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Review

Cell- and gene-therapy approaches to inner ear repair

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Summary. Sensorineural hearing loss is the most common sensory disorder in humans. It is primarily due to the degeneration of highly specialised mechano-sensory cells in the cochlea, the so-called hair cells. Hearing problems can also be caused or further aggravated by the death of auditory sensory neurons that convey the information from the hair cells to the brain stem. Despite the discovery of stem/progenitor cells in the mammalian cochlea, no regeneration of either damaged hair cells or auditory neurons has been observed in mammals, in contrast to what is seen in avians and non-mammalian vertebrates. The reasons for this divergence have not yet been elucidated, although loss of stem cells and/or loss of their phenotypic plasticity in adult mammals have been put forward as possible explanations. Given the high incidence of this disorder and its economic and social implications, a considerable number of research lines have been set up aimed towards the regeneration of cochlear sensory cell types. This review summarizes the various routes that have been explored, ranging from the genetic modification of endogenous cells remaining in the inner ear in order to promote their transdifferentiation, to the implantation of exogenous stem or progenitor cells and their subsequent differentiation within the host tissue. Prophylactic treatments to fight against progressive sensory cell degeneration in the inner ear are also discussed.

Key words: Cochlea, Hair cells, Auditory neurons, Hearing loss

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Introduction

Worldwide, 500 million people are estimated to be affected by some form of hearing loss, and the World Health Organization has estimated that this number will rise to 900 million by 2050. This makes hearing impairment the most common sensory disorder in humans, with serious social and economic implications.

In the majority of the cases, the cause for hearing loss is directly or indirectly linked to degeneration and death of specialized mechanosensory hair cells (HCs). These cells translate the incoming sound waves into electrical signals that are then carried to the brain via their associated auditory neurons (spiral ganglion neurons, SGNs), bipolar neurons that extend one process peripherally (to the HC in the sensory epithelium) and one process centrally (to auditory centres in the brain). HC loss may result from excessive exposure to loud stimuli, bacterial and viral infections, ototoxic drugs, and mechanical trauma, with aging being an additional risk factor. Furthermore, over a hundred genetic loci have by now been identified that either cause deafness or convey susceptibility to some environmental factors that affect hearing (Hilgert et al., 2009; Dror and Avraham, 2010). Work carried out using animal models has shown that SGN degeneration may occur as a primary event, but it most often follows HC death, probably due to the lack of electric stimulation and/or some trophic factor normally provided to them by the HCs.

In the mammalian cochlea, HCs and SGNs are only produced during the fetal stages and the capacity to replace any of these sensory cells following damage is lost soon after birth. This is in contrast to what is seen in avians and non-mammalian vertebrates that are capable of regenerating HCs throughout life (Brignull et al., 2009). Studies in birds have shown that non-sensory supporting cells (SCs) adjacent to damaged HCs may transdifferentiate into new HCs. This regeneration process can occur either directly, in the absence of mitosis, or after re-entering the cell cycle (Roberson et

al., 2004; Stone and Cotanche, 2007; Brignull et al., 2009). Very importantly, afferent neurons establish functional synaptic contacts with the new HCs, resulting in significant functional recovery (Bermingham-McDonogh and Rubel, 2003). On the other hand, although Kelley et al. (1995) demonstrated a limited capacity for regeneration of HCs in embryonic mouse cochleae, cochlear SCs in the adult appear unable to reconstitute degenerated HCs (Yamasoba and Kondo, 2006). Instead, their apical borders expand to fill the lesion site and form a "scar", with no transdifferentiation into a HC phenotype taking place.

The reasons for the lack of regenerative capacity of the adult mammalian cochlea are unkown. A few possible explanations have been put forward. Warchol (2011) has proposed that it may be the result of a trade-off between phenotypic plasticity of the SC and sensitive high-frequency hearing, with a concomitant reduction in the number of HCs and further specialization of cochlear SCs into morphologically different subtypes. The complex cytoarchitecture observed in the mammalian cochlea may have resulted in the development of mechanisms that restrict the ability of SCs to divide and transdifferentiate following HC loss. In general it is considered that the regenerative ability of the sensory organ (so-called Organ of Corti in mammals, OC) is closely linked to the proliferative capacity of its SCs. SCs in the mature OC in mammals are post-mitotic cells and it has been hypothesized that their proliferative ability is perhaps lost due to the absence of certain mitogen receptors in these cells or the expression of cyclin-dependent kinase inhibitors such as p27, and/or changes in the actin cytoskeleton (White et al., 2006; Burns et al., 2008; Warchol, 2011). It has also been argued that pluripotent stem cells present in regenerating organs such as skin are absent in the mature cochlea. While stem-like cells have been isolated from the vestibular organs of mature mice (Li et al., 2003a) and even from the cochlea of neonatal mice (Oshima et al., 2009), tissues where a limited amount of regeneration occurs, this type of cell has not been identified in the cochleae of adult mice (Oshima et al., 2007).

Hearing loss: Therapeutical approaches

At present, the only available treatments for patients suffering from severe hearing loss are based on sound amplification (hearing aids) and/or cochlear implants (CIs). Cochlear implants are electronic devices that bypass the degenerated HCs and directly stimulate the remaining auditory neurons. Although SGNs have been reported to survive for many years in the absence of most or all the HCs, progressive degeneration of these neurons takes place and evidence from animal studies indicates that this degeneration may compromise the efficacy of CIs (Shepherd et al., 2004). Therefore, the success of CIs relies on the preservation of healthy sensory neurons that transmit the signal to the brain.

Although still far from the clinic, different avenues are being explored in order to tackle the problem of hearing loss, the two main objectives being the regeneration of missing cell types and the maintenance of remaining cochlear cells. Studies pursuing either of these aims have been carried out using cell or gene therapy, and/or the application of trophic factors. Critical factors to be considered when contemplating possible therapeutic approaches are the type of cell(s) damaged, the cause and extent of damage, and the overall state of the host tissue, influenced by parameters such as the time passed since the otic injury.

Regeneration of missing cell types

There are two possible routes aimed to regenerate damaged HCs or SGNs. One consists in differentiating other cell types into the sought-for phenotype or an earlier progenitor and subsequently transplanting these cells into the inner ear. Alternatively, already existing endogenous cells may be stimulated to become replacement cells. A number of excellent reviews have been recently published on this subject (Brigande and Heller, 2009; Groves, 2010; Jonkamonwiwat et al., 2010; Shibata and Raphael, 2010).

Cell therapy approaches: Implantation of exogenous cells

Considerations on the host cell type to be replaced and the status of the host tissue

When evaluating the use of exogenous cells to replace damaged HCs a number of important problems emerge, such as: (a) the difficulty to access the auditory epithelium in humans, which would imply invasive methods for the administration of exogenous cells, (b) the exposure of the implanted cells to a hostile environment in the form of the endolymph, an extracellular fluid with high potassium content that is necessary for the correct functioning of the cochlea, and, primarily, (c) the challenge of trying to insert exogenous cells into such a highly organized structure as the OC. Studies have shown the critical importance of maintaining the correct numbers as well as the organization of both HCs and SCs for the proper functioning of the OC. Furthermore, the cells in this epithelium are linked by tight junctions that prevent mixing between the endolymph and the perilymph (Kim and Raphael, 2007), but also complicate the insertion and functional integration of exogenous cells into the epithelium. In contrast, replacing damaged SGNs requires considerably less surgical invasion and precise integration of the transplanted cells *in situ*. It has already been shown that transplants of exogenous embryonic stem cells (ESCs) or ESC-derived neural progenitors into the modiolus results in survival and migration of these cells, and their differentiation into neurons that extend processes towards appropriate targets in the periphery (i.e. HC) and centrally, to the brain (Okano et

al., 2005; Corrales et al., 2006; Sekiya et al., 2006; Shi et al., 2007). Importantly, differentiation of implanted ESCs into excitatory glutamatergic neurons has already been shown *in vivo* (Reyes et al., 2008). While acquisition of this phenotype is the *sine qua non* of auditory nerve replacement, it is possible that other features of auditory neurons (e.g. specific ion channels) do not need to be faithfully replicated in order to transmit some information from the HCs via the SGN to the cochlear nucleus. Overall, these results hold promise for an improved treatment of patients who either suffer from primary neuronal degeneration or use CIs. Since degeneration of SGNs following HC damage occurs over several years after HC loss (Groves, 2010), a reasonably large time window for therapy is offered.

The state of the host tissue is a critical factor to be considered when contemplating any therapeutical approach. Of note, increased survival and differentiation of transplanted cells has been shown in damaged inner ears, compared to uninjured controls (Ulfendahl et al., 2007; Matsuoka et al., 2007). For example, Hu et al. (2005c) reported higher survival rates of adult NSCs implanted into the scala tympani (ST) of neomycin-treated guinea pigs, compared to those implanted in normal-hearing animals. Okano and colleagues (2005) saw that approximately twice the number of transplanted cells survived in injured ears compared to normal ears. These data point to the release of some factor(s) from the injured inner ear that enhances both survival and differentiation of the exogenous cells. Regarding the replacement of missing SGNs, damage clearly leads to increased survival and migration of implanted cells. This is thought to result not only from an augmented production of trophic and chemotactic factors produced by the host tissue, but also from an increase in the space available for the implant, left behind by the degenerated SGN bodies and auditory nerves (Sekiya et al., 2006; Ogita et al., 2010).

Various groups have also demonstrated that the timing of the transplant following injury plays a critical role in the survival and engraftment of the donor cells. Overall, studies have shown that the number of engrafted cells decreases as the latency between damage and exogenous cell injection increases (Parker et al., 2007). Lang and co-workers (2008) confirmed that there is an optimal time window for engraftment and survival of ESCs during the early post-injury period (within 1-3 days after injury). This effect of timing of transplantation of the exogenous cells following injury to the inner ear is thought to be due to increased metabolism in the early post-injury cochlear micro-environment, with the concomitant production and release of factors that are otherwise not present in the healthy cochlea. In agreement with this hypothesis is the observation by Tan and colleagues (2008) that infiltration of labelled bone marrow (BM)-derived cells into the damaged cochlea during the first week after deafening coincides with a transient increase in the expression of stromal-derived factor 1 (SDF-1).

Considerations on the cell types to be used as donor cells

Regarding the cell type to be used for implants, a set of different variables must again be considered. Cells are required that can survive and migrate to appropriate locations once they are implanted. Survival of implanted cells has in general been reported to be very low (Hu et al., 2004, 2005c; Ulfendahl et al., 2007). Some variability has been observed among different cell types, and cell differentiation status prior to implantation appears to also play a part. It is widely accepted that differentiated donor cell types are more likely to elicit an adverse immune response than undifferentiated ESCs, since these latter do not express human leukocyte antigen (HLA) proteins. On the other hand, different cell types have been used as donor cells in transplantation studies in the cochlea and no adverse immune reactions have been reported, leading to claims that this organ is an immunoprivileged site that will allow xenotransplantation (Hildebrand et al., 2005; Regala et al., 2005; Ulfendahl et al., 2007; Reyes et al., 2008).

The implanted cells must also be capable of differentiating into the desired phenotype(s) as well as integrating and establishing adequate functional connections within the tissue. This will likely depend on both the local environment and the ability of the implanted cells to receive and respond to the signals sent by the host tissue. Various degrees of integration of the implanted cells into the host tissue and subsequent differentiation of these cells have been reported (Corrales et al., 2006; Altschuler et al., 2008). Some groups have observed the localization of donor cells to target areas but in the absence of any sign of differentiation or integration (Hildebrand et al., 2005). However a few studies have demonstrated the establishment of new functional connections between the donor cells and the host tissue. Martínez-Monedero et al. (2006) confirmed the ability of denervated HCs, explanted from post-natal cochleae, to attract new processes from neurons growing in their proximity, as well as the formation of synapsin-expressing terminal swellings at the contact points between neurons and HCs. Formation of neurite projections from differentiating donor cells implanted into the cochlear nerve trunk toward the denervated OC was also shown *in vivo* (Corrales et al., 2006). However, formation of functional synapses has not been demonstrated yet, and electrophysiological data are generally lacking.

Besides these considerations, a human source of exogenous cells is required that allows the systematic production of clinical-grade cells for transplants under well-controlled conditions, amenable to standardization and scaling-up (Rivolta, 2010). To date, the majority of studies have been carried out using stem-like cells of various origins. Nevertheless, these are still relatively heterogeneous cell populations and in some cases their availability implies going back to primary tissues as their source. Consequently, efforts are currently being made

by some groups to render the use of conditionally immortal cell lines a safe option for possible therapies (Pollock et al., 2006; Sekiya et al., 2007; Stevanato et al., 2009). Safe and effective transplants of such cells have been carried out in animals, with no reports of tumour formation (Nicholl et al., 2005; Pollock et al., 2006; Sekiya et al., 2007) In fact, some of these cell lines have already been produced under good manufacturing practice (GMP) conditions required for clinical applications, and tested for the treatment of disorders such as stroke (Pollock et al., 2006; Stevanato et al., 2009).

