[Frontiers in Bioscience S4, 121-132, January 1, 2012]

Neural crest stem cells and their potential application in a therapy for deafness

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1. ABSTRACT

Neurosensory hearing loss is a common condition that has major social and economic implications. Recent advances in stem cell research and in cochlear implantation are offering renewed hopes to people suffering from damage to the auditory hair cells and their associated neurons. Several putative donor cell types are currently being explored, including embryonic stem cells, different types of adult stem cell and the recently described induced-pluripotent stem cells. In this review, we draw attention to the potential application of neural crest stem cells for the treatment of deafness. This population shares a similar developmental origin with the cells of the otic placode, the molecular machinery controlling their maturation and differentiation is comparable and they can produce related sensory neurons. More importantly, pockets of neural crest stem cells remain in the adult body in regions of relatively easy access, facilitating their use for autologous transplantation and therefore avoiding the need for immunosuppression and the problems of tissue rejection. Their exploration and application to hearing conditions could facilitate the development of a clinically-viable, cell-based therapy for deafness.

2. DEAFNESS: ITS SOCIAL IMPACT AND THE LACK OF A CURATIVE TREATMENT

Hearing is a sense of paramount importance for verbal communication, pleasure and awareness. The impact of a hearing deficit, especially during childhood, is huge. It can lead to problems with the development of speech and language which has implications for social integration, and affects quality of life as a whole. According to 2005 estimates by the World Health Organization (WHO), 278 million people worldwide have moderate to profound hearing loss in both ears (*www.who.int/mediacentre/factsheets/fs300/en/index.html*)(1).

Many individuals, each with their variable genetic background, are predisposed to hearing loss through environmental factors, such as noise or ototoxic drugs. This leads to age-related hearing loss affecting over 50% of the population by the age of 65.

The most common reason why people become deaf is because the sensory hair cells in the auditory organs -the cochlea- become damaged and die. Since the

mammalian inner ear lacks the capacity to regenerate cochlear hair cells, the loss of hearing is permanent. Injury to the hair cells leads to the degeneration of their unstimulated nerve fibres and subsequently, to the death of the second-level cells in the sensory pathway, the auditory neurons (2). This neural degeneration process is irreversible, although the application of neurotrophic growth factors or electrical stimulation may have some protective effects (3). Today, the only therapy for hearing-impaired people is the use of prosthetics, such as hearing aids, bone-anchored hearing devices and the cochlear implant (CI). These devices help many hearing-impaired people although, in spite of the many benefits of a CI for the profoundly deaf, its performance in some patients can be suboptimal. These limitations are partly due to the number of available channels, and partly due to the low resolution of the CI. Improvement of the resolution with better signal processing or more electrodes is hardly possible due to the relatively large physical distance between the CI electrodes and the degenerated auditory nerve (4). Since the CI functionally replaces the hair cells, it is also crucial that a critical number of healthy auditory neurons remain present for its adequate performance (4, 5).

The auditory sensory neurons, also known as spiral ganglion neurons (SGNs), are located in the conical-shaped, spongy bone of the central axis of the cochlea, the modiolus. The main population (90-95%) of the SGNs are bipolar and myelinated and are commonly named type 1 SGN. These neurons participate in the afferent innervation of the inner hair cells (IHC), thus directing most of the afferent input to the brainstem. The thick, myelinated axons of these SGNs bundle with the vestibulocochlear nerve which synapses, in the brainstem, on the cochlear nucleus complex, constituting a link to the auditory cortex. A small part of the SGN population (~10%) are the type 2 cells. These are bipolar or pseudo-unipolar and their central and peripheral processes are mostly thin and unmyelinated. Type 2 SGNs innervate the OHCs and their central projections reach the cochlear nucleus area. The arrangement of the type 2 fibres within the cochlea, vestibulocochlear nerve and cochlear nucleus is in general similar to that of type 1 fibres.

Cell-replacement therapy could provide benefits in conjunction with a CI (6). Neural progenitor/stem cells are promising candidates for this type of therapy as they have the potential to provide large numbers of replacement neurons to the degenerating auditory nerve (7). Moreover, it has been shown that neural cells are reactive to chronic electrical stimulation, which may be supportive to the regeneration process (8). Encouraging demonstrations of stem cells as a valid therapeutic option come from *in vivo* experiments whereas transplanted ESC-derived neural progenitors were able to differentiate into neurons with extensions arborizing into the organ of Corti (9) (10). It has also been established that stem cells implanted in the cochlea are able to reach functionally relevant regions beyond the actual transplantation site. This migratory behavior is of importance because it is extremely complicated to operate the tiny inner ear, and some of its regions are almost inaccessible surgically. The migration of stem cells can apparently surpass the transitional zone between the central and the peripheral nervous part of the auditory nerve; embryonic stem cells grafted on the transected auditory nerve at the base of the cochlea migrated not only peripherally to the nerve fibres in the cochlea but also in the opposite direction, centrally, close to the cochlear nucleus in the brain stem (11).

However, the promising results obtained in early transplantation experiments could be compromised in prolonged follow up studies, when long term cell survival may well become a relevant issue. The causes for limited cell survival are complex and most probably a combination of different factors: it may not be due solely to the traumatic effects of the transplantation procedure but the microenvironment within the cochlea may also be hostile to stem cells. The fluid-filled scalae might not facilitate adequate attachment and survival of the transplanted cells. The loss of hair cells and their trophic influence on the sensory neurons may also limit the survival of exogenous neurons. Cell transplantation could benefit from the combined application with growth factors, which when applied in the scala tympani of deaf animals either via a mini osmotic pump, by a sponge positioned at the entrance of the cochlea or by using nanoparticles (12, 13) (14) led to an increased survival of sensory neurons. Unfortunately, the beneficial effect of growth factors was not permanent since after the cessation of the therapy, survival decreased.

Another important survival-limiting factor might also be that the donor cells are xeno- or allogenic. As a result, the transplanted cells will be subjected to a graft-versus-host response in the recipient animal. Embryonic or fetal neural progenitor/stem cells should trigger a minimal immune response in the inner ear because of their immature antigenic profile and thus are favorable candidates for a cell-based therapy.

Many reports have demonstrated that ESCs are a good choice in animal models. Still, in a clinical setting, these cells would be subjected to allogenic transplantations which would influence their survival. The question then arises whether ESCs are really immuno-privileged, as it has been shown, for instance, that human ESCs induced a similar immune response as human fibroblast cells on naïve and immunized T-cells, and did not inhibit immune responses during direct or indirect antigen presentation (15). Recently, auditory stem cells were harvested from the human fetal cochlea (hFASCs) (16). These stem cells could be expanded *in vitro* and differentiated into functional auditory neurons and hair cell-like cells, bringing the advances made in animal models closer to a clinical application. However, the use of human embryonic and fetal stem cells is subject to ethical restrictions and moral objections from some groups, making the search for a less controversial source of human cells highly relevant.

