) 30-G needle; 2) vinyl tube; 3) prefilled cannula	Normal hearing	Neuronal differentiation	1) Low survival rate; 2) few surviving cells in the scala media	1) Scala media is a hostile environment; 2) modiolar approach is more appropriate for SGN restoration; 3) xenotransplantation

BMSC indicates bone marrow SCs; HC, hair cell; IE, inner ear; NGN, neurogenin; NSC, neural SC; OC, organ of Corti; SC, stromal cell; SGN, spiral ganglion neuron.

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TABLE 4. *Study characteristics regarding sensory cell regeneration and inner ear trauma prevention*

Type of study	Level of evidence	Type of therapy	Injection site	Technical details	Type of mammals	Reported advantages	Reported disadvantages	Remarks
Prospective control (17)	IIa	Transdifferentiation	Scala media	Microcannula	Deafened *Math1*-treated experimental/deafened non–*Math1*-treated and contralateral ear controls	1) Restoration of the normal cochlear cytoarchitecture; 2) induction of HCs; 3) substantial improvement in HTs; 4) stable functional recovery	OHCs incompletely differentiated	1) Normal morphology and orientation of induced HCs; 2) proportional improvement of HTs and induced HCs 3) cochlear amplifier may be dysfunctioning
Double-blinded prospective control (18)	Ib	NT infusion	Scala tympani	1) Microcannula; 2) osmotic pump	Deafened NT-treated experimental/deafened non–NT-treated controls	1) NTs support SGC survival; 2) systematic decrease in HTs	None reported	Improved hearing sensitivity was accomplished
Prospective control (19)	IIa	AOs	Scala tympani	1) Intracochlear cannula; 2) osmotic pump	Deafened AO-treated experimental/deafened non–AO-treated controls	1) AOs enhance SGC survival; 2) systematic decrease in HTs	None reported	AOs increase electric sensitivity of the cochlear nerve after deafness

AO indicates antioxidant; HC, hair cell; HT, hearing threshold; *Math1*, mouse atonal homologue 1; NT, neurotrophin; OHC, outer hair cell; SGC, spiral ganglion cell.

capsule, and a small piece of fascia is placed around the wall opening to seal the bony defect. A superficial subcutaneous pocket is made over the back of the animal between the scapulae in case a micropump is attached to the system.

Problems that may be encountered during this procedure are basically restricted to a small amount of intrascalar bleeding after the drilling of the basal turn of the cochlea, which, however, quickly resolves, possibly due to the constriction of the cochlear vessels (28). Middle ear infection that is observed in some animals cannot be attributed to inappropriate technique because the inoperated ear is also frequently affected (27). Prophylactic administration of antibiotics is also part of the standard surgical protocol (25–27).

Another alternative might use completely implantable drug delivery systems, which are based on the suitable fit of a micropump within the patient's temporal bone. A catheter positioned near the round-window membrane can be used to deliver the designated substances (29,30). Spiral computed tomographic scans are quite valuable in determining the volume of the mastoid cavity, which is the basic parameter to be considered in these cases, during preoperative planning of the surgical implantation (31).

The feasibility of chronic local inner ear treatment in humans was also tested by using a modified cochlear implant array (32). The original electrode, self-curling by construction, was preserved in a straightened fashion by a stylet inserted in its built-in lumen. In the modified array, the tip of the electrode was cut, and a steel connector linked the electrode to a tube fitted within a micropump (or an infusion pump, alternatively). Temporal bone studies showed that the modified array could be inserted into the cochlea with the same ease as the standard electrode. Potential obstacles that may need to be addressed before using this technique in a larger scale include connective tissue growth, higher viscosity of the solutions in use, and crystallization, which could be caused by low flow rates (not observed at the flow rate of 0.5 μl/h, which presumably meets the requirements for standard human application).

Finally, it should be noted that the use of glass micropipettes in animal studies can be achieved by using a 2- to 3-pm glass electrode tip. The micropipette can be efficiently controlled with the aid of a micromanipulator. Twenty to 30 pounds of pressure can be applied to the micropipette in brief pulses, whereas the pulse duration and total time of the injection determine the final volume of every drop of soluble material (33).