Route of implantation

Besides the cell type to be replaced and the source of exogenous cells to be implanted, one must also consider the route of implantation to be used (Jonkamonwiwat et al., 2010). Minimal damage to the cochlear architecture, together with implantation into locations that are as close as possible to the target sites and that allow a widespread distribution of the donor cells throughout the auditory organ are desired features. Integrity of the membranes that seal the endolymphatic and perilymphatic chambers is critical for normal hearing and damage to these membranes must therefore be avoided or minimized.

A considerable number of studies have been carried out that delivered the donor cells into the fluid-filled compartments of the cochlea, mostly into the easily accessible ST. This latter approach results in widespread distribution of the implanted cells while minimizing mechanical trauma to the cochlear cytoarchitecture. However, the flow of perilymph and concomitant dispersal of donor cells into the cerebrospinal fluid has been suggested as one of the likely reasons for the decline in the number of surviving cells observed at the injection site (Hildebrand et al., 2005; Coleman et al., 2006). In addition, injections of donor cells in the ST has yielded very low densities of exogenous cells in target areas such as Rosenthal's Canal (RC), where the spiral ganglion cells are located, making this delivery route too inefficient for SGN replacement therapy (Coleman et al., 2006; Matsuoka et al., 2006; Altschuler et al., 2008). Consequently, direct injection of donor cells into the auditory nerve has been very often adopted as an alternative route, that requires minimal surgical trauma and results in extensive migration of the exogenous cells, not only along the entire nerve but also to the more distal scala media, where the HCs reside (Sekiya et al., 2006; Lang et al., 2008).

The route of implantation and subsequent distribution patterns of the injected cells are thought to play a role in the survival of donor cells. Overall, it appears that cells implanted intra-neurally survive better than those transplanted peri-neurally (Sekiya et al., 2006). As mentioned before, in a number of studies the donor cells were transplanted into the ST, with very low survival rates. An example of this is the work carried out by Ulfendahl et al. (2007) using ESCs. The authors hypothesized that although the ST is an easily accessible location, it probably lacks essential factors required for the survival of transplanted cells. Importantly, survival of the ESCs was greatly enhanced when these cells were implanted together with small pieces of E13-E14 dorsal root ganglion (DRG) tissue (Hu et al., 2005a), pointing to the lack of some factor(s) in the adult cochlea that might be critical for the survival of the exogenous cells. Additionally, different groups (Hildebrand et al., 2005; Lang et al., 2008) have shown that the survival of implanted cells is higher in the perilymph than in the endolymph.

Some studies have demonstrated that the location of the implanted cells within the tissue also affects their differentiation. Parker and colleagues (2007) observed that NSCs transplanted into hearing-damaged mice differentially regulated their gene and protein expression depending on their location within the OC, demonstrating an influence of the cochlear micro-environment on NSC differentiation. Different morphology of neuralized ESCs depending on their location within the host tissue was also reported by Sekiya and co-workers in the inner ears of deafened rats (2006). Carrying out transplants of neuralized ESCs into the cochleae of deafened gerbils, Lang et al. (2008) showed that a small number of donor cells that were localised within the RC had differentiated into cells that expressed neurofilament-200, a marker of mature neurons, while no signs of neuronal differentiation were found outside this region. These are encouraging observations in support of cell therapy approaches for the treatment of hearing loss, as they indicate that the mature mammalian cochlea, despite its inability to regenerate lost cells, may retain the signals necessary to drive differentiation of stem cells towards cochlear cell phenotypes.

Cell types used for cell therapy in the inner ear

Having outlined some of the most important issues to be addressed when considering a cell therapy approach for treating hearing loss, we will now proceed to summarize the main findings obtained with the various types of cells tested.

(a) Embryonic stem cells (ESCs). In a series of studies undifferentiated ESCs have been directly injected into the cochlea. One argument in favour of this approach is the plasticity of these cells, which might translate into a better response to the different micro-environmental cues the implanted ESCs will encounter within the various cochlear compartments; this would open the door to a possible regeneration of several cellular phenotypes, derived from the same transplant (Shi et al., 2007). Another advantage of using undifferentiated ESCs is their low immunogenicity, which makes a host immune response less likely.

Mouse ESCs transplanted into injured vestibulo-cochlear nerves of adult rats and guinea pigs survived up

to at least 9 weeks (Hu et al., 2004; Regala et al., 2005) and a fraction of these cells differentiated into either glial or neuronal lineages. Importantly, implanted cells were detected not only at the site of injection but also within the brain stem. When ESCs were implanted into the ST (Ulfendahl et al., 2007), surviving cells were identified close to the spiral ganglion region and along the nerve fibers projecting to OC, as well as in the scala vestibuli, indicating migration towards functionally relevant locations. Nevertheless, the survival rate of the transplanted cells was very low (1.1%-1.5%). Cell survival and neuronal differentiation of ESCs injected in the cochlea was greatly enhanced when the stem cells were implanted together with small pieces of embryonic mouse DRG tissue (Hu et al., 2005a). Moreover, formation of neurite-like projections from ßIII-tubulin-expressing donor cells towards peripheral SGN processes was only observed in the presence of the DRG co-graft. However, no differentiation of the ESCs to myosin VIIa-positive cells (a protein typically expressed by HCs) took place.

Altschuler et al. (2008) took the studies on survival and differentiation of ESCs implanted into the cochlea one step further and reported that continuous intrascalar administration of the neurotrophic factor (NF) glial-derived neurotrophic factor (GDNF) following injection of mouse ESCs into the cochleae of hearing-damaged guinea pigs resulted in increased survival of the implanted cells and a much higher rate of neuronal differentiation, compared to implants that did not receive GDNF. Moreover, the ESC-derived neurons were mostly of an excitatory glutamatergic phenotype (indicated by the expression of vesicular glutamate transporters, VGLUT1/2), and thus appropriate for functional replacement of auditory neurons. Most of the processes extended by the ESC-derived neurons were directed towards the OC or towards remaining SGNs in the modiolus. Unfortunately, no functional studies were conducted.

The data obtained from the aforementioned studies clearly indicate that successful differentiation and long-term integration of implanted ESCs will likely require either the co-grafting of some other tissue, or the simultaneous application of appropriate NFs. Alternatively, ESCs can be pre-differentiated *in vitro* in order to promote their final progression towards the desired phenotypes following implantation. One of the clear advantages of these latter methods, compared to the implants of undifferentiated ESC, is the diminished risk of tumour formation *in vivo*. The appearance of teratomas is always a major concern when injecting undifferentiated ESCs (Erdo et al., 2003; Sell, 2004), although it has not been reported by any of the groups cited above (Hu et al., 2005a; Regala et al., 2005; Altschuler et al., 2008). Other advantages are the fact that, unlike ESCs, ESC-derived populations may not be totally dependent on the cochlear environment for their survival and correct differentiation. Additionally, pre-differentiation may allow the selection of a more homogenous and appropriate population of cells for implants. Since lineage analysis studies have provided evidence that SGNs and HCs are derived from a common neural precursor cell, a very frequently adopted approach has been the differentiation of ESCs to neuroectodermal progenitors prior to transplantation (Hildebrand et al., 2005; Coleman et al., 2007; Lang et al., 2008). These cells, albeit committed to the neural lineage, can still integrate into inner ear tissue and respond to various signals present within the cochlear environment (Corrales et al., 2006). Survival of these cells after implantation into either the ST (Coleman et al., 2006) or the auditory nerve (Sekiya et al., 2006) has already been demonstrated. Regarding their differentiation, divergent results have been obtained using various animal models (Hildebrand et al., 2005; Sekiya et al., 2006). Hildebrand and co-workers (2005) failed to detect any further differentiation of ESCs that were partially pre-differentiated to the neuroectodermal lineage following their implantation into deafened guinea pigs, in spite of long survival times and localization close to the damaged OC. In contrast, Corrales and colleagues (2006) reported progressive neuronal differentiation of ESC-derived mouse neural progenitor cells after injection into the cochlear nerve of immunosuppressed gerbils, with formation of abundant neuronal processes that traversed RC and grew towards the HCs in the OC. The observed projection pattern appeared to indicate that the newly formed neurites followed the former pathways of degenerated SGNs, reponding to cues probably sent to them from the denervated OC. Moreover, these neurons expressed peripherin, a protein expressed in sensory neurons. Unfortunately, the authors could not carry out any test to assess functional recovery in the implanted animals, due to the formation of thick scar tissue at the site of surgery. Promising results were obtained by Okano and colleagues (2005), who implanted mouse ESC-derived neural progenitors into the cochlear modiolus of guinea pigs. This group confirmed survival and neural differentiation of the donor cells, which were shown to extend neurite projections toward peripheral and central auditory targets. Furthermore, auditory brain stem response (ABR) measurements indicated the potential of the transplanted cells for functional recovery of the damaged cochleae, although no direct evidence was presented for the establishment of synapses. Later on, Matsumoto and co-workers (2008) demonstrated the potential of ESC-derived neurons to form synaptic contacts with auditory HCs.

An alternative to driving ESCs into the neuroectodermal lineage prior to transplantation into the inner ear is to specifically pre-differentiate ESCs *in vitro* into the sought-after cellular type, i.e. auditory neurons or HCs. Coleman et al. (2007) co-cultured ESC-derived neural progenitors (grown in the form of floating aggregates termed embryoid bodies) with HC explants and observed that a proportion of the ESCs differentiated into neurofilament-68-protein-positive bipolar neuron-

like cells, analogous to mammalian auditory neurons grown *in vitro*. However although neuronal processes were observed, the authors did not observe any significant growth of these processes toward the HC explants. An interesting study carried out by Shi and colleagues (2007) demonstrated that, although human ESCs (hESCs) could be induced to differentiate into neurons, specification of sensory neurons required the addition of BMP-4 to hESC-derived neural progenitor cultures. The addition of BMP-4 to the cultures led to significant sensory neuron differentiation, characterized by the expression of peripherin, GATA3, neurogenin 1 (ngn1), Brn3a, TrkB and TrkC. The newly obtained neurons were shown to make contacts with HCs in denervated mouse OC explants and expressed synapsin, suggesting synapse formation. The same observation was made *in vivo* using a gerbil model: hESC-derived neurons engrafted in the auditory nerve trunk expressed peripherin, and 60 days after implantation had sent out abundant neurites that grew both peripherally toward the cochlea and centrally toward the brain stem. Unfortunately, the authors could not demonstrate synapse formation between the transplanted cells and the endogenous HCs.

Regarding *in vitro* differentiation of ESCs into HCs, work carried out by Heller and his group (Li et al., 2003b; Oshima et al., 2010) established a series of culture conditions that resulted in the expression of markers typical of HCs (e.g. Math1, Brn3c, Myosin VIIa) or SCs (Jagged-1, p27) in a proportion of ESC-derived neural progenitors. Importantly, further differentiation of these cells was accomplished by either co-culturing them with stromal cells derived from the chicken utriculus or *in vivo*, by engrafting them into developing chick otic vesicles. Under these conditions, the authors observed formation of hair-bundle-like protrusions that stained for espin and displayed electrophysiological responses similar to those obtained from immature HCs.

An interesting alternative to differentiating ESCs towards either auditory neurons or HCs prior to their implantation into a host tissue has been presented by Reyes and colleagues (2008). Making use of the already available knowledge on the cues necessary for SGN differentiation *in vivo*, this group employed a mouse ESC line that carries a tet-inducible ngn1 gene and showed that transient expression of this gene promoted the neuronal differentiation of cultured ESCs. Subsequent treatment with brain derived neurotrophic factor (BDNF) and GDNF resulted in 75% of these cells exhibiting a glutamatergic phenotype after 5 days *in vitro*. This inducible system allowed the authors to implant undifferentiated ESCs into deafened guinea pig cochleae and induce them to become neurons by forcing them to transiently express ngn1.The approach resulted in high rates of neuronal differentiation of the implanted ESCs. Upon subsequent infusion of BDNF and GDNF a high proportion (approx. 79%) of the ESC-derived neurons acquired a glutamatergic phenotype. However, no electrophysiological data were presented.

As an alternative to the differentiation of ESCs into neurons, Olivius and colleagues transplantated embryonic DRG neurons into the cochlea (Hu et al., 2004; Regala et al., 2005; Ulfendahl et al., 2007). They showed survival and migration of the implanted cells to the spiral ganglion, along the nerve fibers projecting to the OC and towards the brain stem. The rate of cell survival was very low, although a clear improvement was observed following the application of nerve growth factor (NGF) (Hu et al., 2005b) or a combination of BDNF and ciliary neurotrophic factor (CNTF). Moreover, in the presence of NGF, the DRG cells formed extensive neurite projections that in some cases appeared to contact the host SGN, although no functional interaction between the donor tissue and the host neurons was demonstrated in this work.

(b) Adult or tissue-derived (somatic) stem cells. Several types of stem or progenitor populations are found in the adult organism that may serve as an alternative to the use of ESCs. The former cell types have been attributed a more limited ability for multi-lineage differentiation compared to ESCs. Nevertheless, they offer important advantages such as the possibility of autologous transplants, thereby avoiding problems of immune rejection, and the fact that they can be isolated from the adult organism without facing ethical conflicts raised by the use of ESCs. Below we summarize the efforts made towards achieving the regeneration of inner ear sensory cell types using different sources of adult stem/progenitor cells.