Autologous stem cell transplants might lead to increased survival of the cells, but neural progenitor/stem cells from autologous sources are often very difficult to harvest. In this context, an attractive and theoretically

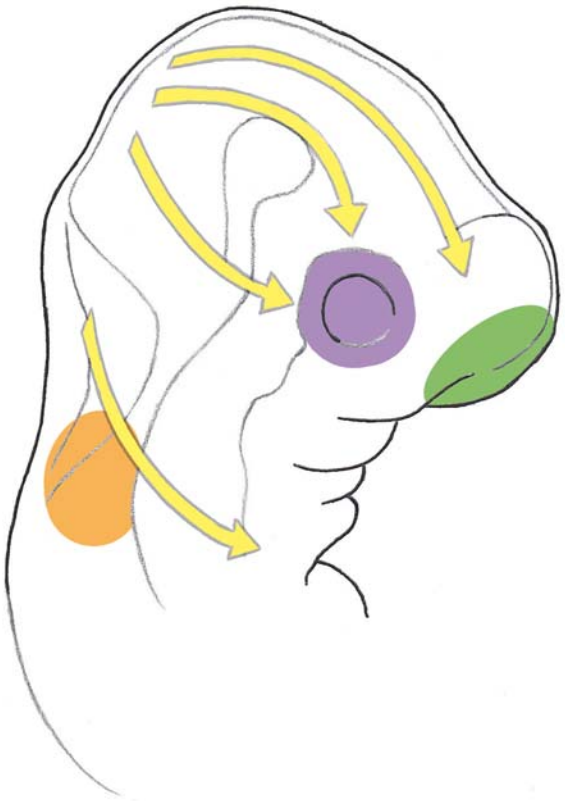

Figure 1. Schematic representation of the migratory behaviour of the neural crest. Waves of neural crest cells wild populate different regions of the body, primary in the upper trunk and face. In color are the location of the paired, sensory placodes (orange, otic; purple, optic and green, olfactory).

useful source would be the induced-pluripotent stem cells (iPSCs).

The use of iPSCs circumvents all ethical discussions about the use of human embryonic material and shows great potential. Since the first reports on iPSCs generated from mouse fibroblasts, many laboratories have reprogrammed human and mouse somatic cells into a pluripotent stem cell-like state by the forced expression of different sets of transcription factors (Klf4, Oct4, Sox2, and c-Myc or Oct4, Sox2, Nanog, and Lin28) (17) (18). A plethora of somatic cells such as murine hepatocytes, gastric cells, keratinocytes, hematopoietic cells and murine neural stem cells can be turned into iPSCs (19-22). With respect to inner ear regeneration, mouse iPSCs appear to be able to generate auditory lineages (23, 24). However, reprogramming by defined transcription factors is inefficient (from 0.0005 % to 0.1 %) and requires expression of the transcripts for approximately 14 days (25, 26). This is achieved by gene delivery through lentiviral or retroviral vectors, consecutive transient transfections, adenoviral vectors, episomally replicating DNA, or membrane penetrating fusion proteins (19, 27, 28) (29-31). The most efficient reprogramming method seems to be retroviral transduction (19). As a result the generated iPSCs harbor numerous viral integration sites in their genomes, which increase the probability of oncogenesis**.** In addition, the *in vivo* safety of iPSCs established by different means has not been analyzed thoroughly (26). Clinical applications of iPSCs can only be considered if the cells generated have been validated extensively, and other safety issues with respect to the transplantation of pluripotent stem cell derivatives have been solved (32). One important aspect besides the safety is the efficient generation of the desired cell type from induced pluripotent stem cells, but the improvement of their number and purity remains a great challenge. At the moment, iPSCs are solely a research tool holding promise as a therapeutic agent.

The pre-requisite of a genuine, continuous and sufficient population of well characterized adult human neural precursor cells with the ability to differentiate into glial cells and neurons also holds true for stem cells derived from adult tissues. Several investigators have claimed success in isolating a purified population of neural precursors from different tissues, but the procedures often require selective, extensive *in vitro* cell expansion. This has been shown for neural progenitors isolated from several sources, such as blood, bone marrow, neuroblasts from the olfactory bulb and fat tissue (33-36). Multiple passages, in order to enhance the number of a certain type of stem cell from a heterogeneous cell population, carry the risk of genetic alterations and stem cell senescence/exhaustion (37). However, the prerequisite of a genuine, continuous and sufficient population could be better met for some types of adult neural progenitor stem cells that can be isolated from adult tissues such as teeth, palatum or hair follicles (38-41). These stem cells are part of a rich source of a multipotent stem cell population: the neural crest (NC), which has been found in various, easily accessible locations in the body. The differentiation of NC from adult tissue, under neurogenic conditions, results in the production of many cells that fulfill most criteria for neuronal differentiation (42). The NC has also been reported to be able to differentiate into non-neural lineages, thus showing pluripotency. For use in inner ear therapy, neural crest stem cells (NCSCs) could have some advantages when compared to neural progenitors from other origins, because during embryogenesis, development of the neural crest is closely related to the generation of the otic placode.

3. THE NEURAL CREST AND THE OTIC PLACODE: DIFFERENT STRUCTURES BUT SIMILAR PROGENY

3.1. The Neural Crest

The neural crest is a multipotent population of cells, unique to vertebrates, born at the interface of the neural tube and the dorsal ectoderm (Figure 1). Neural crest cells undergo epithelial-to-mesenchymal transition and delaminate from the developing neural tube and overlying ectoderm early in development. They then migrate through spatially and temporally coordinated changes in cell-substratum adhesiveness to different destinations, where the cells differentiate into a wide variety of derivatives.

There used to be a fundamental question in developmental biology about whether the neural crest was a

homogenous population of multipotent stem cells whose fates are determined post-migration by localized environmental cues, or whether it was composed of a heterogeneous mixture of precursor cells whose individual fates are specified by intrinsic mechanisms prior to their migration from the neural tube. Although considerable transplantation evidence supported the first theory, lineage-tracing studies provided evidence for the presence of both restricted subpopulations of precursor cells and multipotent stem cells whose fates could be altered by local environmental cues (43-47). Back-transplantation studies were consistent with the latter idea, such that although phenotypically distinct precursor cells may be pre-specified, many of them are not committed and their fate and/or those of their progeny can be determined by cues in their local environment.

In the last five to ten years it was recognized that the developmental program that regulates neural crest cell fate is both plastic and fixed. Specifically, as a cohort of interacting cells, neural crest cells carry information that directs the axial patterning and species-specific morphology of the head and face. As individual cells, neural crest cells are responsive to signals from each other as well as from non-neural crest tissues in the environment. Depending on which tissues they contact during their migration and which signals are received when they reach their final resting place, these highly migratory cells form diverse derivatives including sensory and autonomic neurons for the peripheral nervous system, glia as well as bones, cartilage, and connective tissues of the face. Because this review is focussed on one particular neural crest derivative, the sensory neurons, we refer the reader to the reviews of Kelusa *et al.* (48) and Davies (49), for insight into the contribution of the neural crest to the autonomic nervous system.

A complex gene regulatory network mediates the various processes of delamination from the neural tube, emigration of the neural crest progenitors along distinct pathways, overt differentiation into diverse cell types and maintenance of the neural crest pool (50). Timing plays a key role in determining the types of progeny that are generated (43). This is illustrated by the three successive waves of NC migration, leading to differential sensory neurogenesis (51). The large proprioceptive neurons are born first, followed then by small neurons. The third wave generates mainly small neurons that consist largely of nociceptors, contributing ~5% of adult mouse DRG neurons (51).

The derivatives of the neural crest population in the hindbrain region can be broadly divided into two sub-populations: ectomesenchymal and neuroglial. The ecto-mesenchymal population fills the branchial arches to produce the head cartilage and bone, while the neuroglial population remains above the branchial arches to produce the neurons of the proximal sensory ganglia and the Schwann cells that ensheath the nerves (52). The two populations can be distinguished by gene expression, for example using Sox10 to label neuro-glial cells and Dlx2 to label ectomesenchymal cells.