Embryonic SCs

Transplanted undifferentiated and partially differentiated embryonic SCs (ESCs), which were delivered in the scala media of deafened guinea pigs, may survive in the cochlea for at least 9 weeks in positions near the spiral ligament, the stria vascularis, and, in some cases, near the damaged structure of the organ of Corti (5) (Fig. 1). However, integration of the transplanted cells into the endogenous tissue was not observed despite their propensity to exist as aggregations rather than as single cells.

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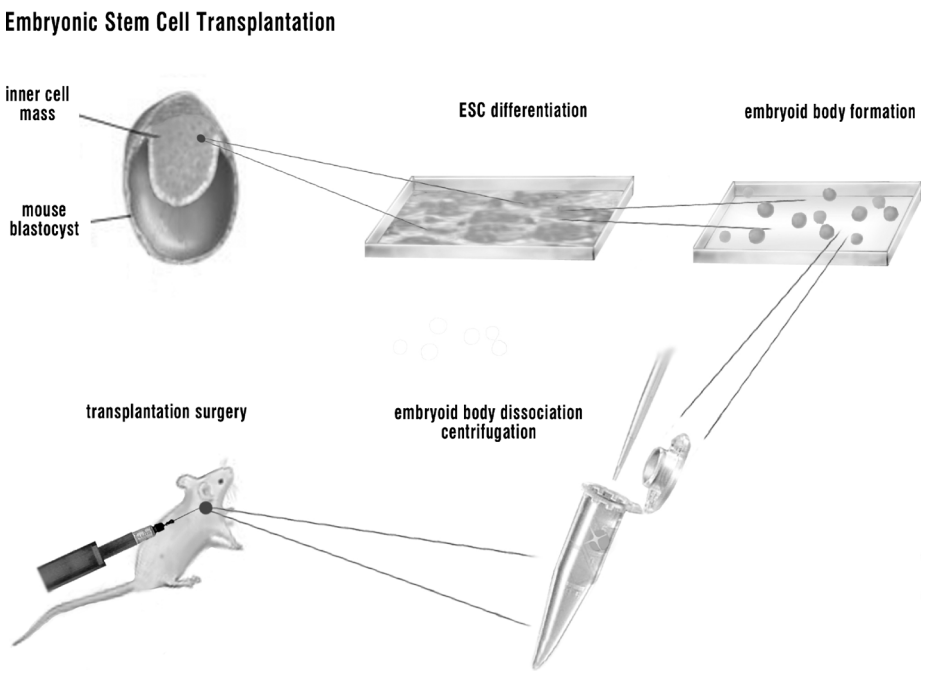

FIG. 1. Laboratory and surgical steps for ESC transplantation into the mouse inner ear.

Nevertheless, the reported survival rate was quite higher than in other studies with similar methodology and reached 19.1%. This percentage, however, might have actually been lower because only 14% of the surviving cells were located in the scala media, whereas surviving cells from the other two cavities were also included into the cell count. The survival rate was also inversely proportional to the duration of transplantation. Nonetheless, the results were promising and may very well be attributed to the surgical approach both in the chosen injection site and the surgical technique used, which minimized tissue trauma to the delicate structures of the cochlea. Predifferentiation of the cells in vitro could also have been partially responsible for the observed survival rate. However, advanced predifferentiation is not always a positive factor because survival rate may be inversely proportional to the level of differentiation of the transplanted cells.

Other experiments suggest that survival and differentiation of transplanted cells might also be influenced by the expression of some kind of scaffolding on behalf of the injured auditory epithelium. This is supported by the fact that otocyst cells from rat fetuses, which were grafted in young Sprague-Dawley rats previously exposed to intense sound, survived inside the inner ear and migrated into the organ of Corti in the form of supporting-like cells, potentially following specific cues (12). However, the disruption of the endolymph microenvironment prevented the extraction of safe conclusions with regard to the potential survival of these cells within the normal cochlear duct (6).

Furthermore, although the damaged inner ear displays some activity in inducing ESCs to develop into ectodermal cells, as was demonstrated by the expression of specific cell markers for epidermal cells and neurons but not for mesodermal cells, this effect is insufficient to induce inner hair cells (IHCs) (7). Therefore, it seems that we still face great difficulties in achieving HC production from transplanted embryonic cells.