(b1) Neural stem cells. Despite the ability of ESCs to give rise to a plethora of cellular phenotypes, ESCs implanted into the cochlea of experimental animals most commonly differentiate into glial cells rather than into neurons (Altschuler et al., 2008; Lang et al., 2008). A number of groups have therefore employed neural stem cells (NSCs) instead of ESCs in an attempt to facilitate the differentiation of the transplanted cells into the auditory sensory lineage. The ability of NSCs to give rise to SGN-like neurons was demonstrated *in vitro* by Wei et al. (2008), who also reported the formation of functional synapses of these cells with HCs and deafferented SGNs. *In vivo*, NSCs have been shown to differentiate into neural, glial and HC-like phenotypes (Tateya et al., 2003; Hu et al., 2005c; Parker et al., 2007). Hu and colleagues (2005c) infused adult mouse NSCs into the ST of guinea pig cochleae and reported that survival and neuronal differentiation appeared to be enhanced within injured inner ears, as compared to normal controls (Hu et al., 2005c); nevertheless, overall survival was very poor in both groups (Ito et al., 2001; Hu et al., 2005c). The authors reported migration of the injected cells along the auditory nerve tract to locations near functionally important structures, such as the OC and the spiral ganglion. However, no contact was observed between the implanted cells and the host tissue (Ulfendahl et al., 2007). Differently from the results

obtained by Hu et al. (2005c), Parker and co-workers (2007) implanted a mouse clonal NSC line into sound-damaged mouse and guinea pig cochleae and reported the differentiation of these cells into a wide range of phenotypes, depending on their localization within the cochlea. Two to six weeks after implantation, a proportion of these cells was found in the spiral ganglion area and appeared to be differentiating into satellite cells, Schwann cells, and even SGNs. Very importantly, some of these cells also labelled for synapsin, suggesting a possible competence for synaptic transmission. Altogether, these data point towards an influence of the microenvironment of the cochlea on the differentiation of the implanted cells.

In another set of experiments Hu and colleagues (2005c) transduced NSCs with neurogenin2 (ngn2) to further promote their differentiation towards a neuronal phenotype. They observed better survival rates of ngn2-expressing NSCs implanted into normal hearing guinea pig cochleae, compared to control NSCs, although the numbers of surviving cells were very low in both groups. Neuronal differentiation was also enhanced by ngn-2 transduction upon which NSC-derived ,III-tubulin-expressing cells were identified in all inner ears that contained surviving NSCs. On the other hand, embryonic NSCs transduced with the Atoh1 gene gave rise to both neurons and HCs, following their implantation into normal guinea pig cochleae (Han et al., 2010).

(b2) Bone marrow stem cells. Mesenchymal and hematopoietic stem cells. Initially isolated from BM and later on from other tissues such as muscle, synovial membranes, peripheral blood, umbilical cord blood and adipose tissue, mesenchymal stem cells (MSCs) are a heterogeneous population of stem/progenitor cells with pluripotent capacity to differentiate into cell types of all three embryonic layers (Krabbe et al., 2005). They are more easily harvested and expanded *ex vivo* than NSCs. Importantly, they have been extensively used in clinical applications, so their safety is known.

There have been a considerable number of reports on the neural differentiation of MSCs (Sánchez-Ramos et al., 2000; Woodbury et al., 2000; Deng et al., 2001). Interpretation of these data must nonetheless be carried out with caution, as it has been shown in some instances that rapid phenotypic changes of treated MSCs towards a neuronal morphology, are often the result of a cytotoxic effect (Jin et al., 2003; Lu et al., 2004). Furthermore, expression of neuronal or glial proteins (e.g. ßIII-tubulin, MAP-2, GFAP) has been demonstrated in standard MSC cultures, in the absence of any specific induction protocol (Tondreau et al., 2004). In addition, cell fusion phenomena have been observed between transplanted MSCs and host tissue. Consequently, confirmation that a transdifferentiation process of MSCs towards neural lineages has taken place requires not only that the possibility of cell fusion has been excluded, but also that morphological, immunocytochemical and electro-physiological studies are carried out (Jin et al., 2003; Krabbe et al., 2005).

The use of MSCs as donor cells for transplantations is further complicated by the fact that it is not yet clear which cell population is responsible for the observed cases of MSC transdifferentiation (Song and Sánchez-Ramos, 2003). There is some evidence for the presence of a set of quiescent primordial stem cells in adult tissues such as the BM and the brain that can differentiate into mesodermal, neuroectodermal and endodermal cell types, and thus might lead to an assumption of transdifferentiation of the cell cultures they are contaminating (Jiang et al., 2002b; Krabbe et al., 2005). These stem cells have been termed multipotent adult progenitor cells (MAPCs) (Jiang et al., 2002a). In a recent publication, Kuroda and colleagues (2010) have also reported the isolation of a type of human MSC that can give rise to cells corresponding to the three germ layers. These cells are present at very low frequencies in primary cultures of BM aspirates and have been named multilineage differentiating stress enduring (Muse) cells, as their numbers increase during passaging of BM cultures and exposure to stress conditions. An alternative hypothesis that has been proposed is that adult tissues might contain mixed populations of progenitor cells derived from distinct embryonic germ layers.

Regarding the transplants of BM-derived stem cells into the cochlea, survival and migration of these cells have been demonstrated (Naito et al., 2004; Matsuoka et al., 2007) and there are some reports on the differentiation of injected MSCs into neuronal-like cells *in vivo* (Naito et al., 2004). In order to investigate the potential for transdifferentation of BM-derived cells in the cochlea, Tan et al. (2008) isolated these cells from GFP-transgenic donor mice and engrafted them into lethally irradiated mice. Shortly after acoustic trauma, prominent GFP-positive cell infiltration was observed in the cochlea, outside the sensory epithelial regions. This infiltration was most intense during the first week after deafening, coinciding with an up-regulation of SDF-1. However, most of the infiltrated BM-derived cells were identified as macrophages and GFP-positive cells did not exhibit any cochlear characteristics. No sign of ABR threshold recovery was observed up to 8 weeks after deafening. The main conclusion from this work was that, although acoustic damage resulted in a clear increase of the homing ability of the BM-derived cells to the cochlea, compared to control cochleae, these cells did not transdifferentiate into any cochlear cell type and did not contribute to its repair. As possible explanations for these results, the authors suggested that the damaged cochlea might not be sending out the necessary cues needed to attract pluripotent cells from the BM-derived cell pool and/or it might not provide the regenerative signals required for BM-derived cells to trans-differentiate into appropriate cell types. Similar results were obtained by Lang and co-workers (2006), who confirmed homing of BM-derived cells to the inner ear of irradiated mice, outside the epithelial regions; although some implanted cells expressed proteins typical

of specialized fibrocytes involved in ion transport, the authors did not observe transdifferentiation of any donor cell into either HCs or neurons.

Overall, MSCs have been shown to survive in and migrate to multiple sites within the cochlea following implantation (Matsuoka et al., 2007). However, the rates of neuronal differentiation of these cells are reportedly very low, both in normal and in hearing-damaged animal models. In light of these results, Ogita et al. (2010) adopted a method for the neural induction of BM-MSCs isolated from adult guinea pigs prior to their transfer into the modiolus of adult guinea pigs. Neuronal differentiation of the MSCs was observed in 18,6 ± 6,4% and 24.1±5.3% of the transplants in the normal and damaged cochleae, respectively. However, no difference was found between transplanted and control animals in the number of surviving SGNs following ouabain treatment, indicating that the transplanted cells did not promote the survival of host SGNs. This was consistent with the ABR results, showing that no significant functional recovery of the damaged cochleae had occurred. This result differed from the recovery of ABR thresholds observed by the same group when using mouse ESC-derived neural progenitors (Okano et al., 2005). It is possible that the MSCs did not differentiate into the appropriate neuronal subtype (glutamatergic), and thus lacked the characteristics of auditory neurons. The ability of MSCs to differentiate into glutamatergic neurons was demonstrated *in vitro* by Kondo and colleagues (2005). These authors observed that, although a marked up-regulation of neuronal markers was observed in mouse BM-MSCs exposed to bFGF/Forskolin, expression of sensory neuronal markers was only detected when the cells were also treated with a combination of Shh and RA, two molecules that are secreted in the vicinity of peripheral sensory ganglia during embryogenesis. Co-culture experiments with prenatal mouse OC explants revealed that the BM-MSC-derived neurons extended processes towards HCs.

Not only have BM-MSCs been shown to differentiate into glutamatergic neurons *in vitro,* but also into HC-like cells, as reported by Jeon and colleagues (2007). This group used a combination of growth factors to drive mouse BM-MSCs into a neurosensory progenitor phenotype. As observed by Heller's group (Li et al., 2003b) with ESCs, further differentiation of the progenitors into a mature HC phenotype (e.g. expression of markers such as myosin VIIa, espin, and presence of protrusions resembling stereociliary bundles) was observed when these cells were either co-cultured with embryonic day 3 (E3) chick otocyst cells or injected ex vivo into E3 chick otocysts. Alternatively, further maturation of the progenitors was achieved by forced expression of the transcription factor Atoh1, required for HC formation in the inner ear.

(b3) Olfactory bulb precursor cells. The olfactory neuroepithelium is the only tissue in the body where damaged or dead neurons are replaced throughout life, and this is due to the presence of multipotent stem cells (Roisen et al., 2001; Othman et al., 2005). These cells can be easily and abundantly obtained, making this tissue a very important source of stem cells for autologous transplantation. Following culture of mouse olfactory precursor cells in the presence of conditioned medium from adult mouse cochlear cultures or in co-culture with these cells, Doyle and colleagues (2007) reported differentiation of a proportion of these precursors into cells that expressed proteins found in HCs (i.e. myosin VIIa, espin, calretinin, prestin). These results pointed to the presence of soluble factors in the cochlear cultures capable of inducing differentiation of non-auditory cells.

(b4) Ependymal cells. A population of proliferative cells that exhibit morphological and functional characteristics similar to those of HCs was isolated by Wei and co-workers (2008) from the ependymal layer of the lateral brain ventricle of adult rodents and humans. These cells expressed various HC proteins (e.g. myosin VIIa, myosin VI, the HC synaptic protein ribeye) and large-conductance FM1-43 permeable channels, possessed stereociliary and kinociliary bundles, and were capable of establishing functional synapses with primary SGNs *in vitro*. Interestingly, these cells incorporated well into the sensory epithelia of explanted cochleae from which HCs had been eliminated. The authors thus proposed ependymal cells as candidates to take over functional roles of HCs in the damaged inner ear.

(b5) Inner ear stem cells. Stem cells of the inner ear have been isolated from the vestibular organs of mice (Li et al., 2003a; Oshima et al., 2007), from the mouse OC (Oshima et al., 2007; Savary et al., 2007, 2008) and from the spiral ganglion (Rask-Andersen et al., 2005; Oshima et al., 2007), applying a sphere-formation assay routinely used with CNS stem cells (Martínez-Monedero et al., 2007). Li and colleagues (2003a) showed that inner ear stem cells isolated from the adult mouse utricle could give rise to cells from the three germ layers. Moreover they could also differentiate into auditory neurons and HC-like cells that expressed multiple HC markers, presented hair-bundle-like protrusions, and expressed functional ion channels similar to those of embryonic HCs. Martínez-Monedero and co-workers (2008) demonstrated that this differentiation could occur spontaneously *in vitro,* in the absence of any added growth factor. Inner ear stem cells gave rise to HC-like and glial cells, as well as to auditory-like neurons that responded to glutamate, fired action potentials and sent out processes towards explants of denervated OCs, where they formed contacts with HCs. Very relevant is also the finding by Rask-Andersen and colleagues (2005) who described a stem/progenitor cell population present in adult human and guinea pig spiral ganglia. These cells formed nestin-expressing spheres *in vitro* and could differentiate into neurons and glial cells. The newly emerged neurons expressed TrkB and TrkC, receptors for the NFs BDNF and neurotrophin-3 (NT-3). These data support the notion that inner ear stem cells keep some kind of memory of their tissue of origin, and

seem to follow an innate program to differentiate towards the corresponding auditory phenotypes. Oshima and co-workers (2007) pointed out intrinsic differences in the potential of stem cells isolated from distinct inner ear tissues to give rise to auditory phenotypes. Accordingly, spiral ganglion-derived spheres appear to give rise mostly to neurons and glial cell types, while spheres derived from the OC or vestibular sensory epithelia most frequently give rise to HCs.

An interesting population of auditory progenitors was identified by Rivolta and his group (Chen et al., 2007, 2009) in cochleae from 9-11-week-old human fetuses (human fetal auditory stem cells or hFASCs). In contrast to some of the progenitors described above (Rask-Andersen et al., 2005; Savary et al., 2007), these cells were capable of undergoing long-term *in vitro* expansion (i.e. for at least up to 1 year). Furthermore, hFASCs could differentiate into HC-like and sensory neuron-like cells that exhibited functional and electrophysiological properties similar to those of developing cochlear HCs and auditory neurons *in vivo*; cells expressing SC markers were also obtained from hFASCs.