Neural crest cells are also involved in the integration of placodal neuroblasts with the hindbrain. Peripheral neurons involved in cephalic sensory systems are born at a distance from the neural tube. These neuroblasts migrate internally, coalesce to form ganglia and extend axons to the central nervous system. The coordination of this migration and the integration on these cells with the hindbrain occurs through interaction with neural crest cells, where the migrating placodal neuroblasts would follow the pathway of the neuroglial crest cells (53). Hence the neural crest plays a key role in neuroblast migration, axon guidance and the subsequent neuronal ensheathing process and is therefore of great importance in generating a functional sensory circuit.

3.2. The otic placode

The inner ear in the vertebrate arises from a simple ectodermal thickening in the head region called the otic placode. Placodes are discrete areas of columnar epithelium derived from non-neural ectoderm. They give rise not only to the paired sensory organs (olfactory, lens, auditory-vestibular) and, in aquatic vertebrates to the lateral line system, but they also make vital contributions to the peripheral sensory nervous system. Some placodes invaginate and form a pit (olfactory), or a vesicle (lens, otic), or placodal cells can delaminate and migrate to a secondary position (cranial ganglia, lateral line). Depending upon their placode of origin, the cells are capable of adopting a variety of fates including glia, sensory receptor cells, neurons and supporting/structural cells. Placodes can, to a point, be compared to the neural crest because both arise from the neural plate border, and both contribute to the peripheral nervous system (54). It has often been suggested that both are derived from a common neural plate border region, being the interface between future neural and non-neural ectoderm. In this neural plate border model, additional signals subsequently induce dedicated neural crest and pre-placodal ectoderm (55, 56). Recently, an alternative model was proposed, which questions this common origin and hypothesizes that the competence to form neural crest and pre-placodal ectoderm is restricted to neural and non-neural ectoderm respectively, and inductive signals acting on this border induce neural crest and pre-placodal ectoderm at opposite sides of the border (57). There are ‘for’ and ‘against’ arguments for the complex succession of events involved in both models and they are currently under intensive investigation.

Transcriptional networks, influenced by extrinsic signals, drive the simple spheroid otocyst into a complex construction in which neural and non-neural portions are regionally patterned. The mechanosensory and neuronal lineages of the inner ear appear to be generated in different regions of the inner ear placode, with only a small region of overlap in areas giving rise to the utricular and saccular sensory organs (58). The otic placode gives rise to both the auditory and vestibular parts of the entire inner ear, including the mechanosensory hair cells, all supporting cells, the biomineralized otoliths and the neurons that will form the auditory/vestibular ganglia and the VIIth cranial nerve (54, 59). The neural crest stem cells, however, will

produce the Schwann cells to myelinate the neuronal processes in the cochlea (60).

4. MOLECULAR COMPARISON BETWEEN NEUROGENESIS IN THE NC AND OTIC PLACODE.

4.1. Neural induction from the neural crest

Although there may be species-specific differences, it is generally thought that neural induction is driven within the ectoderm by mutually antagonistic signalling molecules that promote an epidermal fate (54, 61). Several BMP-antagonists are secreted from the underlying mesoderm to promote neural plate formation. Noggin and Chordin each bind directly to BMPs as well as wingless (WNT), Nodal ligands and fibroblast growth factor (FGF) (62-69). Several studies indicate that at intermediate concentrations of BMP antagonists, neural crest formation is induced (70-72). Since these factors presumably diffuse locally from the different dorsal midline tissues, it has been proposed that a concentration gradient of neural inducers patterns the embryonic ectoderm into several subdomains: neural plate at the highest concentration of BMP (low antagonist), epidermis at the lowest BMP concentration (high antagonist) and neural crest in between (54, 62). Dependent on the BMP gradient, FGFs and Wnt produced in the dorsal neural tube induce the expression of *Pax3* and *Zic1*, which in turn upregulate neural crest critical genes such as *Snail1*, *Slug* and *FoxD3* (61). Further definition of neural crest identity is conferred by a set of transcription factors, termed NC-specifiers, such as *Twist, c-Myc*, *id*-family members, *AP-2 and* the SoxE transcription factors: *sox-8,-9* and-*10* (73). These transcription factors guide a complex series of events to specify the NC cells to their fates. These events lead to an epithelial- mesenchymal transition, which allows the neural crest to segregate and delaminate from the neuroepithelium and migrate away from the neural tube (74). During this migration, NC cells travel along precise paths determined by cell adhesion molecules that integrate internal and environmental guidance signals (75, 76). *In vivo* gain-of-function studies indicate that WNT and β-catenin direct the specification of early neural crest towards the sensory lineage (77). Data on WNT signalling in clonal neural crest cultures corroborate this conclusion. Interestingly, exposure to BMP2 induces an autonomic fate (78). The exposure to WNT in the presence of BMP2, however, maintains the pluripotency of the neural crest cells preventing the formation of sensory neurons. In any case, early migratory NCSCs are multipotent and able to generate both sensory and autonomic lineages.

The process of neural crest differentiation involves a small cohort of genes in which NC specifiers, such as SoxE transcription factors, often regulate effector genes that give derivative cells their terminally differentiated characteristics. During sensory neuron fate specification, *Sox10* is involved in the upregulation of neurogenin1 (*Neurog1*) and neurogenin2 (*Neurog2*) expression, while it also directly regulates gliogenesis via Schwann cell-specific genes such as protein zero (79, 80).

Neural crest neurogenesis and specification to the sensory lineage are linked, driving neurogenesis in sequential waves and leading to the appearance of specific sensory subtypes in the dorsal root ganglia (DRG). The basic helix-loop-helix transcription factors *Neurog1* and *Neurog2* are required for neurogenesis and the specification of peripheral sensory neurons (51, 81-85). In the first wave, SOX10+ cells migrate and express *Neurog2*, which biases them towards a sensory fate (51). Cells with high levels of *Neurog2* subsequently commit to a sensory neuronal fate as defined by the expression of the forkhead transcription factor *Foxs1* during migration (46). The newly created, postmigratory, neurons of the first wave express *Brn3a* and form large proprioceptive and mechanoreceptive neurons that will also express the runt-related transcription factor 3 (Runx3) and the neurotrophic tyrosine receptor kinase C (*TrkC*) at early developmental stages (46, 51, 81, 86-88). In conclusion, the first *Neurog2*-mediated wave of neurogenesis produces mainly TrkB/TrkC mechanoreceptive and proprioceptive neuronal subtypes, arising from an initial TrkC+ pool(51). In the second wave of neurogenesis, a subset of NC cells, characterized by the continuous expression of *Sox10* throughout migration, starts to express *Foxs1*, *Brn3a* and *Neurog1* in the DRG, before it expresses RUNX factors (89). This wave probably produces both the small TrkA+ and TrkC+ populations of neurons by expressing *Runx1* or *Runx3*, respectively (90-92). The third wave of neurogenesis arises from boundary cap cells expressing *Sox10* and *Krox20*; they contribute mainly to the RUNX1/TrkA population of neurons and to glia (93).

4.2. Sensory neurons from the otic placode

Although the auditory sensory neurons are derived from the otic placode and not from the neural crest like the sensory neurons described above, they share similar molecular events during their specification and differentiation.

The ear develops from the otic placode and undergoes morphogenesis to form the otocyst through the interaction of several diffusible factors from the surrounding ectoderm and the underlying mesoderm and endoderm, such as FGFs, sonic hedgehog, WNTs and BMP (94-98).