Partially differentiated ESCs might also assist to the functional recovery of the spiral ganglion neurons (SGNs) if they are also affected by SNHL. Moreover, ESC transplantation can provide a more effective approach to the target site of SGNs (namely, the Rosenthal canal) than other delivering methods such as exogenous administration of neurotrophins (NTs). Thus, partially differentiated mouse ESCs, which were delivered into the scala tympani of deafened adult guinea pigs, survived in the transplantation cavity for up to 4 weeks, albeit in small numbers, and were capable of widespread dispersal throughout the cochlea. A proportion of these cells also retained expression of a 68kDa-neurofilament protein in vivo (8). Quantitative data revealed a significant decline in the number of surviving cells between 2 and 4 weeks after transplantation. This decline was attributed by the authors to the potential dispersal of the transplanted cells from the cochlea into the cerebrospinal fluid through the patent cochlear aqueduct. Furthermore, the authors suggested that despite the minimal mechanical trauma to the cochlear cytoarchitecture caused by the delivery technique, a more direct delivery of these cells into the Rosenthal canal using biocompatible matrices to minimize their dispersal and/or the coadministration of trophic support could have yielded better results. The latter notion is also supported by in vitro studies in which neurally differentiated mouse ESCs cocultured with auditory epithelium explants of mice exhibited massive elongation of neurites toward auditory HCs. The expression of

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synaptophysin was also demonstrated, suggesting the potential of ESC-derived neurons for synapse formation with the auditory HCs and reinnervation of the auditory epithelia (34).

In addition, fetal dorsal root ganglion (DRG) neurons have survived either as allografts (9) or as xenografts (10) in 15 of 24 albino guinea pigs and 25 of 44 Sprague-Dawley rats after transplantation into the scala tympani, indicating the potential intrinsic capacity of fetal DRG neurons to survive in the transplanted sites and migrate to the area of the primary auditory sensory neurons. However, neither structural nor functional integration of the donor cells into inner ear structures has actually been demonstrated. The latter may be an important challenge with regard to the feasibility of embryonic neuronal transplantation for the replacement of damaged SGNs. Survival of the implanted DRGs may also rely on endogenous factors such as vascular supply or neurotrophic factors produced by various inner ear structures. Hence, the placement of the implant beneath the organ of Corti and attached to the modiolus might ensure the necessary blood supply. Moreover, neurotrophic factors from the SGNs (35) or the sensory epithelium (36) might not only support DRG neuronal survival but also guide the neurons from the implanted location toward SGNs and the organ of Corti (10).

Similar results were obtained after the infusion of nerve growth factor into the scala tympani of Sprague-Dawley rats, leading to enhanced survival of implanted DRG neurons compared with controls ($p < 0.01$) (36). Neurite outgrowth from the surviving DRG neurons toward functionally important areas in the inner ear was also induced (11). However, although the total number of surviving grafted DRG neurons increased significantly when it was combined with the supply of exogenous neurotrophic factors, the number of implanted animals with surviving DRGs did not increase as a result of this treatment (9). Hence, surviving DRG neurons were found in 5 of 9 NT-treated animals and 10 of 15 animals not receiving NTs, which is an almost identical percentage.

The fact that only 62.5% of the allografts and 57% of the xenografts survived in the host animals can be attributed to the poor viability of fetal DRG neurons before implantation; inadequate graft integration with the host tissue, for example, because of ototoxin-associated cochlear pathologic findings; and/or insufficient vascularization at the site of the implant. The sectioning procedure itself can also result in loss of the implant if it is not well attached to the cochlea (9,10).

Neural SCs

The use of neural SCs (NSCs) has also yielded promising results. Thus, adult NSCs infused into the scala tympani of adult guinea pigs have survived in the mature inner ear in approximately 50% of the implanted animals during the first 2 weeks after transplantation. The results seemed especially encouraging in chemically deafened and neurogenin (Ngn)-treated animals compared with the normal-hearing group. In addition, the surviving cells have migrated to functionally important structures such as the sensory epithelium and the SGNs. However, NSC survival dramatically decreased 4 weeks after implantation, resulting in 0% survival in the normal-hearing group and 33% survival in the other 2 groups. The cell counting also revealed a relatively poor number of surviving NSCs at 2 weeks, ranging between 0.4 and 0.7‰ in the 3 groups, without statistically significant differences. The relatively low survival rate was attributed by the authors to the lack of nutrition and/or essential growth factors in the scala tympani (13).