The presence of stem cells in the post-natal inner ear appears contradictory with the observed lack of regeneration in the mature cochlea following otic damage. However, lower numbers of sphere-forming cells in early postnatal cochleae compared to embryonic tissue have been reported by Savary and co-workers (2008), and Oshima and colleagues (2007) recently demonstrated that there is a radical loss of stem cells in the mammalian cochlea during postnatal maturation of the auditory system. While cochlear tissues isolated from mice not older than 3 weeks of age harbour sphere-forming cells, this capacity appears to be practically absent in the cochlea of older mice. This seems to be due to either death of these stem/progenitor types or to a loss of their stem cell features. Regarding this latter possibility, in the young mammalian cochlea it is not yet clear from which cell population stem cells are derived from. Zhai and co-workers (2005) isolated a population of HC progenitors from the lesser epithelial ridge (LER) of neonatal rats. This tissue is juxtaposed to the outer hair cells and, together with the medially located greater epithelial ridge (GER), has been proposed as a potential source for HC progenitors, as both of these tissues may give rise to HCs when forced to express Atoh1 (Zheng and Gao, 2000; Shou et al., 2003; Zhai et al., 2005). Zhai and colleagues (2005) demonstrated that isolated LER cells grown as spheres in the presence of EGF proliferated and could differentiate into HC-like and SC-like cells when co-cultured with utricular mesenchymal cells, in a pattern reminiscent of the sensory epithelium *in vivo*. In another set of experiments, Malgrange and colleagues (2002) detected nestin expression in the GER region and below the IHC of rats between embryonic day 19 (E19) and postnatal day 7 (P7); they observed that *in vitro* non-adherent culture of nestin-positive cells isolated from the newborn rat OC led to their differentiation into HC-like and SC-like cells. Interestingly, nearly one third of the HC-like cells present after 2 days of suspension culture were produced following cell division, as indicated by myosin VIIa/BrdU double labeling.

It has also been hypothesized that HC progenitor cells in the early postnatal period might in fact be SCs, as it has been shown that SCs isolated from neonatal mice can divide and transdifferentiate into HC-like cells *in vitro* (White et al., 2006). Interestingly, an Abcg2-expressing side population (SP) of progenitor cells has been identified within the population of SCs in the P3 mouse cochlea and can be isolated by FACS sorting (Savary et al., 2007). These cells also express another stem cell marker, Musashi1, proliferate and give rise to HCs and SCs *in vitro*. Of note, they have a very limited capacity for self-renewal (i.e. only two passages), in contrast to stem cells isolated from the vestibular system, where a limited amount of HC regeneration has been observed. Intrinsic changes in the ability of SCs to give rise to HCs over time have also been reported. White and co-workers (2006) demonstrated that SCs isolated from P14 mouse cochleae failed to down-regulate the cyclin-dependent kinase inhibitor p27, compared to cultures of SCs isolated from neonatal mice. This inability to re-enter the cell cycle was associated with lower numbers of cells trans-differentiating into HCs. In addition to a loss of phenotypic plasticity of progenitor cells during maturation of the mammalian cochlea, the observed lack of regeneration of lost cell types in the injured auditory system might also be related with the presence of *in vivo* mechanisms that are inhibitory for cell proliferation. Data from different groups (White et al., 2006; Savary et al., 2007) point to the existence of pools of progenitor cells present in the OC of post-natal animals that are capable of proliferating under the appropriate *in vitro* culture conditions following their isolation from the original tissue.

Regeneration by endogenous cell types

As already mentioned above, one of the main hurdles when approaching inner ear cell therapy is the correct integration of the transplanted cells into the host tissue. Transdifferentiation of endogenous inner ear cell types might overcome this problem (Shibata and Raphael, 2010). Studies on avians and other non-mammalian systems (Stone and Cotanche, 2007; Cotanche, 2008; Yu et al., 2010), and the similarities found with embryonic and neonatal mammalian auditory organs (Fekete and Wu, 2002; Daudet and Lewis, 2005) have led to the hypothesis that de-differentiation of SCs, non-sensory cells that are adjacent to HCs and that share a common precursor with them, might be a potential route to the replacement of HCs. While continued expression of the cell cycle inhibitor p27 in HCs and SCs of the mature avian sensory organ does not appear to inhibit regeneration (Cotanche and Kaiser, 2010),

failure of the adult mammalian SCs to re-enter the cell cycle has been attributed, among other reasons, to their inability to suppress this inhibitor (White et al., 2006; Ono et al., 2009). Therefore, some of the attempts to obtain new HCs have focused on the down-regulation of this and other inhibitors such as retinoblastoma (Rb), which is also plays a role in maintaining the postmitotic state of SCs and HCs (Sage et al., 2005; Laine et al., 2007). Indeed, inactivation of these proliferation inhibitors in mammalian SCs has led to cell cycle re-entry of some SC populations (Minoda et al., 2007; Ono et al., 2009; Yu et al., 2010). Moreover, while some groups have not observed transdifferentiation of the cycling SCs into HCs, others have reported the production of excess SCs as well as supernumerary HCs (Chen et al., 2003; Sage et al., 2005, 2006; Weber et al., 2008) following mitosis. However, this abnormal proliferation of SCs ultimately resulted in markedly increased apoptotic rates of the newly generated cells which in turn led to a disruption of the sensory epithelium and subsequent hearing loss (Sage et al., 2005; Laine et al., 2007; Weber et al., 2008; Ono et al., 2009; Groves, 2010). Similarly, inactivation of Rb in postmitotic HCs was shown to result in cell cycle re-entry, although these cells died at different stages before mitosis was complete (Weber et al., 2008).

Failure of SCs that have re-entered the cell cycle to give rise to HCs has led to the hypothesis that additional factors are required. A main candidate is Math1/Atoh1, encoding a basic helix-loop-helix transcription factor and considered to be a master gene for the specification of the HC lineage (Woods et al., 2004; Jeon et al., 2007; Han et al., 2010). Absence of Atoh1 in knock-out mice results in the loss of HCs in the sensory epithelium and disrupted differentiation of cochlear SCs (Bermingham et al., 1999; Woods et al., 2004). On the other hand, it has been reported that over-expression of this gene may lead to the formation of supernumerary HC-like cells (Zheng and Gao, 2000; Zheng et al., 2000; Gubbels et al., 2008) both *in vitro* and *in vivo*, and this may occur in the absence of proliferation (Shou et al., 2003). These cells have been shown to express HC proteins such as myosin VIIa and calretinin and display HC morphology and stereociliary bundles (Zheng et al., 2000; Kawamoto et al., 2003; Shou et al., 2003; Izumikawa et al., 2005). In some cases, the newly emerged HCs exhibited electrophysiological properties similar to those of already existing HCs and some appeared to attract neurofilament-bearing processes from either the OC or the cochlear nucleus (Kawamoto et al., 2003; Gubbels et al., 2008). Furthermore, Izumikawa and co-workers (2005) reported reduced ABR thresholds in the ears of hearing-damaged adult guinea pigs that had been transduced with an Atoh1-GFP construct, compared to non-inoculated control ears. This was associated with the appearance of a substantial number of mature HC-like cells in the deafened cochlea. The same group observed that, while novel HCs detected within the OC exhibited normal HC-like morphology and orientation, these features were not maintained in neighbouring ectopic HCs. In fact, some of these cells displayed a mixed phenotype between HCs and SCs, pointing to transdifferentiation of SCs to HCs following misexpression of Atoh1 (Izumikawa et al., 2005). As mentioned above, other non-sensory inner ear cell types have been shown to give rise to new HCs following Atoh1 over-expression; this is the case for cells present in the GER and LER (Zheng et al., 2000; Shou et al., 2003).

With regards to transdifferentiation of non-sensory cells to HCs via their genetic manipulation it is very important to bear in mind the status of the damaged tissue before its repair (Izumikawa et al., 2005). SCs in damaged cochleae undergo a series of marked morphological changes, expanding and forming scars that prevent the perilymph and endolymph from mixing (Oesterle and Campbell, 2009). Extensive otic injury can ultimately result in the absence of differentiated SCs, which are replaced by a simple epithelium with cuboidal or flat appearance; forced expression of Atoh1 in this epithelium does not lead to any morphological changes and HCs are not regenerated, indicating that the presence of differentiated SCs is a prerequisite for Atoh1-mediated transdifferentiation (Izumikawa et al., 2008).

Together with Atoh1, the Notch signalling pathway also plays a key role in regulating the numbers of HCs that form in the OC. It has been shown that inhibition of this pathway in the mammalian inner ear leads to the formation of supernumerary HCs derived from either the transdifferentiation of SCs into HCs and/or the differentiation of sensory progenitor cells still present in early postnatal cochlear tissue (Zine et al., 2000; Kiernan et al., 2005; Yamamoto et al., 2006; Doetzlhofer et al., 2009; Groves, 2010). Nevertheless, this effect gradually diminishes during development in mammals (Zine et al., 2000) and is practically absent in adults. This is thought to result from changes in the expression of Notch pathway components (Hori et al., 2007; Batts et al., 2009; Groves, 2010). Work carried out by Ito's group (Hori et al., 2007) has revealed very low or absent Notch1 and Jagged1 expression in the cochlea of adult guinea pigs, in contrast with the higher expression levels found in the GER and SCs of embryonic auditory epithelia. In addition, and in agreement with the results obtained by Batts and co-workers (2009), Hori and colleagues (2007) observed up-regulation of both genes in the inner sulcus region of the auditory epithelium following ototoxic treatment of adult animals. Notch inhibition in these animals led to the formation of ectopic HCs, in contrast to its effects on the cochleae of normal-hearing control animals. Damage prior to Notch inhibition was therefore required for ectopic HC formation to take place. Importantly, a time window was identified in mammals when interference with Notch pathway activation may lead to an increase in HC production in damaged auditory epithelia. This appears to be dependent on the species as well as on the severity of the lesion (Oesterle et al., 2008; Batts et al., 2009).

Promoting the survival of cell types that remain in the cochlea following otic injury

Cell therapy approaches: Introduction of exogenous cells

A very important aspect of inner ear therapy consists in promoting the survival of cell types that remain following otic injury. This is especially critical in the case of SGNs, given the requirement for a certain population size of healthy SGNs that ensure the efficacy of cochlear implants in hearing-damaged patients. Several NFs have been identified that can promote regeneration and enhance the function of surviving cells (see below). Maintenance of SGNs in the damaged inner ear may require long-term administration of these factors; however, the osmotic pumps employed for intracochlear infusion of NFs have a finite life-span (Gillespie et al., 2003; Shepherd et al., 2008). Repeated replacement of these pumps conveys the serious risk of infection within the cochlea, which makes these devices unsuitable for clinical applications. On the other hand, use of viral vectors to deliver therapeutic molecules may yield high and sustained expression of the gene of interest (Liu et al., 2005; Konishi et al., 2008), but carries the risk of potential toxicity. *Ex vivo* gene manipulation of appropriate cell types and their subsequent use as a source of NFs following their transplantation into the inner ear has emerged as an alternative therapeutic option. Various cell types have already been tested, such as HSCs, NSCs, fibroblasts and Schwann cells; the latter are especially interesting, since they are amenable to genetic manipulation, can be easily obtained and used autologously. Importantly, and differently from the setting when differentiation of exogenous cells into specific inner ear cell types is sought-after, the implantation of donor cells that act as delivery vectors for the production of trophic factors should not require precise morphological integration of these cells into the host tissue (Sekiya et al., 2006). Additional support for the use of cell/gene therapy versus the direct infusion of relevant trophic factors is underlined by studies such as that carried out by Pettingill and colleagues (2008), who demonstrated that Schwann cells genetically modified to over-express BDNF elicited a greater effect on the survival of rat SGNs in vitro, compared to the direct application of recombinant BDNF. These results indicate that Schwann cells produce some additional factor(s) that acted synergistically with the secreted BDNF.

Okano and colleagues (2006) demonstrated for the first time the possibility of using genetically modified cells as vectors for the local and sustained delivery of therapeutic agents into the cochlea. Prior to transplantation, this group transfected the fibroblast cell line NIH3T3 with the BDNF gene, whose product had previously been shown to efficiently protect HCs and SGNs from various ototoxic insults. Okano and co-workers confirmed survival and settlement of the engineered cells in the perilymphatic space of the cochlea and vestibule and a significant increase in BDNF protein levels in the inner ear. Subsequent work by Pettingill and co-workers (2008) demonstrated that rat Schwann cells genetically modified to over-express BDNF or NT-3 enhanced rat SGN survival *in vitro* compared to both control Schwann cells or recombinant neurotrophin proteins.