The HMG-domain-containing transcription factor Sox9, a member of the SoxE subfamily of Sox genes, is expressed early in the otic placode in several species and studies in Zebrafish and Xenopus have provided evidence that Sox9 is essential for otic formation and specification (99). In addition to Sox9, Sox8 and Sox10, the two other members of the SoxE subfamily, are also expressed during ear development but expression of both starts later than that of Sox9, and Sox8 is expressed at much lower levels. Interestingly, it has been reported that the expression of *Sox10* in the otic vesicle is similar to that in the NC, being affected when FGF or Wnt8 activity is perturbed, suggesting that the same molecular mechanisms that induce neural crest could be important in specifying the placode regions (100).

Neurons and hair cells arise from adjacent and partially overlapping areas and may in certain cases share a

clonal relationship. Neuronal and hair cell differentiation is driven by the upregulation of specific bHLH genes, Neurog1 for neurons and Atoh1 for hair cells (101, 102). This process is enhanced through the interaction with other factors such as FOXG and RUNX (94, 101). Neurog1 is the bHLH gene which is found to be upregulated first in the mammalian inner ear and it activates the downstream genes Neurod1, Nhlh1 and Nhlh2, which govern further neuronal development (101-105). Neurod1 primarily functions in regulating neuronal differentiation and survival (104, 106). It has been reported that Nhlh2 cooperates with Neurod1 in neuronal differentiation, while the function of Nhlh1 in neuronal differentiation has not yet been fully explored (105). In this time window of otic development, Brn3a has been shown to be expressed in the facial-stato-acoustic ganglion prior to sensory neuron differentiation and innervation of the otocyst (107). Loss of Brn3a leads to downregulation of *TrkC*, *Brn3b* and *Parvalbumin* in the spiral ganglion, suggesting that these are downstream targets of Brn3a. Brn3a is required for the proper growth and migration of inner ear neuroblasts and is critically involved in target innervation and axon guidance by spiral and vestibular ganglion neurons by regulating different downstream genes. This parallels NC neurogenesis, in which RUNX and FOXG are also involved, although it is not yet clear, whether this is before or after Brn3a expression (51, 90).The onset of neurogenesis and the genes and morphogens involved in NC and otic vesicle are very similar, from the early beginning (WNT, BMP, FGF), through different time intervals (SOXE subfamily) to later in neuronal development (Neurog1, Brn3a). This would suggest that NCSCs and their early neuroblastic lineages as identified by the expression of Snail, Slug and SOX10, could be plausible candidates for SGN regeneration.

5. POTENTIAL SOURCES FOR NCSC ISOLATION

Neural crest stem cells persist into adulthood in the tissues that were originally derived from it. This allows their potential isolation from various sources such as the gut, dorsal root ganglion, heart, hair follicles, olfactory sheath and craniofacial tissue (35, 38-40, 108-112). For stem cell-based therapy it is obviously of importance to harvest these NCSC from a minimally invasive, easily accessible source. When the possibility of autologous transplantation is added to these criteria, olfactory sheath, palatum, dental pulp or the hair follicle are considered the most advantageous sources (35, 38-40, 111, 112). Taking into consideration that extensive expansion of cell numbers requiring multiple passages could scale up the potential costs of the procedures and also increase the chances of introducing unwanted chromosomal anomalies, the sources are further narrowed down to the craniofacial tissue and the hair follicle. The isolation of stem cells from the palatum requires several steps which might affect the quality of cells, such as mechanical chipping and the use of a high concentration of proteolytic enzymes (39). On the other hand, isolation of stem cells from the dental pulp and hair follicles do not need a pre-treatment, since they can be cultured directly by allowing them to migrate out of their natural niches. The soft tissue of the dental pulp can easily been taken out of the centre of the tooth while entire hair follicles can be cultured with subsequent outgrowth of NCSCs (113, 114). However, the harvest of dental pulp is invasive because it requires the extraction of a tooth; we therefore believe that the hair follicle would be the most useful source of NCSCs.

It has been shown that hair follicle NCSCs are slightly more restricted in their fates than the "true" pluripotent NCSCs, although there is a considerable degree of heterogeneity (115). Depending on the species, the isolation procedure and the culture methods, stem cells with different molecular characteristics have been generated. This heterogeneity is also reflected also in the nomenclature for hair follicle stem cells. They are known variously as skin derived precursor cells, epidermal neural crest stem cells, follicular stem cells and hair follicle neural crest stem cells (40, 41, 116, 117).

The diversity in the hair follicle NCSC population is also confounded by the lack of a universal, useful marker. In the mouse, CD34 has been recognized as a reliable marker for hair follicle NCSCs, but its expression is controversial in humans (40, 118). Conversely, these differences underline the exceptionally diverse and dynamic stem cell population located in distinct regions of the hair follicle (119).

Hair follicle stem cells can generate all ectodermal derivatives, including neurons, nerve supporting cells, smooth muscle cells, bone/cartilage cells and melanocytes (118, 120). However, a potential caveat of their use for the generation of sensory neurons lies in the fact that early exposure of migrating NCSCs to BMP2 appears to suppress sensory neurogenesis (78). In agreement with this observation, adult skin-derived precursors appear to differentiate primarily into autonomic neurons (41, 42). It is possible then that the competence of adult skin neural crest cells to produce sensory neurons has been lost during development, although this is not entirely clear for, on the other hand, epidermal neural crest stem cells can differentiate into Brn3a-expressing cells (121) and can elicit recovery of sensory function when transplanted into a model of spinal cord injury (122). Moreover, an independent group has obtained functional neurons displaying some features of sensory cells, like sodium channels. (123). As mentioned above, a more precise definition of the population under exploration using surface markers and the exploration of purpose-defined, specific culture conditions could help to establish if sensory lineages can indeed be obtained from adult skin neural crest derivatives.

Despite their ectodermal origin but in agreement with their neural crest nature, hair follicle stem cells can also generate cell types that are typically derived from the mesoderm, such as endothelial and hematopoietic cells (124, 125). Besides the contribution of Hu *et al.* (122), further application of hair follicle stem cells for neural repair has been demonstrated by Amoh *et al.* in an exceptional experiment where newly generated Schwann cells supported the recovery of injured peripheral nerve fibres (117).

6. CONCLUSIONS

A regenerative treatment for the inner ear is needed to restore the damaged population of sensory cells. Since the currently available cochlear implant can substitute, albeit partially, for the role of the hair cells, a promising strategy could concentrate on replacing the lost auditory neurons with stem cells. This aim will probably be facilitated by the use of autologous cells, which should improve the survival of the grafted cells by removing the problem of immunogenic rejection. Adult neural crest stem cells, available from several easily accessible sources, have so far not been applied to the inner ear regenerative field. They are, in our opinion, worthy of exploration. Hair follicle stem cells are NCSC descendants, likely to retain the potential to differentiate towards functional sensory neurons. We postulate that hair follicle stem cells are highly suitable as a donor cell type and could be of great use in the development of a cell-based therapy to treat deafness. Ongoing experiments in our laboratories are aiming to test their potential.

7. ACKNOWLEDGEMENTS

MAH and MNR were supported by a grant from the British Council Partnership Programme in Science. MNR is supported by the MRC, the RNID and a DRUK Centre of Excellence Award. We are indebted to Leila Abbas for critically reading the manuscript.