In addition to the survival rate, the morphology of the implanted cells is also considered to be a critical issue. Thus, hippocampal adult NSCs injected into the perilymphatic space of newborn rats were found to be attached to the inner surface of the cochlear cavity 2 weeks after implantation and to form cell clusters in some cases. By the fourth week after the implantation, many surviving cells migrated as far as the space of the organ of Corti; however, no morphologic change to the cochlea was observed despite the well-established integration with the organ of Corti. Furthermore, some cells had adopted the morphologic phenotypes of outer or inner HCs (as demonstrated by phalloidin labelling), whereas no clear evidence of hippocampal SC-derived spiral ganglion–like cells was observed (12). These are promising results with regard to the main objective of HC restoration because the transplanted cells were able to morphologically develop phenotypes of HCs.

In support of the results extracted from the animal models, in vitro studies using immature neural progenitors have also established the potential of the latter to differentiate into HC immunophenotypes, as was demonstrated by the expression of both HC markers Brn-3c and myosin VIIa (37). Interestingly, no evidence of transdifferentiation of neural progenitors to the epithelial lineage was found, potentially suggesting that other mechanisms such as a hitherto unrevealed competence for alternative cell fates influenced by epigenetic factors may have been involved in these processes (38).

Fetal NSCs are also possible candidates for the restoration of SGNs. Indeed, injection of these cells into the cochlea of chemically injured mice resulted in robust survival of the injected cells in all experimental animals. Neural SC–derived cells were located either in the modiolus or inside the scala tympani. Furthermore, migrational activity of NSCs into the modiolus was also suggested because cells injected in its basal portion were eventually found in the apical end and the osseous spiral lamina (14). However, neuronal differentiation of the implanted NSCs was relatively poor, and that was mainly attributed to a trend of differentiation into glial cells rather than neurons (80% of NSC-derived cells compared with 10%, respectively). The latter obstacle can be overcome through the ectopic expression of Ngn2, as demonstrated in in vitro studies. Indeed, NSC cultures transduced with a vesicular stomatitis virus G–pseudotyped retrovirus showed a 90% differentiation toward a neuronal fate when the viral vector expressed the Ngn2 protein compared with 1% when a control retrovirus was used. In

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addition, very few of the Ngn2-transduced cells expressed glial markers, in comparison to cells receiving no virus, or the control virus, in which astrocytes were the majority (39).

Bone Marrow SCs

The use of bone marrow SCs (BMSCs) has also been proposed for SC treatment. The observation that transplanted adult BMSCs have the ability to migrate into the brain and differentiate into cells that express neuron-specific antigens in both rodent models and human patients (40–42) suggested that these cells can be used to prevent the development or progression of degenerative diseases in the inner ear or to repair damaged tissue. Indeed, robust survival of grafted autologous BMSCs was established in multiple regions within the cochlea and in the modiolus of gentamicin-treated chinchillas after modiolar injection through the round window. Transplant-derived cells were found in every turn of the cochlea, including its apical end, in all animals. These cells were predominantly located in the perilymphatic space, whereas few were also located in the scala media and in the lateral wall. The paucity of transplant-derived cells in the scala media suggests that the local environment was less preferable for the survival of BMSCs potentially due to the high potassium concentration. Overall, the proportion of transplant-derived cells that expressed neuronal or glial phenotypes was 0.4 and 1.2%, respectively. In addition, cells that were implanted in the basal turn of the cochlea migrated as far as the apical end or into the spiral ligament (15). However, because most of the transplanted cells eventually evolve into nonneuronal cells (40,41), additional studies are required to identify factors that promote the differentiation of BMSCs into distinct neural cell types and provide adequate numbers of cells that can actually enhance cochlear function.

The aim of BMSCs taking over SGN properties might also require modification of the delivering techniques into the cochlea. Indeed, although BMSCs have the potential to migrate (15), the anatomic barrier between the perilymphatic space and the modiolus might be responsible for the absence of these cells from the latter after injection in the scala tympani of gerbils. In contrast, when direct injection of mouse BMSCs into the modiolus was performed, transplanted cells were also found at this site. Interestingly, only a small number of transplanted cells were located in the scala media, irrespective of the delivering technique that was followed. Moreover, the average survival rate was similar for both types of injection (0.54% for scala tympani injection compared with 0.59% for modiolar injection) (16).