Interestingly, it has been observed that besides genetically modified cells, also normal, unmodified cells such as stem cells of various types, e.g. HSCs, BM-MSCs or NSCs may contribute to the survival and function of remaining inner ear cell types (Krabbe et al., 2005; Yoshida et al., 2007; Whitlon et al., 2009). In this regard, the beneficial effects observed following transplantation of MSCs into injured tissue may be at least partially mediated by their production of trophic and protective factors (Krabbe et al., 2005; Caddick et al., 2006). In line with this argument are also the data by Yoshida and colleagues (2007), who reported that treatment with HSCs ameliorated progressive HC damage caused by transient cochlear ischemia in gerbils and prevented a shift in ABR thresholds. While no transdifferentiation of the implanted cells into cochlear cells or fusion events were observed, HSC injection appeared to up-regulate the expression of GDNF in the OC following ischemia.

Hakuba and co-workers (2005) observed that injection of NSCs into the inner ear of gerbils that had suffered cochlear ischemia resulted in a markedly reduced injury-induced ABR threshold shift and decreased inner HC damage, compared to the non-transplanted side. These effects were also considered to be the result of increased production of NFs or cytokines by the implanted cells. In an *in vitro* study, Chen and colleagues (2010) showed increased proliferation of auditory cell cultures grown in NSC-conditioned medium, compared to controls, and correlated this effect with higher concentrations of leukaemia inhibitory factor (LIF) in the NSC-conditioned medium, leading to activation of the LIF/JAK/STAT signalling pathway. LIF had already been previously shown to improve survival of SGNs (Marzella et al., 1999; Whitlon et al., 2006). Regarding the use of NSCs for repair of inner ear damage, it is also worth taking into account the observations made by Ourednik and colleagues (2002) in the CNS. They observed that transplanted NSCs appeared to have an inherent ability to migrate to damaged areas and, rather than differentiating into "replacement" cells, rescue the damaged host neurons from undergoing permanent functional impairment. They attributed this positive effect of undifferentiated NSCs to the secretion of important trophic and/or neuroprotective factors, such as GDNF. On the other hand, work carried out by Ito and co-workers (Iguchi et al., 2003) showed that a population of NSCs injected into mouse inner ears spontaneously differentiated into glial cells, and started expressing GDNF and BDNF, thereby contributing to SGN survival.

In addition to the production of trophic factors,

implanted cells may also exert beneficial effects on remaining auditory cell types through other means, such as the secretion of extracellular matrix proteins and the expression of cell adhesion molecules on their surface. Whitlon et al. (2006) reported improved survival of SGNs that had been co-cultured with Schwann cells, as compared to controls, and attributed this positive outcome not only to the production of NFs by the glial cells (Hansen et al., 2001) but also to the establishment of direct cell-cell contacts. The Schwann cells appeared to provide a favourable microenvironment for the regeneration of auditory neurons and served as a substrate for neuronal attachment and growth (Whitlon et al., 2009). Similar observations have been recently made with olfactory ensheathing cells (OECs): conditioned medium from these cells promoted the survival and proliferation of cultured SGNs (Liu et al., 2010; Yu et al., 2010), in agreement with the reports on the ability of these cells to secrete NFs such as NGF, BDNF, NT-3 and GDNF. Nevertheless, survival of the plated SGNs was most markedly enhanced when the neurons were cultured in direct contact with the OECs themselves (Liu et al., 2010). Interestingly, expression of adhesion molecules implicated in neuronal survival (e.g. NCAM) was detected in OECs (Liu et al., 2010).

Protection via administration of survival factors

As mentioned above, maintenance of a certain population of functional SGNs is necessary for CIs to exert a beneficial effect following HC loss. SGN development and survival is dependent on the presence of various NFs as well as neural activity (Schimmang et al., 2003; Alam et al., 2007; Hildebrand et al., 2008; Shepherd et al., 2008) which are provided to a large extent by HCs and SCs within the OC. While HC degeneration is mostly irreversible by the time it is detected, SGN loss is usually a slow process in humans, offering a reasonably wide therapeutic window. Regarding HC loss, factors such as GDNF and NT-3, as well as anti-oxidants have been identified (Shoji et al., 2000a,b; Hakuba et al., 2003; Liu et al., 2008) that could exert a prophylactic effect on the survival and function of HCs if administered prior to otic damage. Application of these factors may be useful for some groups of patients who require aminoglycoside treatment (e.g. gentamicin) and are at risk of developing hearing loss (Talaska et al., 2006; Bitner-Glindzicz et al., 2010; Prayle and Smyth, 2010). Regarding the protection/regeneration of SGNs, administration of NFs, electrical stimulation and anti-oxidative treatment are the approaches most extensively studied (Pettingill et al., 2007; Shepherd et al., 2008). It has been demonstrated that expression of NF receptors is maintained in SGNs of deafened animals (Gillespie et al., 2004; Hurley et al., 2004) and NF administration has resulted in improved survival of auditory neurons (Meen et al., 2009). Of note, improved SGN survival was also obtained with some NF combinations in studies where the administration of the treatment was delayed to a timepoint when degenerative and apoptotic processes were already ongoing (Yamagata et al., 2004; Miller et al., 2007; Pettingill et al., 2007). In addition to improved survival, some studies have reported enhanced resprouting of auditory peripheral processes (Altschuler et al., 1999; Wise et al., 2005). A considerable amount of work has been done on the delivery of BDNF and/or NT-3 into hearing-damaged animal models, using osmotic pumps or alginate beads (Gillespie et al., 2004; Yamagata et al., 2004; Noushi et al., 2005; Richardson et al., 2005; Wise et al., 2005). Other factors that have been administered are NGF, GDNF, CNTF, LIF, transforming growth factor-ß (TGF-ß), and fibroblast growth factor (FGF), alone or in different combinations (Marzella et al., 1999; Shoji et al., 2000b; Shinohara et al., 2002; Gillespie et al., 2004; Gillespie and Shepherd, 2005; Miller et al., 2007). Some studies used viral vectors for NF expression (Yagi et al., 2000; Kanzaki et al., 2002; Nakaizumi et al., 2004), since it has been shown that long-term administration of these compounds is necessary in order to obtain a significant effect on SGN survival (Gillespie et al., 2003; Richardson et al., 2005). Very recent work has focused on the specific targeting of nanoparticles to SGNs, using organotypic explant cultures of the inner ear (Roy et al., 2010). This might be a new therapeutic option for the selective delivery of NFs that should reduce the risk of non-specific effects of the administered factors on other cell types. Unregulated NF administration might lead to errors in the re-innervation patterns of the surviving SGNs, since it is known that NFs not only exert an effect on neuron survival but also on axon guidance (Tessarollo et al., 2004).

Following the same trend as other studies on NF administration, intracochlear infusion of GDNF or a combination of BDNF and CNTF (Shinohara et al., 2002) has been shown to enhance SGN survival in damaged inner ears (Gillespie and Shepherd, 2005; Fransson et al., 2010). Importantly, these two studies associated increased SGN survival rates to significantly lower ABR thresholds, compared to non-treated damaged controls. Interestingly, while there have been some reports on an accelerated loss of SGNs following BDNF treatment withdrawal (Gillespie et al., 2003; Shepherd et al., 2008), Ulfendahl and co-workers (Maruyama et al., 2008; Fransson et al., 2010) reported that the beneficial effects of GDNF administration on SGN survival were maintained for at least two to four weeks after the treatment had ceased. Although differential effects of GDNF and BDNF and/or the production of endogenous survival factors after a certain period of time following otic injury could explain this divergence, it must also be mentioned that the data on the effects following BDNF withdrawal do not appear to be consistent (Miller et al., 2006; Agterberg et al., 2009).

Chronic electrical stimulation (ES) has been demonstrated to enhance the effects of NFs on SGN survival (Miller and Altschuler, 2004; Shepherd et al.,

2005). Work done by Shepherd and colleagues (2005) revealed that while chronic ES of damaged guinea pig cochleae did not enhance SGN survival compared to non-treated controls, co-treatment with both ES and BDNF led to increased SGN survival and reduced ABR thresholds when compared both to deafened controls and also to cochleae that had only received BDNF. These results pointed to a synergistic interaction between both treatment regimes. Interestingly, it was shown that, following an initial period of ES and BDNF co-treatment, ES could on its own sustain SGN survival, overcoming the requirement for continuous BDNF administration (Shepherd et al., 2008). Highly relevant for the possible application of NF therapy to humans is the observation that combined ES/NT therapy protects SGNs from degenerating and preserves functional responses even when the application of the treatment is delayed (Yamagata et al., 2004; Song et al., 2008; Scheper et al., 2009) and significant neurodegeneration has already taken place.

Another therapeutic approach consisted in the application of anti-oxidants, based on the observation that otic damage of different etiologies leads to formation of reactive oxygen species that ultimately results in apoptotic or necrotic processes (Henderson et al., 2006; Poirrier et al., 2010). In this context, Maruyama and colleagues (2007) demonstrated that anti-oxidant treatment increased both survival and electrical responsiveness of SGNs in hearing-damaged animals.

Conclusions

The majority of sensorineural hearing loss cases are due to the degeneration of the mechanotransducing cells in the inner ear, the hair cells. Their loss is irreversible and any therapeutical approach based on the implantation of exogenous cells into the cochlea will face the formidable task of integrating these cells into the highly organized and complex cytoarchitecture of the Organ of Corti. Therefore, it is likely that current efforts directed to the ex vivo differentiation of exogenous cells into HC-like cells will find their most immediate use in drug testing and expression profiling studies. The latter studies will be crucial for the improvement of the current HC differentiation protocols and, especially, the establishment of future therapies, as they should shed light on the factors that control HC specification and differentiation *in vitro*, processes that have not yet been well characterized. At present it appears that the best approach towards HC replacement strategies would be based on the differentiation of endogenous inner ear cells towards the sensory lineage, as shown by the promising results obtained by Izumikawa and colleagues (2005) following Atoh1 overexpression in hearing-damaged cochleae. Some evidence exists pointing to the capacity of some supporting cell (SC) populations to act as the sought-for stem-like cells in the inner ear (Savary et al., 2007); the loss of their plasticity in the adult organism has been linked to changes in their phenotype and/or changes in the surrounding tissue. Induced alterations in some of their properties, such as their cytoskeletal arrangement or their quiescent status, via Rho inhibitors (Meyers and Corwin, 2007; Burns et al., 2008) and/or cyclin-dependent kinase inhibitors, could perhaps potentiate the effects observed upon ectopic expression of genes such as Atoh1.

Cell therapy approaches might be more easily applied to protocols aimed at either promoting the survival of cochlear cell types or replacing degenerated auditory neurons, processes that will likely not require such a highly precise integration of the donor cells into the host tissue. A wide range of cell types have already been evaluated for their possible use in cell-based therapies. Most of them were not human in their origin and therefore very extensive characterisation of possible human cell sources will still be required prior to their use in the clinic. In this regard, results obtained in our lab using human MSCs suggest that compared to murine MSCs there may exist important differences between their *in vitro* responses to differentiation cues aimed at driving them towards auditory sensory lineages. On the other hand, current work on induced pluripotent stem cells (Takahashi and Yamada, 2006; Takahashi et al., 2006, 2007; Tsuji et al., 2010) is yielding promising results in the auditory system (Nishimura et al., 2009; Oshima et al., 2010); these cells might represent a compromise between critical parameters such as ease of isolation and expansion, phenotypic plasticity and availability for autologous transplantation.

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References

Agterberg M.J., Versnel H., van Dijk L.M., de Groot J.C. and Klis S.F. (2009). Enhanced survival of spiral ganglion cells after cessation of treatment with brain-derived neurotrophic factor in deafened guinea pigs. J. Assoc. Res. Otolaryngol. 10, 355-367.

Alam S.A., Robinson B.K., Huang J. and Green S.H. (2007). Prosurvival and proapoptotic intracellular signaling in rat spiral ganglion neurons in vivo after the loss of hair cells. J. Comp. Neurol. 503, 832-852.

Altschuler R.A., Cho Y., Ylikoski J., Pirvola U., Magal E. and Miller J.M. (1999). Rescue and regrowth of sensory nerves following deafferentation by neurotrophic factors. Ann. N. Y. Acad. Sci. 884, 305-311.

Altschuler R.A., O'Shea K.S. and Miller J.M. (2008). Stem cell transplantation for auditory nerve replacement. Hear. Res. 242, 110-116.

Batts S.A., Shoemaker C.R. and Raphael Y. (2009). Notch signaling and Hes labeling in the normal and drug-damaged organ of Corti. Hear. Res. 249, 15-22.

Bermingham N.A., Hassan B.A., Price S.D., Vollrath M.A., Ben-Arie N., Eatock R.A., Bellen H.J., Lysakowski A. and Zoghbi H.Y. (1999). Math1: an essential gene for the generation of inner ear hair cells. Science 284, 1837-1841.

Bermingham-McDonogh O. and Rubel E.W. (2003). Hair cell regeneration: winging our way towards a sound future. Curr. Opin.

Neurobiol. 13, 119-126.

Bitner-Glindzicz M., Osei-Lah V., Colvin I., Sirimanna T., Lucas D., Mac Ardle B., Webb D., Shankar A., Kingston J., Jenkins L. and Rahman S. (2010). Aminoglycoside-induced deafness during treatment of acute leukaemia. Arch. Dis. Child. 95, 153-155.