8. REFERENCES

1. N. Jongkamonwiwat, A. Zine and M. N. Rivolta: Stem cell based therapy in the inner ear: appropriate donor cell types and routes for transplantation. *Curr Drug Targets*, 11(7), 888-97 (2010)

2. H. C. Dodson and A. Mohuiddin: Response of spiral ganglion neurones to cochlear hair cell destruction in the guinea pig. *J Neurocytol*, 29(7), 525-37 (2000)

3. L. N. Gillespie and R. K. Shepherd: Clinical application of neurotrophic factors: the potential for primary auditory neuron protection. *Eur J Neurosci*, 22(9), 2123-33 (2005)

4. J. J. Briaire and J. H. Frijns: The consequences of neural degeneration regarding optimal cochlear implant position in scala tympani: a model approach. *Hear Res*, 214(1-2), 17-27 (2006)

5. J. B. Nadol, Jr.: Degeneration of cochlear neurons as seen in the spiral ganglion of man. *Hear Res*, 49(1-3), 141-54 (1990)

6. S. Maini, H. Lisnichuk, H. Eastwood, D. Pinder, D. James, R. T. Richardson, A. Chang, T. Connolly, D. Sly, G. Kel and S. J. O'Leary: Targeted therapy of the inner ear. *Audiol Neurootol*, 14(6), 402-10 (2009)

7. B. Coleman, M. G. de Silva and R. K. Shepherd: Concise review: the potential of stem cells for auditory neuron generation and replacement. *Stem Cells*, 25(11), 2685-94 (2007)

8. A. N. Ide, A. Andruska, M. Boehler, B. C. Wheeler and G. J. Brewer: Chronic network stimulation enhances evoked action potentials. *J Neural Eng*, 7(1), 16008 (2010)

9. C. E. Corrales, L. Pan, H. Li, M. C. Liberman, S. Heller and A. S. Edge: 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(13), 1489-500 (2006)

10. N. Jongkamonwiwat, W. Chen and M. N. Rivolta: Functional Recovery Obtained by Human ESCs-Derived Otic NeuroProgenitor Cells (ONPs) Transplanted Into the Deafened Gerbil Cochlea. In: *Assoc. Res. Otolaryngol.*, (2010)

11. M. Ulfendahl, Z. Hu, P. Olivius, M. Duan and D. Wei: A cell therapy approach to substitute neural elements in the inner ear. *Physiol Behav*, 92(1-2), 75-9 (2007)

12. M. J. Agterberg, H. Versnel, L. M. van Dijk, J. C. de Groot and S. F. Klis: Enhanced survival of spiral ganglion cells after cessation of treatment with brain-derived neurotrophic factor in deafened guinea pigs. *J Assoc Res Otolaryngol*, 10(3), 355-67 (2009)

13. S. Takemoto, N. Morimoto, Y. Kimura, T. Taira, T. Kitagawa, K. Tomihata, Y. Tabata and S. Suzuki: Preparation of collagen/gelatin sponge scaffold for sustained release of bFGF. *Tissue Eng Part A*, 14(10), 1629-38 (2008)

14. S. Roy, A. H. Johnston, T. A. Newman, R. Glueckert, J. Dudas, M. Bitsche, E. Corbacella, G. Rieger, A. Martini and A. Schrott-Fischer: 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(2), 214-24 (2010)

15. K. H. Grinnemo, M. Kumagai-Braesch, A. Mansson-Broberg, H. Skottman, X. Hao, A. Siddiqui, A. Andersson, A. M. Stromberg, R. Lahesmaa, O. Hovatta, C. Sylven, M. Corbascio and G. Dellgren: Human embryonic stem cells are immunogenic in allogeneic and xenogeneic settings. *Reprod Biomed Online*, 13(5), 712-24 (2006)

16. W. Chen, S. L. Johnson, W. Marcotti, P. W. Andrews, H. D. Moore and M. N. Rivolta: Human fetal auditory stem cells can be expanded *in vitro* and differentiate into functional auditory neurons and hair cell-like cells. *Stem Cells*, 27(5), 1196-204 (2009)

17. K. Takahashi and S. Yamanaka: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, 126(4), 663-76 (2006)

18. J. Yu, M. A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J. L. Frane, S. Tian, J. Nie, G. A. Jonsdottir, V. Ruotti, R. Stewart, Slukvin, II and J. A. Thomson: Induced pluripotent stem cell lines derived from human somatic cells. *Science*, 318(5858), 1917-20 (2007)

19. T. Aoi, K. Yae, M. Nakagawa, T. Ichisaka, K. Okita, K. Takahashi, T. Chiba and S. Yamanaka: Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science*, 321(5889), 699-702 (2008)

20. T. Aasen, A. Raya, M. J. Barrero, E. Garreta, A. Consiglio, F. Gonzalez, R. Vassena, J. Bilic, V. Pekarik, G. Tiscornia, M. Edel, S. Boue and J. C. Izpisua Belmonte: Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol*, 26(11), 1276-84 (2008)

21. J. Hanna, S. Markoulaki, P. Schorderet, B. W. Carey, C. Beard, M. Wernig, M. P. Creyghton, E. J. Steine, J. P. Cassady, R. Foreman, C. J. Lengner, J. A. Dausman and R. Jaenisch: Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell*, 133(2), 250-64 (2008)

22. D. Duinsbergen, M. Eriksson, P. A. t Hoen, J. Frisen and H. Mikkers: Induced pluripotency with endogenous and inducible genes. *Exp Cell Res*, 314(17), 3255-63 (2008)

23. K. Nishimura, T. Nakagawa, K. Ono, H. Ogita, T. Sakamoto, N. Yamamoto, K. Okita, S. Yamanaka and J. Ito: Transplantation of mouse induced pluripotent stem cells into the cochlea. *Neuroreport*, 20(14), 1250-4 (2009)

24. K. Oshima, K. Shin, M. Diensthuber, A. W. Peng, A. J. Ricci and S. Heller: Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells. *Cell*, 141(4), 704-16 (2010)

25. M. Wernig, A. Meissner, R. Foreman, T. Brambrink, M. Ku, K. Hochedlinger, B. E. Bernstein and R. Jaenisch: *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature*, 448(7151), 318-24 (2007)

26. L. Shao and W. S. Wu: Gene-delivery systems for iPS cell generation. *Expert Opin Biol Ther*, 10(2), 231-42 (2010)

27. Y. Ma, A. Ramezani, R. Lewis, R. G. Hawley and J. A. Thomson: High-level sustained transgene expression in human embryonic stem cells using lentiviral vectors. *Stem Cells*, 21(1), 111-7 (2003)

28. K. Kaji, K. Norrby, A. Paca, M. Mileikovsky, P. Mohseni and K. Woltjen: Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature*, 458(7239), 771-5 (2009)

29. K. Tashiro, M. Inamura, K. Kawabata, F. Sakurai, K. Yamanishi, T. Hayakawa and H. Mizuguchi: Efficient adipocyte and osteoblast differentiation from mouse induced pluripotent stem cells by adenoviral transduction. *Stem Cells*, 27(8), 1802-11 (2009)

30. J. Yu, K. Hu, K. Smuga-Otto, S. Tian, R. Stewart, Slukvin, II and J. A. Thomson: Human induced pluripotent stem cells free of vector and transgene sequences. *Science*, 324(5928), 797-801 (2009)

31. H. Zhou, S. Wu, J. Y. Joo, S. Zhu, D. W. Han, T. Lin, S. Trauger, G. Bien, S. Yao, Y. Zhu, G. Siuzdak, H. R. Scholer, L. Duan and S. Ding: Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell*, 4(5), 381-4 (2009)