Nevertheless, the discovery of cells displaying neuronal phenotypes in the area around the spiral ganglion is clinically important and suggests that marrow cell transplantation can increase the number of SGNs. Migration of transplant-derived cells into the lateral wall of the cochlea possibly indicates that transplantation of BMSCs may be also used to restore the damaged spiral ligament (15).

Immune Barriers

A critical issue that should be addressed before SC transplantation can be considered as a therapeutic option is the possibility of immune rejection. Although immunosuppression was seldom administered in the previously analyzed experimental models (9,10), regardless of the type of grafts that was used (i.e., allografts, xenografts), no evidence of significant immunologic rejection or inflammatory tissue response toward the implanted cells was observed (5,8). This suggests that the adult auditory system can accept foreign nervous tissue (at least in very early stages of differentiation), which is a prerequisite for clinical treatment based on a biological implantation (10). However, no matter how encouraging the responses may seem on behalf of the host animals, the use of SC therapy in humans should take into consideration the possibility of immune rejection due to differences in the major histocompatibility complexes among humans and between species. Interestingly, Olivius et al. (9) have also reported that most of the animals without survival of implanted cells showed signs of leukocyte invasion or hemorrhage.

In addition, it should be analyzed whether the observed immunological tolerance of the inner ear can be attributed to the immune privilege that exists in certain sensitive organs such as the eye, the brain, and the reproductive organs (43). In any case, the use of autologous grafts (i.e., BMSCs) bypasses the previously mentioned immune barriers along with the problems that arise from species-specific signaling.

SENSORY CELL REGENERATION

Another therapeutic approach, which is theoretically available, is based on expressing or modifying a number of developmental genes. These genes can either stimulate differentiation of the potentially existing inner ear progenitor cells (i.e., IESCs) (44) or induce a phenotypic transdifferentiation of the nonsensory cells that remain in the deaf cochlea (17). Local adenoviral gene therapy in the inner ear may be successfully used in this context (45). Indeed, viral vectors incorporating the mouse atonal homologue 1 (*Math1*) gene, which were inoculated into the scala media of chemically deafened adult guinea pigs, were found to restore the normal cytoarchitecture in the organ of Corti. This restoration was made possible through *Math1* transgene expression and transduction of the nonsensory cells in the deaf cochlea and was better near the site of inoculation and in the first and second cochlear turns, within the normal boundaries of the organ of Corti. The "new" HCs showed normal morphology and orientation, whereas ectopic HCs outside the organ of Corti were neither well differentiated nor correctly oriented. The number of HCs in the *Math1*-treated ears was significantly greater than in contralateral ears ($p < 0.0006$).

With regard to hearing, a substantial improvement in hearing thresholds was demonstrated by auditory brainstem response measurements in the *Math1*-treated ears compared with the contralateral side at the high-frequency

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region of the guinea pig cochlea (4–24 kHz), in agreement with the restoration of inner HC morphology in this area ($p < 0.004$) (17). Hence, this study was another milestone toward the objective of clinically measured results in hearing restoration because the extracted data provided the first demonstration of a therapeutic approach that led to substantial recovery of hearing in deaf mammalian ears.

The release from the p27kip1-induced cell cycle arrest may also prove efficient in allowing postnatal supporting cell proliferation to occur (46,47). However, by reducing the inhibition of the cell cycle on the organ of Corti, pathologic findings and dysfunction may occur. Therefore, manipulations to regulate the spatiotemporal pattern of p27 inhibition will be necessary before inducing functionally useful HC regeneration (48). In the same context, the Notch signaling may provide a general developmental tool to influence the morphogenesis of the inner ear (49), and supplementation of growth factors may induce renewed HC proliferation and differentiation (50–52). Furthermore, the release of intrinsic neurotrophic factors such as activity-dependent brain-derived neurotrophic factor secretion may also be important for HC development (53).