Brigande J.V. and Heller S. (2009). Quo vadis, hair cell regeneration? Nat. Neurosci. 12, 679-685.

Brignull H.R., Raible D.W. and Stone J.S. (2009). Feathers and fins: non-mammalian models for hair cell regeneration. Brain Res. 1277, 12-23.

Burns J., Christophel J.J., Collado M.S., Magnus C., Carfrae M. and Corwin J.T. (2008). Reinforcement of cell junctions correlates with the absence of hair cell regeneration in mammals and its occurrence in birds. J. Comp. Neurol. 511, 396-414.

Caddick J., Kingham P.J., Gardiner N.J., Wiberg M. and Terenghi G. (2006). Phenotypic and functional characteristics of mesenchymal stem cells differentiated along a Schwann cell lineage. Glia 54, 840-849.

Chen H.C., Ma H.I., Sytwu H.K., Wang H.W., Chen C.C., Liu S.C., Chen C.H., Chen H.K. and Wang C.H. (2010). Neural stem cells secrete factors that promote auditory cell proliferation via a leukemia inhibitory factor signaling pathway. J. Neurosci. Res. 88, 3308-3318.

Chen P., Zindy F., Abdala C., Liu F., Li X., Roussel M.F. and Segil N. (2003). Progressive hearing loss in mice lacking the cyclin-dependent kinase inhibitor Ink4d. Nat. Cell Biol. 5, 422-426.

Chen W., Cacciabue-Rivolta D.I., Moore H.D. and Rivolta M.N. (2007). The human fetal cochlea can be a source for auditory progenitors/stem cells isolation. Hear. Res. 233, 23-29.

Chen W., Johnson S.L., Marcotti W., Andrews P.W., Moore H.D. and Rivolta M.N. (2009). Human fetal auditory stem cells can be expanded in vitro and differentiate into functional auditory neurons and hair cell-like cells. Stem Cells 27, 1196-1204.

Coleman B., Fallon J.B., Pettingill L.N., de Silva M.G. and Shepherd R.K. (2007). Auditory hair cell explant co-cultures promote the differentiation of stem cells into bipolar neurons. Exp. Cell Res. 313, 232-243.

Coleman B., Hardman J., Coco A., Epp S., de Silva M., Crook J. and Shepherd R. (2006). Fate of embryonic stem cells transplanted into the deafened mammalian cochlea. Cell Transplant. 15, 369-380.

Corrales C.E., Pan L., Li H., Liberman M.C., Heller S. and Edge A.S. (2006). Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti. J. Neurobiol. 66, 1489-1500.

Cotanche D.A. (2008). Genetic and pharmacological intervention for treatment/prevention of hearing loss. J. Commun. Disord. 41, 421-443.

Cotanche D.A. and Kaiser C.L. (2010). Hair cell fate decisions in cochlear development and regeneration. Hear. Res. 266, 18-25.

Cotanche D.A., Lee K.H., Stone J.S. and Picard D.A. (1994). Hair cell regeneration in the bird cochlea following noise damage or ototoxic drug damage. Anat. Embryol. (Berl) 189, 1-18.

Daudet N. and Lewis J. (2005). Two contrasting roles for Notch activity in chick inner ear development: specification of prosensory patches and lateral inhibition of hair-cell differentiation. Development 132, 541-551.

Deng W., Obrocka M., Fischer I. and Prockop D.J. (2001). In vitro differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP. Biochem. Biophys. Res. Commun. 282, 148-152.

Doetzlhofer A., Basch M.L., Ohyama T., Gessler M., Groves A.K. and Segil N. (2009). Hey2 regulation by FGF provides a Notch-independent mechanism for maintaining pillar cell fate in the organ of Corti. Dev. Cell. 16, 58-69.

Doyle K.L., Kazda A., Hort Y., McKay S.M. and Oleskevich S. (2007). Differentiation of adult mouse olfactory precursor cells into hair cells in vitro. Stem Cells 25, 621-627.

Dror A.A. and Avraham K.B. (2010). Hearing impairment: a panoply of genes and functions. Neuron 68, 293-308.

Erdo F., Buhrle C., Blunk J., Hoehn M., Xia Y., Fleischmann B., Focking M., Kustermann E., Kolossov E., Hescheler J., Hossmann K.A. and Trapp T. (2003). Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. J. Cereb. Blood Flow Metab. 23, 780-785.

Fekete D.M. and Wu D.K. (2002). Revisiting cell fate specification in the inner ear. Curr. Opin. Neurobiol. 12, 35-42.

Fransson A., Maruyama J., Miller J.M. and Ulfendahl M. (2010). Post-treatment effects of local GDNF administration to the inner ears of deafened guinea pigs. J. Neurotrauma 27, 1745-1751.

Gillespie L.N. and Shepherd R.K. (2005). Clinical application of neurotrophic factors: the potential for primary auditory neuron protection. Eur. J. Neurosci. 22, 2123-2133.

Gillespie L.N., Clark G.M., Bartlett P.F. and Marzella P.L. (2003). BDNF-induced survival of auditory neurons in vivo: Cessation of treatment leads to accelerated loss of survival effects. J. Neurosci. Res. 71, 785-790.

Gillespie L.N., Clark G.M. and Marzella P.L. (2004). Delayed neurotrophin treatment supports auditory neuron survival in deaf guinea pigs. Neuroreport 15, 1121-1125.

Groves A.K. (2010). The challenge of hair cell regeneration. Exp. Biol. Med. (Maywood) 235, 434-446.

Gubbels S.P., Woessner D.W., Mitchell J.C., Ricci A.J. and Brigande J.V. (2008). Functional auditory hair cells produced in the mammalian cochlea by in utero gene transfer. Nature 455, 537-541.

Hakuba N., Hata R., Morizane I., Feng G., Shimizu Y., Fujita K., Yoshida T., Sakanaka M. and Gyo K. (2005). Neural stem cells suppress the hearing threshold shift caused by cochlear ischemia. Neuroreport 16, 1545-1549.

Hakuba N., Watabe K., Hyodo J., Ohashi T., Eto Y., Taniguchi M., Yang L., Tanaka J., Hata R. and Gyo K. (2003). Adenovirus-mediated overexpression of a gene prevents hearing loss and progressive inner hair cell loss after transient cochlear ischemia in gerbils. Gene Ther. 10, 426-433.

Han Z., Yang J.M., Chi F.L., Cong N., Huang Y.B., Gao Z. and Li W. (2010). Survival and fate of transplanted embryonic neural stem cells by Atoh1 gene transfer in guinea pigs cochlea. Neuroreport 21, 490-496.

Hansen M.R., Vijapurkar U., Koland J.G. and Green S.H. (2001). Reciprocal signaling between spiral ganglion neurons and Schwann cells involves neuregulin and neurotrophins. Hear. Res. 161, 87-98.

Henderson D., Bielefeld E.C., Harris K.C. and Hu B.H. (2006). The role of oxidative stress in noise-induced hearing loss. Ear Hear. 27, 1-19.

Hildebrand M.S., Dahl H.H., Hardman J., Coleman B., Shepherd R.K. and de Silva M.G. (2005). Survival of partially differentiated mouse embryonic stem cells in the scala media of the guinea pig cochlea. J. Assoc. Res. Otolaryngol. 6, 341-354.

Hildebrand M.S., Newton S.S., Gubbels S.P., Sheffield A.M., Kochhar A., de Silva M.G., Dahl H.H., Rose S.D., Behlke M.A. and Smith R.J. (2008). Advances in molecular and cellular therapies for hearing loss. Mol. Ther. 16, 224-236.

Hilgert N., Smith R.J. and Van Camp G. (2009). Function and

expression pattern of nonsyndromic deafness genes. Curr. Mol. Med. 9, 546-564.

Hori R., Nakagawa T., Sakamoto T., Matsuoka Y., Takebayashi S. and Ito J. (2007). Pharmacological inhibition of Notch signaling in the mature guinea pig cochlea. Neuroreport 18, 1911-1914.

Hu Z., Ulfendahl M. and Olivius N.P. (2004). Central migration of neuronal tissue and embryonic stem cells following transplantation along the adult auditory nerve. Brain Res. 1026, 68-73.

Hu Z., Andang M., Ni D. and Ulfendahl M. (2005a). Neural cograft stimulates the survival and differentiation of embryonic stem cells in the adult mammalian auditory system. Brain Res. 1051, 137-144.

Hu Z., Ulfendahl M. and Olivius N.P. (2005b). NGF stimulates extensive neurite outgrowth from implanted dorsal root ganglion neurons following transplantation into the adult rat inner ear. Neurobiol. Dis. 18, 184-192.

Hu Z., Wei D., Johansson C.B., Holmstrom N., Duan M., Frisen J. and Ulfendahl M. (2005c). Survival and neural differentiation of adult neural stem cells transplanted into the mature inner ear. Exp. Cell Res. 302, 40-47.

Hurley F.M., Costello D.J. and Sullivan A.M. (2004). Neuroprotective effects of delayed administration of growth/differentiation factor-5 in the partial lesion model of Parkinson's disease. Exp. Neurol. 185, 281-289.

Iguchi F., Nakagawa T., Tateya I., Kim T.S., Endo T., Taniguchi Z., Naito Y. and Ito J. (2003). Trophic support of mouse inner ear by neural stem cell transplantation. Neuroreport 14, 77-80.

Ito J., Kojima K. and Kawaguchi S. (2001). Survival of neural stem cells in the cochlea. Acta Otolaryngol. 121, 140-142.

Izumikawa M., Minoda R., Kawamoto K., Abrashkin K.A., Swiderski D.L., Dolan D.F., Brough D.E. and Raphael Y. (2005). Auditory hair cell replacement and hearing improvement by Atoh1 gene therapy in deaf mammals. Nat. Med. 11, 271-276.

Izumikawa M., Batts S.A., Miyazawa T., Swiderski D.L. and Raphael Y. (2008). Response of the flat cochlear epithelium to forced expression of Atoh1. Hear. Res. 240, 52-56.

Jeon S.J., Oshima K., Heller S. and Edge A.S. (2007). Bone marrow mesenchymal stem cells are progenitors in vitro for inner ear hair cells. Mol. Cell. Neurosci. 34, 59-68.

Jiang Y., Jahagirdar B.N., Reinhardt R.L., Schwartz R.E., Keene C.D., Ortiz-Gonzalez X.R., Reyes M., Lenvik T., Lund T., Blackstad M., Du J., Aldrich S., Lisberg A., Low W.C., Largaespada D.A. and Verfaillie C.M. (2002a). Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418, 41-49.

Jiang Y., Vaessen B., Lenvik T., Blackstad M., Reyes M. and Verfaillie C.M. (2002b). Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp. Hematol. 30, 896-904.

Jin K., Mao X.O., Batteur S., Sun Y. and Greenberg D.A. (2003). Induction of neuronal markers in bone marrow cells: differential effects of growth factors and patterns of intracellular expression. Exp. Neurol. 184, 78-89.

Jongkamonwiwat N., Zine A. and Rivolta M.N. (2010). Stem cell based therapy in the inner ear: appropriate donor cell types and routes for transplantation. Curr. Drug Targets 11, 888-897.

Kanzaki S., Stover T., Kawamoto K., Prieskorn D.M., Altschuler R.A., Miller J.M. and Raphael Y. (2002). Glial cell line-derived neurotrophic factor and chronic electrical stimulation prevent VIII cranial nerve degeneration following denervation. J. Comp. Neurol. 454, 350-360.

Kawamoto K., Ishimoto S., Minoda R., Brough D.E. and Raphael Y. (2003). Math1 gene transfer generates new cochlear hair cells in mature guinea pigs in vivo. J. Neurosci. 23, 4395-4400.

Kelley M.W., Talreja D.R. and Corwin J.T. (1995). Replacement of hair cells after laser microbeam irradiation in cultured organs of corti from embryonic and neonatal mice. J. Neurosci. 15, 3013-3026.

Kiernan A.E., Cordes R., Kopan R., Gossler A. and Gridley T. (2005). The Notch ligands DLL1 and JAG2 act synergistically to regulate hair cell development in the mammalian inner ear. Development 132, 4353-4362.

Kim Y.H. and Raphael Y. (2007). Cell division and maintenance of epithelial integrity in the deafened auditory epithelium. Cell. Cycle 6, 612-619.

Kondo T., Johnson S.A., Yoder M.C., Romand R. and Hashino E. (2005). Sonic hedgehog and retinoic acid synergistically promote sensory fate specification from bone marrow-derived pluripotent stem cells. Proc. Natl. Acad. Sci. USA 102, 4789-4794.

Konishi M., Kawamoto K., Izumikawa M., Kuriyama H. and Yamashita T. (2008). Gene transfer into guinea pig cochlea using adeno-associated virus vectors. J. Gene Med. 10, 610-618.

Krabbe C., Zimmer J. and Meyer M. (2005). Neural transdifferentiation of mesenchymal stem cells--a critical review. APMIS 113, 831-844.