32. J. C. Belmonte, J. Ellis, K. Hochedlinger and S. Yamanaka: Induced pluripotent stem cells and reprogramming: seeing the science through the hype. *Nat Rev Genet*, 10(12), 878-83 (2009)

33. A. Jose and L. K. Krishnan: Effect of matrix composition on differentiation of nestin-positive neural progenitors from circulation into neurons. *J Neural Eng*, 7(3), 036009 (2010)

34. K. Jin, X. O. Mao, S. Batteur, Y. Sun and D. A. Greenberg: Induction of neuronal markers in bone marrow cells: differential effects of growth factors and patterns of intracellular expression. *Exp Neurol*, 184(1), 78-89 (2003)

35. P. Durbec and G. Rougon: Transplantation of mammalian olfactory progenitors into chick hosts reveals migration and differentiation potentials dependent on cell commitment. *Mol Cell Neurosci*, 17(3), 561-76 (2001)

36. K. M. Safford, K. C. Hicok, S. D. Safford, Y. D. Halvorsen, W. O. Wilkison, J. M. Gimble and H. E. Rice: Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem Biophys Res Commun*, 294(2), 371-9 (2002)

37. Y. Ruzankina, A. Asare and E. J. Brown: Replicative stress, stem cells and aging. *Mech Ageing Dev*, 129(7-8), 460-6 (2008)

38. W. Techawattanawisal, K. Nakahama, M. Komaki, M. Abe, Y. Takagi and I. Morita: Isolation of multipotent stem cells from adult rat periodontal ligament by neurosphere-forming culture system. *Biochem Biophys Res Commun*, 357(4), 917-23 (2007)

39. D. Widera, C. Zander, M. Heidbreder, Y. Kasperek, T. Noll, O. Seitz, B. Saldamli, H. Sudhoff, R. Sader, C. Kaltschmidt and B. Kaltschmidt: Adult palatum as a novel source of neural crest-related stem cells. *Stem Cells*, 27(8), 1899-910 (2009)

40. M. Sieber-Blum and M. Grim: The adult hair follicle: cradle for pluripotent neural crest stem cells. *Birth Defects Res C Embryo Today*, 72(2), 162-72 (2004)

41. K. J. Fernandes, I. A. McKenzie, P. Mill, K. M. Smith, M. Akhavan, F. Barnabe-Heider, J. Biernaskie, A. Junek, N. R. Kobayashi, J. G. Toma, D. R. Kaplan, P. A. Labosky, V. Rafuse, C. C. Hui and F. D. Miller: A dermal niche for multipotent adult skin-derived precursor cells. *Nat Cell Biol*, 6(11), 1082-93 (2004)

42. K. J. Fernandes, N. R. Kobayashi, C. J. Gallagher, F. Barnabe-Heider, A. Aumont, D. R. Kaplan and F. D. Miller: Analysis of the neurogenic potential of multipotent skin-derived precursors. *Exp Neurol*, 201(1), 32-48 (2006)

43. F. Lefcort and L. George: Neural crest cell fate: to be or not to be prespecified. *Cell Adh Migr*, 1(4), 199-201 (2007)

44. M. Bronner-Fraser and S. E. Fraser: Cell lineage analysis reveals multipotency of some avian neural crest cells. *Nature*, 335(6186), 161-4 (1988)

45. M. Bronner-Fraser and S. Fraser: Developmental potential of avian trunk neural crest cells *in situ*. *Neuron*, 3(6), 755-66 (1989)

46. E. Frank and J. R. Sanes: Lineage of neurons and glia in chick dorsal root ganglia: analysis *in vivo* with a recombinant retrovirus. *Development*, 111(4), 895-908 (1991)

47. M. Sieber-Blum: Clonal analysis of the quail neural crest: pluripotency, autonomous differentiation, and modulation of differentiation by exogenous factors. *Prog Clin Biol Res*, 85 Pt B, 485-96 (1982)

48. P. M. Kulesa, F. Lefcort and J. C. Kasemeier-Kulesa: The migration of autonomic precursor cells in the embryo. *Auton Neurosci*, 151(1), 3-9 (2009)

49. A. M. Davies: Extracellular signals regulating sympathetic neuron survival and target innervation during development. *Auton Neurosci*, 151(1), 39-45 (2009)

50. L. L. Sandell and P. A. Trainor: Neural crest cell plasticity. size matters. *Adv Exp Med Biol*, 589, 78-95 (2006)

51. F. Marmigere and P. Ernfors: Specification and connectivity of neuronal subtypes in the sensory lineage. *Nat Rev Neurosci*, 8(2), 114-27 (2007)

52. J. Begbie: Migration of neuroblasts from neurogenic placodes. *Dev Neurosci*, 30(1-3), 33-5 (2008)

53. J. Begbie and A. Graham: Integration between the epibranchial placodes and the hindbrain. *Science*, 294(5542), 595-8 (2001)

54. S. A. Brugmann and S. A. Moody: Induction and specification of the vertebrate ectodermal placodes: precursors of the cranial sensory organs. *Biol Cell*, 97(5), 303-19 (2005)

55. K. L. McCabe and M. Bronner-Fraser: Molecular and tissue interactions governing induction of cranial ectodermal placodes. *Dev Biol*, 332(2), 189-95 (2009)

56. A. Litsiou, S. Hanson and A. Streit: A balance of FGF, BMP and WNT signalling positions the future placode territory in the head. *Development*, 132(18), 4051-62 (2005)

57. G. Schlosser: Do vertebrate neural crest and cranial placodes have a common evolutionary origin? *Bioessays*, 30(7), 659-72 (2008)

58. R. K. Ladher, P. O'Neill and J. Begbie: From shared lineage to distinct functions: the development of the inner ear and epibranchial placodes. *Development*, 137(11), 1777-85 (2010)

59. S. H. Kil and A. Collazo: A review of inner ear fate maps and cell lineage studies. *J Neurobiol*, 53(2), 129-42 (2002)

60. K. R. Jessen and R. Mirsky: Signals that determine Schwann cell identity. *J Anat*, 200(4), 367-76 (2002)

61. T. Sauka-Spengler and M. Bronner-Fraser: A gene regulatory network orchestrates neural crest formation. *Nat Rev Mol Cell Biol*, 9(7), 557-68 (2008)

62. C. V. Baker and M. Bronner-Fraser: Vertebrate cranial placodes I. Embryonic induction. *Dev Biol*, 232(1), 1-61 (2001)

63. L. B. Zimmerman, J. M. De Jesus-Escobar and R. M. Harland: The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell*, 86(4), 599-606 (1996)

64. J. Larrain, D. Bachiller, B. Lu, E. Agius, S. Piccolo and E. M. De Robertis: BMP-binding modules in chordin: a model for signalling regulation in the extracellular space. *Development*, 127(4), 821-30 (2000)

65. W. Liu, C. Ren, J. Shi, X. Feng, Z. He, L. Xu, K. Lan, L. Xie, Y. Peng, J. Fan, H. Kung, K. T. Yao and R. H. Xu: Characterization of the functionally related sites in the neural inducing gene noggin. *Biochem Biophys Res Commun*, 270(1), 293-7 (2000)

66. S. Piccolo, E. Agius, L. Leyns, S. Bhattacharyya, H. Grunz, T. Bouwmeester and E. M. De Robertis: The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. *Nature*, 397(6721), 707-10 (1999)