PREVENTIVE STRATEGIES FOR INNER EAR TRAUMA

As mentioned earlier, neurotrophic factors also significantly increase the number of surviving implanted DRG neurons (9). Recent studies have also shown that intracochlear administration of a combination of brain-derived neurotrophic factor and ciliary neurotrophic factor (18,54) or NT-3 (55) protects SGNs from degeneration caused by aminoglycoside toxicity and promotes their survival and functional restoration. In addition, overexpressed transgenic glial line–derived neurotrophic factor was shown to have a robust protective effect against acoustic and ototoxic inner ear trauma (56).

Antioxidant therapy has also proved effective in protecting SGNs from deafferentation-induced degeneration. Indeed, local application of a combination of vitamins E and C into the scala tympani of deafened guinea pigs not only enhanced SGN survival but also increased electric sensitivity in the cochlear nerve. Similar results were also obtained after systematic administration of the same combination of antioxidants (19). This suggests that the formation of free radicals and the change in the oxidative state may be an important consequence of the deprivation of neurotrophic inputs from the sensory cells to the auditory neurons.

The characterization of the genes that regulate inner ear response to trauma might help in designing strategies for enhanced inner ear protection and HC regeneration (57). In this context, apoptosis and proliferation may be considered as coupled controlling factors for the regeneration of the auditory epithelium, and antiapoptotic proteins such as Bcl-2 might provide a significant level of protection against cell death (58,59). Neurotrophin factor replacement therapy might also block the initial stages of the apoptotic pathway and prove more effective toward the preservation of the auditory neurons than later stage interventions (19). The latter might include the supplementation of inhibitors against proteases that mediate cell death, namely, caspases, taking into account that the cell death that follows exposure to various ototoxic factors (i.e., aminoglycosides) reportedly occurs in a caspase-dependent manner (60,61). However, it is worth mentioning that the ongoing HC death was shown to stimulate supporting cell proliferation in the mature avian utricle, and that caspase inhibitors reduced the amount of ongoing cell death and supporting cell proliferation in a dose-dependent manner (62).

FUTURE ORIENTATION

Although it sounds exciting as a prospect, the complete restoration of the complex architecture of the cochlea might not prove feasible in the near future. However, a more approachable solution can be the attainment of a monolayer cochlear structure composed of generic HCs in a single plane similar to the organization of the avian basilar papilla (63). However, the potential benefits of such a structure cannot be assessed in advance because they are also linked to the function of the adjacent tectorial membrane. Indeed, mutations in the gene that encodes the α-tectorin protein (one of its major noncollagenous components) have also been associated with hearing impairment (64).

In addition, other mechanisms such as competence for unexpected fate may be involved during the generation of HC immunophenotypes from neural progenitors, and cell transdifferentiation may encounter significant problems because factors that regulate transdifferentiation in 1 species might not be possible to be extrapolated to other species due to the existing differences in the intrinsic properties and mechanisms that regulate gene expression. Moreover, transdifferentiation is normally tightly regulated, and complex manipulations may be required. Otherwise, uncontrolled or ectopic inappropriate differentiation is likely to be harmful (38). The establishment of an in vivo microenvironment, which can provide the biological signals that can direct SC behavior, might eventually help determine the appropriate cell fates.

CONCLUSION

Cochlear HCs are a terminally differentiated cell population that is crucial for hearing and have no possibility of spontaneous regeneration in mammals. Genetic manipulation of the genes that control the exit from the cell cycle, induction of new HCs through gene modification therapy, and introduction of SCs into damaged cochleas offer exciting new alternatives for the treatment of SNHL. There is accumulating evidence that transplanted NSCs can adopt the morphologic phenotypes of outer or inner HCs. There is also evidence that the normal cytoarchitecture in the organ of Corti can be restored through *Math1* transgene expression and transduction of the nonsensory

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cells and produce clinically measured improvement in hearing thresholds. Finally, ESC-derived neurons have the potential for synapse formation with the auditory HCs and reinnervation of the auditory epithelia. With regard to the injection site, the scala media seems more functionally appropriate for the survival of the implanted SCs, whereas a modiolar approach may be indicated for SGN restoration.

However, in-depth understanding of the underlying mechanisms of auditory genetics and continuing investigation of the human genome, along with the refinement of delivering techniques and specification of therapeutic strategies, are essential before functional regeneration of the cochlea can be achieved in clinical practice.

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