Kuroda Y., Kitada M., Wakao S., Nishikawa K., Tanimura Y., Makinoshima H., Goda M., Akashi H., Inutsuka A., Niwa A., Shigemoto T., Nabeshima Y., Nakahata T., Nabeshima Y., Fujiyoshi Y. and Dezawa M. (2010). Unique multipotent cells in adult human mesenchymal cell populations. Proc. Natl. Acad. Sci. USA 107, 8639-8643.

Laine H., Doetzlhofer A., Mantela J., Ylikoski J., Laiho M., Roussel M.F., Segil N. and Pirvola U. (2007). p19(Ink4d) and p21(Cip1) collaborate to maintain the postmitotic state of auditory hair cells, their codeletion leading to DNA damage and p53-mediated apoptosis. J. Neurosci. 27, 1434-1444.

Lang H., Ebihara Y., Schmiedt R.A., Minamiguchi H., Zhou D., Smythe N., Liu L., Ogawa M. and Schulte B.A. (2006). Contribution of bone marrow hematopoietic stem cells to adult mouse inner ear: mesenchymal cells and fibrocytes. J. Comp. Neurol. 496, 187-201.

Lang H., Schulte B.A., Goddard J.C., Hedrick M., Schulte J.B., Wei L. and Schmiedt R.A. (2008). Transplantation of mouse embryonic stem cells into the cochlea of an auditory-neuropathy animal model: effects of timing after injury. J. Assoc. Res. Otolaryngol. 9, 225-240.

Li H., Liu H. and Heller S. (2003a). Pluripotent stem cells from the adult mouse inner ear. Nat. Med. 9, 1293-1299.

Li H., Roblin G., Liu H. and Heller S. (2003b). Generation of hair cells by stepwise differentiation of embryonic stem cells. Proc. Natl. Acad. Sci. USA 100, 13495-13500.

Liu Q., Ye J., Yu H., Li H., Dai C., Gu Y., Zhu Y. and Zhang Z. (2010). Survival-enhancing of spiral ganglion cells under influence of olfactory ensheathing cells by direct cellular contact. Neurosci. Lett. 478, 37-41.

Liu Y., Okada T., Sheykholeslami K., Shimazaki K., Nomoto T., Muramatsu S., Kanazawa T., Takeuchi K., Ajalli R., Mizukami H., Kume A., Ichimura K. and Ozawa K. (2005). Specific and efficient transduction of Cochlear inner hair cells with recombinant adeno-associated virus type 3 vector. Mol. Ther. 12, 725-733.

Liu Y., Okada T., Shimazaki K., Sheykholeslami K., Nomoto T., Muramatsu S., Mizukami H., Kume A., Xiao S., Ichimura K. and Ozawa K. (2008). Protection against aminoglycoside-induced ototoxicity by regulated AAV vector-mediated GDNF gene transfer into the cochlea. Mol. Ther. 16, 474-480.

Lu P., Blesch A. and Tuszynski M.H. (2004). Induction of bone marrow

stromal cells to neurons: differentiation, transdifferentiation, or artifact? J. Neurosci. Res. 77, 174-191.

Malgrange B., Belachew S., Thiry M., Nguyen L., Rogister B., Alvarez M.L., Rigo J.M., Van De Water T.R., Moonen G. and Lefebvre P.P. (2002). Proliferative generation of mammalian auditory hair cells in culture. Mech. Dev. 112, 79-88.

Martinez-Monedero R., Corrales C.E., Cuajungco M.P., Heller S. and Edge A.S. (2006). Reinnervation of hair cells by auditory neurons after selective removal of spiral ganglion neurons. J. Neurobiol. 66, 319-331.

Martinez-Monedero R., Oshima K., Heller S. and Edge A.S. (2007). The potential role of endogenous stem cells in regeneration of the inner ear. Hear. Res. 227, 48-52.

Martinez-Monedero R., Yi E., Oshima K., Glowatzki E. and Edge A.S. (2008). Differentiation of inner ear stem cells to functional sensory neurons. Dev. Neurobiol. 68, 669-684.

Maruyama J., Yamagata T., Ulfendahl M., Bredberg G., Altschuler R.A. and Miller J.M. (2007). Effects of antioxidants on auditory nerve function and survival in deafened guinea pigs. Neurobiol. Dis. 25, 309-318.

Maruyama J., Miller J.M. and Ulfendahl M. (2008). Glial cell line-derived neurotrophic factor and antioxidants preserve the electrical responsiveness of the spiral ganglion neurons after experimentally induced deafness. Neurobiol. Dis. 29, 14-21.

Marzella P.L., Gillespie L.N., Clark G.M., Bartlett P.F. and Kilpatrick T.J. (1999). The neurotrophins act synergistically with LIF and members of the TGF-beta superfamily to promote the survival of spiral ganglia neurons in vitro. Hear. Res. 138, 73-80.

Matsumoto M., Nakagawa T., Kojima K., Sakamoto T., Fujiyama F. and Ito J. (2008). Potential of embryonic stem cell-derived neurons for synapse formation with auditory hair cells. J. Neurosci. Res. 86, 3075-3085.

Matsuoka A.J., Kondo T., Miyamoto R.T. and Hashino E. (2006). In vivo and in vitro characterization of bone marrow-derived stem cells in the cochlea. Laryngoscope 116, 1363-1367.

Matsuoka A.J., Kondo T., Miyamoto R.T. and Hashino E. (2007). Enhanced survival of bone-marrow-derived pluripotent stem cells in an animal model of auditory neuropathy. Laryngoscope 117, 1629-1635.

Meen E., Blakley B. and Quddusi T. (2009). Brain-derived nerve growth factor in the treatment of sensorineural hearing loss. Laryngoscope 119, 1590-1593.

Meyers J.R. and Corwin J.T. (2007). Shape change controls supporting cell proliferation in lesioned mammalian balance epithelium. J. Neurosci. 27, 4313-4325.

Miller J. and Altschuler R. (2004). Neurotrophic factor and electrical stimulation promotes auditory nerve survival and regrowth following deafness. ARO Midwinter meeting, 90.

Miller J., Prieskorn D., Lee S., Kim J., Mitchell A. and Altschuler R. (2006). Auditory nerve survival remains enhanced four weeks following cessation of treatment with glial cell-line derived neurotrophic factor. ARO Midwinter meeting, Abstract 891.

Miller J.M., Le Prell C.G., Prieskorn D.M., Wys N.L. and Altschuler R.A. (2007). Delayed neurotrophin treatment following deafness rescues spiral ganglion cells from death and promotes regrowth of auditory nerve peripheral processes: effects of brain-derived neurotrophic factor and fibroblast growth factor. J. Neurosci. Res. 85, 1959-1969.

Minoda R., Izumikawa M., Kawamoto K., Zhang H. and Raphael Y. (2007). Manipulating cell cycle regulation in the mature cochlea. Hear. Res. 232, 44-51.

Naito Y., Nakamura T., Nakagawa T., Iguchi F., Endo T., Fujino K., Kim T.S., Hiratsuka Y., Tamura T., Kanemaru S., Shimizu Y. and Ito J. (2004). Transplantation of bone marrow stromal cells into the cochlea of chinchillas. Neuroreport 15, 1-4.

Nakaizumi T., Kawamoto K., Minoda R. and Raphael Y. (2004). Adenovirus-mediated expression of brain-derived neurotrophic factor protects spiral ganglion neurons from ototoxic damage. Audiol. Neurootol. 9, 135-143.

Nicholl A.J., Kneebone A., Davies D., Cacciabue-Rivolta D.I., Rivolta M.N., Coffey P. and Holley M.C. (2005). Differentiation of an auditory neuronal cell line suitable for cell transplantation. Eur. J. Neurosci. 22, 343-353.

Nishimura K., Nakagawa T., Ono K., Ogita H., Sakamoto T., Yamamoto N., Okita K., Yamanaka S. and Ito J. (2009). Transplantation of mouse induced pluripotent stem cells into the cochlea. Neuroreport 20, 1250-1254.

Noushi F., Richardson R.T., Hardman J., Clark G. and O'Leary S. (2005). Delivery of neurotrophin-3 to the cochlea using alginate beads. Otol. Neurotol. 26, 528-533.

Oesterle E.C. and Campbell S. (2009). Supporting cell characteristics in long-deafened aged mouse ears. J. Assoc. Res. Otolaryngol. 10, 525-544.

Oesterle E.C., Campbell S., Taylor R.R., Forge A. and Hume C.R. (2008). Sox2 and JAGGED1 expression in normal and drug-damaged adult mouse inner ear. J. Assoc. Res. Otolaryngol. 9, 65-89.

Ogita H., Nakagawa T., Sakamoto T., Inaoka T. and Ito J. (2010). Transplantation of bone marrow-derived neurospheres into guinea pig cochlea. Laryngoscope 120, 576-581.

Okano T., Nakagawa T., Endo T., Kim T.S., Kita T., Tamura T., Matsumoto M., Ohno T., Sakamoto T., Iguchi F. and Ito J. (2005). Engraftment of embryonic stem cell-derived neurons into the cochlear modiolus. Neuroreport 16, 1919-1922.

Okano T., Nakagawa T., Kita T., Endo T. and Ito J. (2006). Cell-gene delivery of brain-derived neurotrophic factor to the mouse inner ear. Mol. Ther. 14, 866-871.

Ono K., Nakagawa T., Kojima K., Matsumoto M., Kawauchi T., Hoshino M. and Ito J. (2009). Silencing p27 reverses post-mitotic state of supporting cells in neonatal mouse cochleae. Mol. Cell. Neurosci. 42, 391-398.

Oshima K., Grimm C.M., Corrales C.E., Senn P., Martinez Monedero R., Geleoc G.S., Edge A., Holt J.R. and Heller S. (2007). Differential distribution of stem cells in the auditory and vestibular organs of the inner ear. J. Assoc. Res. Otolaryngol. 8, 18-31.

Oshima K., Senn P. and Heller S. (2009). Isolation of sphere-forming stem cells from the mouse inner ear. Methods Mol. Biol. 493, 141-162.

Oshima K., Shin K., Diensthuber M., Peng A.W., Ricci A.J. and Heller S. (2010). Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells. Cell 141, 704-716.

Othman M., Lu C., Klueber K., Winstead W. and Roisen F. (2005). Clonal analysis of adult human olfactory neurosphere forming cells. Biotech. Histochem. 80, 189-200.

Ourednik J., Ourednik V., Lynch W.P., Schachner M. and Snyder E.Y. (2002). Neural stem cells display an inherent mechanism for rescuing dysfunctional neurons. Nat. Biotechnol. 20, 1103-1110.

Parker M.A., Corliss D.A., Gray B., Anderson J.K., Bobbin R.P., Snyder E.Y. and Cotanche D.A. (2007). Neural stem cells injected into the sound-damaged cochlea migrate throughout the cochlea and express markers of hair cells, supporting cells, and spiral ganglion

cells. Hear. Res. 232, 29-43.

Pettingill L.N., Richardson R.T., Wise A.K., O'Leary S.J. and Shepherd R.K. (2007). Neurotrophic factors and neural prostheses: potential clinical applications based upon findings in the auditory system. IEEE Trans. Biomed. Eng. 54, 1138-1148.

Pettingill L.N., Minter R.L. and Shepherd R.K. (2008). Schwann cells genetically modified to express neurotrophins promote spiral ganglion neuron survival in vitro. Neuroscience 152, 821-828.

Poirrier A.L., Pincemail J., Van Den Ackerveken P., Lefebvre P.P. and Malgrange B. (2010). Oxidative stress in the cochlea: an update. Curr. Med. Chem. 17, 3591-3604.

Pollock K., Stroemer P., Patel S., Stevanato L., Hope A., Miljan E., Dong Z., Hodges H., Price J. and Sinden J.D. (2006). A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. Exp. Neurol. 199, 143-155.

Prayle A. and Smyth A.R. (2010). Aminoglycoside use in cystic fibrosis: therapeutic strategies and toxicity. Curr. Opin. Pulm. Med. 16, 604-610.

Puligilla C. and Kelley M.W. (2009). Building the world's best hearing aid; regulation of cell fate in the cochlea. Curr. Opin. Genet. Dev. 19, 368-373.

Rask-Andersen H., Bostrom M., Gerdin B., Kinnefors A., Nyberg G., Engstrand T., Miller J.M. and Lindholm D. (2005). Regeneration of human auditory nerve. In vitro/in video demonstration of neural progenitor cells in adult human and guinea pig spiral ganglion. Hear. Res. 203, 180-191.

Regala C., Duan M., Zou J., Salminen M. and Olivius P. (2005). Xenografted fetal dorsal root ganglion, embryonic stem cell and adult neural stem cell survival following implantation into the adult vestibulocochlear nerve. Exp. Neurol. 193, 326-333.

Reyes J.H., O'Shea K.S., Wys N.L., Velkey J.M., Prieskorn D.M., Wesolowski K., Miller J.M. and Altschuler R.A. (2008). Glutamatergic neuronal differentiation of mouse embryonic stem cells after transient expression of neurogenin 1 and treatment with BDNF and GDNF: in vitro and in vivo studies. J. Neurosci. 28, 12622-12631.