67. I. Mason: Neural induction: do fibroblast growth factors strike a cord? *Curr Biol*, 6(6), 672-5 (1996)

68. S. I. Wilson, E. Graziano, R. Harland, T. M. Jessell and T. Edlund: An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo. *Curr Biol*, 10(8), 421-9 (2000)

69. A. Streit, A. J. Berliner, C. Papanayotou, A. Sirulnik and C. D. Stern: Initiation of neural induction by FGF signalling before gastrulation. *Nature*, 406(6791), 74-8 (2000)

70. R. Morgan and M. G. Sargent: The role in neural patterning of translation initiation factor eIF4AII; induction of neural fold genes. *Development*, 124(14), 2751-60 (1997)

71. L. Marchant, C. Linker, P. Ruiz, N. Guerrero and R. Mayor: The inductive properties of mesoderm suggest that

the neural crest cells are specified by a BMP gradient. *Dev Biol*, 198(2), 319-29 (1998)

72. R. Mayor and M. J. Aybar: Induction and development of neural crest in Xenopus laevis. *Cell Tissue Res*, 305(2), 203-9 (2001)

73. D. Meulemans and M. Bronner-Fraser: Gene-regulatory interactions in neural crest evolution and development. *Dev Cell*, 7(3), 291-9 (2004)

74. G. Schlosser and K. Ahrens: Molecular anatomy of placode development in Xenopus laevis. *Dev Biol*, 271(2), 439-66 (2004)

75. M. Cheung, M. C. Chaboissier, A. Mynett, E. Hirst, A. Schedl and J. Briscoe: The transcriptional control of trunk neural crest induction, survival, and delamination. *Dev Cell*, 8(2), 179-92 (2005)

76. S. Nakagawa and M. Takeichi: Neural crest emigration from the neural tube depends on regulated cadherin expression. *Development*, 125(15), 2963-71 (1998)

77. H. Y. Lee, M. Kleber, L. Hari, V. Brault, U. Suter, M. M. Taketo, R. Kemler and L. Sommer: Instructive role of Wnt/beta-catenin in sensory fate specification in neural crest stem cells. *Science*, 303(5660), 1020-3 (2004)

78. M. Kleber, H. Y. Lee, H. Wurdak, J. Buchstaller, M. M. Riccomagno, L. M. Ittner, U. Suter, D. J. Epstein and L. Sommer: Neural crest stem cell maintenance by combinatorial Wnt and BMP signaling. *J Cell Biol*, 169(2), 309-20 (2005)

79. T. J. Carney, K. A. Dutton, E. Greenhill, M. Delfino-Machin, P. Dufourcq, P. Blader and R. N. Kelsh: A direct role for Sox10 in specification of neural crest-derived sensory neurons. *Development*, 133(23), 4619-30 (2006)

80. R. I. Peirano, D. E. Goerich, D. Riethmacher and M. Wegner: Protein zero gene expression is regulated by the glial transcription factor Sox10. *Mol Cell Biol*, 20(9), 3198-209 (2000)

81. Q. Ma, C. Fode, F. Guillemot and D. J. Anderson: Neurogenin1 and neurogenin2 control two distinct waves of neurogenesis in developing dorsal root ganglia. *Genes Dev*, 13(13), 1717-28 (1999)

82. S. E. Perez, S. Rebelo and D. J. Anderson: Early specification of sensory neuron fate revealed by expression and function of neurogenins in the chick embryo. *Development*, 126(8), 1715-28 (1999)

83. D. J. Anderson: Lineages and transcription factors in the specification of vertebrate primary sensory neurons. *Curr Opin Neurobiol*, 9(5), 517-24 (1999)

84. N. Bertrand, D. S. Castro and F. Guillemot: Proneural genes and the specification of neural cell types. *Nat Rev Neurosci*, 3(7), 517-30 (2002)

85. L. Lo, E. Dormand, A. Greenwood and D. J. Anderson: Comparison of the generic neuronal differentiation and neuron subtype specification functions of mammalian achaete-scute and atonal homologs in cultured neural progenitor cells. *Development*, 129(7), 1553-67 (2002)

86. A. I. Chen, J. C. de Nooij and T. M. Jessell: Graded activity of transcription factor Runx3 specifies the laminar termination pattern of sensory axons in the developing spinal cord. *Neuron*, 49(3), 395-408 (2006)

87. I. Kramer, M. Sigrist, J. C. de Nooij, I. Taniuchi, T. M. Jessell and S. Arber: A role for Runx transcription factor signaling in dorsal root ganglion sensory neuron diversification. *Neuron*, 49(3), 379-93 (2006)

88. D. Levanon, D. Bettoun, C. Harris-Cerruti, E. Woolf, V. Negreanu, R. Eilam, Y. Bernstein, D. Goldenberg, C. Xiao, M. Fliegauf, E. Kremer, F. Otto, O. Brenner, A. Lev-Tov and Y. Groner: The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. *Embo J*, 21(13), 3454-63 (2002)

89. A. Montelius, F. Marmigere, C. Baudet, J. B. Aquino, S. Enerback and P. Ernfors: Emergence of the sensory nervous system as defined by Foxs1 expression. *Differentiation*, 75(5), 404-17 (2007)

90. F. Marmigere, A. Montelius, M. Wegner, Y. Groner, L. F. Reichardt and P. Ernfors: The Runx1/AML1 transcription factor selectively regulates development and survival of TrkA nociceptive sensory neurons. *Nat Neurosci*, 9(2), 180-7 (2006)

91. C. L. Chen, D. C. Broom, Y. Liu, J. C. de Nooij, Z. Li, C. Cen, O. A. Samad, T. M. Jessell, C. J. Woolf and Q. Ma: Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. *Neuron*, 49(3), 365-77 (2006)

92. D. Levanon, O. Brenner, V. Negreanu, D. Bettoun, E. Woolf, R. Eilam, J. Lotem, U. Gat, F. Otto, N. Speck and Y. Groner: Spatial and temporal expression pattern of Runx3 (Aml2) and Runx1 (Aml1) indicates non-redundant functions during mouse embryogenesis. *Mech Dev*, 109(2), 413-7 (2001)

93. G. S. Maro, M. Vermeren, O. Voiculescu, L. Melton, J. Cohen, P. Charnay and P. Topilko: Neural crest boundary cap cells constitute a source of neuronal and glial cells of the PNS. *Nat Neurosci*, 7(9), 930-8 (2004)

94. B. Fritzsch, K. W. Beisel and L. A. Hansen: The molecular basis of neurosensory cell formation in ear development: a blueprint for hair cell and sensory neuron regeneration? *Bioessays*, 28(12), 1181-93 (2006)

95. M. M. Riccomagno, L. Martinu, M. Mulheisen, D. K. Wu and D. J. Epstein: Specification of the mammalian cochlea is dependent on Sonic hedgehog. *Genes Dev*, 16(18), 2365-78 (2002)

96. T. J. Wright, E. P. Hatch, H. Karabagli, P. Karabagli, G. C. Schoenwolf and S. L. Mansour: Expression of mouse fibroblast growth factor and fibroblast growth factor receptor genes during early inner ear development. *Dev Dyn*, 228(2), 267-72 (2003)

97. M. M. Riccomagno, S. Takada and D. J. Epstein: Wnt-dependent regulation of inner ear morphogenesis is balanced by the opposing and supporting roles of Shh. *Genes Dev*, 19(13), 1612-23 (2005)

98. H. Morsli, D. Choo, A. Ryan, R. Johnson and D. K. Wu: Development of the mouse inner ear and origin of its sensory organs. *J Neurosci*, 18(9), 3327-35 (1998)

99. N. Saint-Germain, Y. H. Lee, Y. Zhang, T. D. Sargent and J. P. Saint-Jeannet: Specification of the otic placode depends on Sox9 function in Xenopus. *Development*, 131(8), 1755-63 (2004)

100. S. M. Honore, M. J. Aybar and R. Mayor: Sox10 is required for the early development of the prospective neural crest in Xenopus embryos. *Dev Biol*, 260(1), 79-96 (2003)

101. B. Fritzsch, D. F. Eberl and K. W. Beisel: The role of bHLH genes in ear development and evolution: revisiting a 10-year-old hypothesis. *Cell Mol Life Sci* (2010)

102. Q. Ma, Z. Chen, I. del Barco Barrantes, J. L. de la Pompa and D. J. Anderson: neurogenin1 is essential for the determination of neuronal precursors for proximal cranial sensory ganglia. *Neuron*, 20(3), 469-82 (1998)

103. Q. Ma, D. J. Anderson and B. Fritzsch: Neurogenin 1 null mutant ears develop fewer, morphologically normal hair cells in smaller sensory epithelia devoid of innervation. *J Assoc Res Otolaryngol*, 1(2), 129-43 (2000)

104. W. Y. Kim, B. Fritzsch, A. Serls, L. A. Bakel, E. J. Huang, L. F. Reichardt, D. S. Barth and J. E. Lee: NeuroD-null mice are deaf due to a severe loss of the inner ear sensory neurons during development. *Development*, 128(3), 417-26 (2001)

105. M. Kruger, T. Schmid, S. Kruger, E. Bober and T. Braun: Functional redundancy of NSCL-1 and NeuroD during development of the petrosal and vestibulocochlear ganglia. *Eur J Neurosci*, 24(6), 1581-90 (2006)

106. M. Liu, F. A. Pereira, S. D. Price, M. J. Chu, C. Shope, D. Himes, R. A. Eatock, W. E. Brownell, A. Lysakowski and M. J. Tsai: Essential role of BETA2/NeuroD1 in development of the vestibular and auditory systems. *Genes Dev*, 14(22), 2839-54 (2000)

107. E. J. Huang, W. Liu, B. Fritzsch, L. M. Bianchi, L. F. Reichardt and M. Xiang: Brn3a is a transcriptional regulator of soma size, target field innervation and axon pathfinding of inner ear sensory neurons. *Development*, 128(13), 2421-32 (2001)

108. G. M. Kruger, J. T. Mosher, S. Bixby, N. Joseph, T. Iwashita and S. J. Morrison: Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. *Neuron*, 35(4), 657-69 (2002)

109. H. Y. Li, E. H. Say and X. F. Zhou: Isolation and characterization of neural crest progenitors from adult dorsal root ganglia. *Stem Cells*, 25(8), 2053-65 (2007)

110. Y. Tomita, K. Matsumura, Y. Wakamatsu, Y. Matsuzaki, I. Shibuya, H. Kawaguchi, M. Ieda, S. Kanakubo, T. Shimazaki, S. Ogawa, N. Osumi, H. Okano and K. Fukuda: Cardiac neural crest cells contribute to the dormant multipotent stem cell in the mammalian heart. *J Cell Biol*, 170(7), 1135-46 (2005)

111. J. G. Toma, M. Akhavan, K. J. Fernandes, F. Barnabe-Heider, A. Sadikot, D. R. Kaplan and F. D. Miller: Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol*, 3(9), 778-84 (2001)

112. R. Sasaki, S. Aoki, M. Yamato, H. Uchiyama, K. Wada, T. Okano and H. Ogiuchi: Neurosphere generation from dental pulp of adult rat incisor. *Eur J Neurosci*, 27(3), 538-48 (2008)

113. K. L. Yang, M. F. Chen, C. H. Liao, C. Y. Pang and P. Y. Lin: A simple and efficient method for generating Nurr1-positive neuronal stem cells from human wisdom teeth (tNSC) and the potential of tNSC for stroke therapy. *Cytotherapy*, 11(5), 606-17 (2009)

114. R. El Seady, M. A. Huisman, C. W. Lowik and J. H. Frijns: Uncomplicated differentiation of stem cells into bipolar neurons and myelinating glia. *Biochem Biophys Res Commun*, 376(2), 358-62 (2008)

115. J. Biernaskie: Human hair follicles: "bulging" with neural crest-like stem cells. *J Invest Dermatol*, 130(5), 1202-4 (2010)

116. J. G. Nijhof, K. M. Braun, A. Giangreco, C. van Pelt, H. Kawamoto, R. L. Boyd, R. Willemze, L. H. Mullenders, F. M. Watt, F. R. de Gruijl and W. van Ewijk: The cell-surface marker MTS24 identifies a novel population of follicular keratinocytes with characteristics of progenitor cells. *Development*, 133(15), 3027-37 (2006)

117. Y. Amoh, L. Li, R. Campillo, K. Kawahara, K. Katsuoka, S. Penman and R. M. Hoffman: Implanted hair follicle stem cells form Schwann cells that support repair of severed peripheral nerves. *Proc Natl Acad Sci U S A*, 102(49), 17734-8 (2005)

118. V. Jaks, M. Kasper and R. Toftgard: The hair follicle-a stem cell zoo. *Exp Cell Res*, 316(8), 1422-8 (2010)

119. M. Sieber-Blum, M. Grim, Y. F. Hu and V. Szeder: Pluripotent neural crest stem cells in the adult hair follicle. *Dev Dyn*, 231(2), 258-69 (2004)

120. Y. Amoh, Y. Hamada, R. Aki, K. Kawahara, R. M. Hoffman and K. Katsuoka: Direct transplantation of uncultured hair-follicle pluripotent stem (hfPS) cells promotes the recovery of peripheral nerve injury. *J Cell Biochem*, 110(1), 272-7 (2010)

121. Y. F. Hu, Z. J. Zhang and M. Sieber-Blum: An epidermal neural crest stem cell (EPI-NCSC) molecular signature. *Stem Cells*, 24(12), 2692-702 (2006)

122. Y. F. Hu, K. Gourab, C. Wells, O. Clewes, B. D. Schmit and M. Sieber-Blum: Epidermal neural crest stem cell (EPI-NCSC)--mediated recovery of sensory function in a mouse model of spinal cord injury. *Stem Cell Rev*, 6(2), 186-98 (2010)

123. E. V. Sviderskaya, D. J. Easty, M. A. Lawrence, D. P. Sanchez, Y. A. Negulyaev, R. H. Patel, P. Anand, Y. E. Korchev and D. C. Bennett: Functional neurons and melanocytes induced from immortal lines of postnatal neural crest-like stem cells. *Faseb J*, 23(9), 3179-92 (2009)

124. M. Lako, L. Armstrong, P. M. Cairns, S. Harris, N. Hole and C. A. Jahoda: Hair follicle dermal cells repopulate the mouse haematopoietic system. *J Cell Sci*, 115(Pt 20), 3967-74 (2002)

125. A. J. Reynolds, C. Lawrence, P. B. Cserhalmi-Friedman, A. M. Christiano and C. A. Jahoda: Trans-gender induction of hair follicles. *Nature*, 402(6757), 33-4 (1999)

Key Words: Deafness, Spiral Ganglion Neurons, Neural Crest Stem Cells, Hair Follicle, Stem Cell Therapy, Review

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