Richardson R.T., O'Leary S., Wise A., Hardman J. and Clark G. (2005). A single dose of neurotrophin-3 to the cochlea surrounds spiral ganglion neurons and provides trophic support. Hear. Res. 204, 37-47.

Rivolta M.N. (2010). Stem cells and cell lines from the human auditory organ: applications, hurdles and bottlenecks in the development of regenerative therapies for deafness. Drug Discov. Today 15, 283-286.

Roberson D.W., Alosi J.A. and Cotanche D.A. (2004). Direct transdifferentiation gives rise to the earliest new hair cells in regenerating avian auditory epithelium. J. Neurosci. Res. 78, 461-471.

Roisen F.J., Klueber K.M., Lu C.L., Hatcher L.M., Dozier A., Shields C.B. and Maguire S. (2001). Adult human olfactory stem cells. Brain Res. 890, 11-22.

Roy S., Johnston A.H., Newman T.A., Glueckert R., Dudas J., Bitsche M., Corbacella E., Rieger G., Martini A. and Schrott-Fischer A. (2010). Cell-specific targeting in the mouse inner ear using nanoparticles conjugated with a neurotrophin-derived peptide ligand: potential tool for drug delivery. Int. J. Pharm. 390, 214-224.

Sage C., Huang M., Karimi K., Gutierrez G., Vollrath M.A., Zhang D.S., Garcia-Anoveros J., Hinds P.W., Corwin J.T., Corey D.P. and Chen Z.Y. (2005). Proliferation of functional hair cells in vivo in the absence of the retinoblastoma protein. Science 307, 1114-1118.

Sage C., Huang M., Vollrath M.A., Brown M.C., Hinds P.W., Corey D.P., Vetter D.E. and Chen Z.Y. (2006). Essential role of retinoblastoma protein in mammalian hair cell development and hearing. Proc. Natl. Acad. Sci. USA 103, 7345-7350.

Sanchez-Ramos J., Song S., Cardozo-Pelaez F., Hazzi C., Stedeford T., Willing A., Freeman T.B., Saporta S., Janssen W., Patel N., Cooper D.R. and Sanberg P.R. (2000). Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp. Neurol. 164, 247-256.

Savary E., Hugnot J.P., Chassigneux Y., Travo C., Duperray C., Van De Water T. and Zine A. (2007). Distinct population of hair cell progenitors can be isolated from the postnatal mouse cochlea using side population analysis. Stem Cells 25, 332-339.

Savary E., Sabourin J.C., Santo J., Hugnot J.P., Chabbert C., Van De Water T., Uziel A. and Zine A. (2008). Cochlear stem/progenitor cells from a postnatal cochlea respond to Jagged1 and demonstrate that notch signaling promotes sphere formation and sensory potential. Mech. Dev. 125, 674-686.

Scheper V., Paasche G., Miller J.M., Warnecke A., Berkingali N., Lenarz T. and Stover T. (2009). Effects of delayed treatment with combined GDNF and continuous electrical stimulation on spiral ganglion cell survival in deafened guinea pigs. J. Neurosci. Res. 87, 1389-1399.

Schimmang T., Tan J., Muller M., Zimmermann U., Rohbock K., Kopschall I., Limberger A., Minichiello L. and Knipper M. (2003). Lack of Bdnf and TrkB signalling in the postnatal cochlea leads to a spatial reshaping of innervation along the tonotopic axis and hearing loss. Development 130, 4741-4750.

Sekiya T., Holley M.C., Kojima K., Matsumoto M., Helyer R. and Ito J. (2007). Transplantation of conditionally immortal auditory neuroblasts to the auditory nerve. Eur. J. Neurosci. 25, 2307-2318.

Sekiya T., Kojima K., Matsumoto M., Kim T.S., Tamura T. and Ito J. (2006). Cell transplantation to the auditory nerve and cochlear duct. Exp. Neurol. 198, 12-24.

Sell S. (2004). Stem cell origin of cancer and differentiation therapy. Crit. Rev. Oncol. Hematol. 51, 1-28.

Shepherd R.K., Roberts L.A. and Paolini A.G. (2004). Long-term sensorineural hearing loss induces functional changes in the rat auditory nerve. Eur. J. Neurosci. 20, 3131-3140.

Shepherd R.K., Coco A., Epp S.B. and Crook J.M. (2005). Chronic depolarization enhances the trophic effects of brain-derived neurotrophic factor in rescuing auditory neurons following a sensorineural hearing loss. J. Comp. Neurol. 486, 145-158.

Shepherd R.K., Coco A. and Epp S.B. (2008). Neurotrophins and electrical stimulation for protection and repair of spiral ganglion neurons following sensorineural hearing loss. Hear. Res. 242, 100-109.

Shi F., Corrales C.E., Liberman M.C. and Edge A.S. (2007). BMP4 induction of sensory neurons from human embryonic stem cells and reinnervation of sensory epithelium. Eur. J. Neurosci. 26, 3016-3023.

Shibata S.B. and Raphael Y. (2010). Future approaches for inner ear protection and repair. J. Commun. Disord. 43, 295-310.

Shinohara T., Bredberg G., Ulfendahl M., Pyykko I., Olivius N.P., Kaksonen R., Lindstrom B., Altschuler R. and Miller J.M. (2002). Neurotrophic factor intervention restores auditory function in deafened animals. Proc. Natl. Acad. Sci. USA 99, 1657-1660.

Shoji F., Miller A.L., Mitchell A., Yamasoba T., Altschuler R.A. and Miller J.M. (2000a). Differential protective effects of neurotrophins in the attenuation of noise-induced hair cell loss. Hear. Res. 146, 134-142.

Shoji F., Yamasoba T., Magal E., Dolan D.F., Altschuler R.A. and Miller J.M. (2000b). Glial cell line-derived neurotrophic factor has a dose

dependent influence on noise-induced hearing loss in the guinea pig cochlea. Hear. Res. 142, 41-55.

Shou J., Zheng J.L. and Gao W.Q. (2003). Robust generation of new hair cells in the mature mammalian inner ear by adenoviral expression of Hath1. Mol. Cell. Neurosci. 23, 169-179.

Song B.N., Li Y.X. and Han D.M. (2008). Effects of delayed brain-derived neurotrophic factor application on cochlear pathology and auditory physiology in rats. Chin. Med. J. (Engl) 121, 1189-1196.

Song S. and Sanchez-Ramos J. (2003). Brain as the Sea of Marrow. Exp. Neurol. 184, 54-60.

Stevanato L., Corteling R.L., Stroemer P., Hope A., Heward J., Miljan E.A. and Sinden J.D. (2009). c-MycERTAM transgene silencing in a genetically modified human neural stem cell line implanted into MCAo rodent brain. BMC Neurosci. 10, 86.

Stone J.S. and Cotanche D.A. (2007). Hair cell regeneration in the avian auditory epithelium. Int. J. Dev. Biol. 51, 633-647.

Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomoda K. and Yamanaka S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861-872.

Takahashi K. and Yamanaka S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676.

Talaska A.E., Schacht J. and Fischel-Ghodsian N. (2006). Molecular and genetic aspects of aminoglycoside-induced hearing loss. Drug Discovery Today: Disease Mechanisms 3, 119-124.

Tan B.T., Lee M.M. and Ruan R. (2008). Bone-marrow-derived cells that home to acoustic deafened cochlea preserved their hematopoietic identity. J. Comp. Neurol. 509, 167-179.

Tateya I., Nakagawa T., Iguchi F., Kim T.S., Endo T., Yamada S., Kageyama R., Naito Y. and Ito J. (2003). Fate of neural stem cells grafted into injured inner ears of mice. Neuroreport 14, 1677-1681.

Tessarollo L., Coppola V. and Fritzsch B. (2004). NT-3 replacement with brain-derived neurotrophic factor redirects vestibular nerve fibers to the cochlea. J. Neurosci. 24, 2575-2584.

Tondreau T., Lagneaux L., Dejeneffe M., Massy M., Mortier C., Delforge A. and Bron D. (2004). Bone marrow-derived mesenchymal stem cells already express specific neural proteins before any differentiation. Differentiation 72, 319-326.

Tsuji O., Miura K., Okada Y., Fujiyoshi K., Mukaino M., Nagoshi N., Kitamura K., Kumagai G., Nishino M., Tomisato S., Higashi H., Nagai T., Katoh H., Kohda K., Matsuzaki Y., Yuzaki M., Ikeda E., Toyama Y., Nakamura M., Yamanaka S. and Okano H. (2010). Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc. Natl. Acad. Sci. USA 107, 12704-12709.

Ulfendahl M., Hu Z., Olivius P., Duan M. and Wei D. (2007). A cell therapy approach to substitute neural elements in the inner ear. Physiol. Behav. 92, 75-79.

Warchol M.E. (2011). Sensory regeneration in the vertebrate inner ear: Differences at the levels of cells and species. Hear. Res. 273, 72-79.

Weber T., Corbett M.K., Chow L.M., Valentine M.B., Baker S.J. and Zuo J. (2008). Rapid cell-cycle reentry and cell death after acute inactivation of the retinoblastoma gene product in postnatal cochlear hair cells. Proc. Natl. Acad. Sci. USA 105, 781-785.

Wei D., Levic S., Nie L., Gao W.Q., Petit C., Jones E.G. and Yamoah E.N. (2008). Cells of adult brain germinal zone have properties akin to hair cells and can be used to replace inner ear sensory cells after damage. Proc. Natl. Acad. Sci. USA 105, 21000-21005.

White P.M., Doetzlhofer A., Lee Y.S., Groves A.K. and Segil N. (2006). Mammalian cochlear supporting cells can divide and trans-differentiate into hair cells. Nature 441, 984-987.

Whitlon D.S., Ketels K.V., Coulson M.T., Williams T., Grover M., Edpao W. and Richter C.P. (2006). Survival and morphology of auditory neurons in dissociated cultures of newborn mouse spiral ganglion. Neuroscience 138, 653-662.

Whitlon D.S., Tieu D., Grover M., Reilly B. and Coulson M.T. (2009). Spontaneous association of glial cells with regrowing neurites in mixed cultures of dissociated spiral ganglia. Neuroscience 161, 227-235.

Wise A.K., Richardson R., Hardman J., Clark G. and O'leary S. (2005). Resprouting and survival of guinea pig cochlear neurons in response to the administration of the neurotrophins brain-derived neurotrophic factor and neurotrophin-3. J. Comp. Neurol. 487, 147-165.

Woodbury D., Schwarz E.J., Prockop D.J. and Black I.B. (2000). Adult rat and human bone marrow stromal cells differentiate into neurons. J. Neurosci. Res. 61, 364-370.

Woods C., Montcouquiol M. and Kelley M.W. (2004). Math1 regulates development of the sensory epithelium in the mammalian cochlea. Nat. Neurosci. 7, 1310-1318.

Yagi M., Kanzaki S., Kawamoto K., Shin B., Shah P.P., Magal E., Sheng J. and Raphael Y. (2000). Spiral ganglion neurons are protected from degeneration by GDNF gene therapy. J. Assoc. Res. Otolaryngol. 1, 315-325.

Yamagata T., Miller J.M., Ulfendahl M., Olivius N.P., Altschuler R.A., Pyykko I. and Bredberg G. (2004). Delayed neurotrophic treatment preserves nerve survival and electrophysiological responsiveness in neomycin-deafened guinea pigs. J. Neurosci. Res. 78, 75-86.

Yamamoto N., Tanigaki K., Tsuji M., Yabe D., Ito J. and Honjo T. (2006). Inhibition of Notch/RBP-J signaling induces hair cell formation in neonate mouse cochleas. J. Mol. Med. 84, 37-45.

Yamasoba T. and Kondo K. (2006). Supporting cell proliferation after hair cell injury in mature guinea pig cochlea in vivo. Cell Tissue Res. 325, 23-31.

Yoshida T., Hakuba N., Morizane I., Fujita K., Cao F., Zhu P., Uchida N., Kameda K., Sakanaka M., Gyo K. and Hata R. (2007). Hematopoietic stem cells prevent hair cell death after transient cochlear ischemia through paracrine effects. Neuroscience 145, 923-930.

Yu Y., Weber T., Yamashita T., Liu Z., Valentine M.B., Cox B.C. and Zuo J. (2010). In vivo proliferation of postmitotic cochlear supporting cells by acute ablation of the retinoblastoma protein in neonatal mice. J. Neurosci. 30, 5927-5936.

Zhai S., Shi L., Wang B.E., Zheng G., Song W., Hu Y. and Gao W.Q. (2005). Isolation and culture of hair cell progenitors from postnatal rat cochleae. J. Neurobiol. 65, 282-293.

Zheng J.L. and Gao W.Q. (2000). Overexpression of Math1 induces robust production of extra hair cells in postnatal rat inner ears. Nat. Neurosci. 3, 580-586.

Zheng J.L., Shou J., Guillemot F., Kageyama R. and Gao W.Q. (2000). Hes1 is a negative regulator of inner ear hair cell differentiation. Development 127, 4551-4560.

Zine A., Van De Water T.R. and de Ribaupierre F. (2000). Notch signaling regulates the pattern of auditory hair cell differentiation in mammals. Development 127, 3373-3383.

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