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Neural Crest-Derived Stem Cells Display a Wide Variety of Characteristics

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ABSTRACT

A recent burst of findings has shown that neural crest-derived stem cells (NCSCs) can be found in diverse mammalian tissues. In addition to their identification in tissues that are known to be derived from the neural crest, recent studies have revealed NCSCs in tissues that are not specifically derived from the neural crest, such as bone marrow. NCSCs can express a wide range of characteristics, and which properties are expressed mainly depends on their tissue sources and the ontogenic stage of the animal. The identification of NCSCs in various tissues opens an entirely new avenue of approach to developing autologous cell replacement therapies for use in regenerative medicine. In this review, we discuss the origin, migration, and lineage potential of NCSCs from various mammalian tissue sources. J. Cell. Biochem. 107: 1046–1052, 2009. © 2009 Wiley-Liss, Inc.

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The neural crest is a transient embryonic tissue that originates at the neural folds during vertebrate development. The neural crest-derived cells delaminate from the dorsal neural tube and migrate to various locations, where they differentiate into a vast array of cell types, from neural to mesenchymal [Le Douarin and Kalcheim, 1999]. In addition, some neural crest-derived cells are maintained in an undifferentiated state as neural crest-derived stem cells (NCSCs) throughout the life of the animal. Although how NCSCs are defined has varied among reports to date, it is clear that NCSCs have a self-renewal ability and the potential to differentiate into several different neural-crest lineages, including neurons, glial cells, myofibroblasts, melanocytes, adipocytes, chondrocytes, osteocytes, and connective tissues [Crane and Trainor, 2006; Delfino-Machin et al., 2007]. Recent studies have demonstrated the presence of NCSCs in a number of different tissues. This review will consider the present status of NCSC research, focusing on the origin, migration, and characteristics of mammalian NCSCs, and highlighting the differences in the phenotypes of NCSCs derived from different tissues.

ISOLATION AND CHARACTERIZATION OF EMBRYONIC NCSCs

Stemple and Anderson [1992] first described mammalian NCSCs, which they isolated separately from the rat embryonic neural tube. These NCSCs were isolated by flow cytometry set to select cells expressing low-affinity nerve growth factor (NGF) receptor ($p75^{LNTR}$). The frequency of colony formation was significantly higher in the $p75^+$ fraction than the $p75^-$ one. The $p75^+$ colony-forming cells had self-renewal activity and gave rise to neurons, glial cells, and myofibroblasts. These colony-forming cells are now well accepted as being the NCSCs.

Cells with similar properties to the NCSCs have been isolated from rat embryonic sciatic nerve in the post-migratory phase of neural crest development [Morrison et al., 1999]. Because glial cells in the sciatic nerve also express p75, the authors selected the cell fraction that was both positive for p75 and negative for P0 (peripheral myelin protein). The isolated NCSCs showed a significant enrichment of colony-forming cells, with self-renewal and multipotent

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differentiation potential. An in vivo study in mouse revealed that NCSCs in the peripheral nerve generate Schwann cells and endoneurial fibroblasts during embryonic development [Joseph et al., 2004]. These reports demonstrate that multipotent NCSCs persist at least until late gestation, after the onset of neural crest migration, and suggest that they persist in other tissues as well, during embryogenesis.

Rat NCSCs were also isolated from the gut at E14.5 by selecting for p75^{+} and α4 integrin^{+} fractions [Bixby et al., 2002]. The authors compared the characteristics of the NCSCs from the gut and sciatic nerve, and demonstrated that gut NCSCs are sensitive to neurogenic factors, while sciatic nerve NCSCs are sensitive to gliogenic factors in vitro. Upon transplantation of both types of NCSCs into the developing peripheral nerve in vivo, gut NCSCs gave rise primarily to neurons, while sciatic nerve NCSCs gave rise to glial cells. These results suggest that the phenotype of NCSCs mainly depends on their post-migratory tissue source. Furthermore, NCSCs express characteristics in a combinatorial manner that reflects regional environmental differences as well as cell-intrinsic differences [Bixby et al., 2002]. However, the mechanisms that control the lineage determination and/or the timing of differentiation among the tissue sources remain unclear. Therefore, of interest will be the clarification of the molecular mechanisms operating on epigenetic modifications of a differentiation-related gene that is involved in the regulation of the spatial and temporal specifications of NCSCs.

INSTRUCTIVE SIGNALS FOR NCSCs

After migration, the NCSCs undergo specific differentiation steps that are influenced by environmental signals. BMP2 acts as a growth factor and instructs embryonic NCSCs to become autonomic neurons of the peripheral nervous system [Shah et al., 1996]. Wnt signaling instructs embryonic NCSCs to adopt a sensory neuronal phenotype [Lee et al., 2004]. Interestingly, the combined action of BMP2 and Wnt1 does not induce the NCSCs to differentiate into a particular cell fate, but rather maintains the undifferentiated state [Kleber et al., 2005]. Other factors involved in embryonic NCSC differentiation are glial growth factors, which induce the generation of peripheral glia, and transforming growth factor-β (TGF-β), which promotes the generation of smooth muscle cells [Le Douarin and Dupin, 2003]. Thus, the developmental processes of differentiation and proliferation in NCSCs require precise coordination and control.

MIGRATORY PATHWAYS OF NCSCs

Once the neural crest-derived cells detach from the dorsal neural tube, they migrate along specific pathways. The neural crest-derived cells of both the branchial region and the trunk of vertebrate embryos migrate in segmentally restricted streams. The cephalic neural crest cells migrate into the branchial arches, where they differentiate into specific bones, cartilages, and cranial ganglia [Kontges and Lumsden, 1996]. The trunk neural crest cells travel two pathways, dorsolaterally, between the somites and the overlying ectoderm, and ventromedially, through the somatic mesoderm or between the neural tube and the somites (Fig. 1) [Le Douarin and

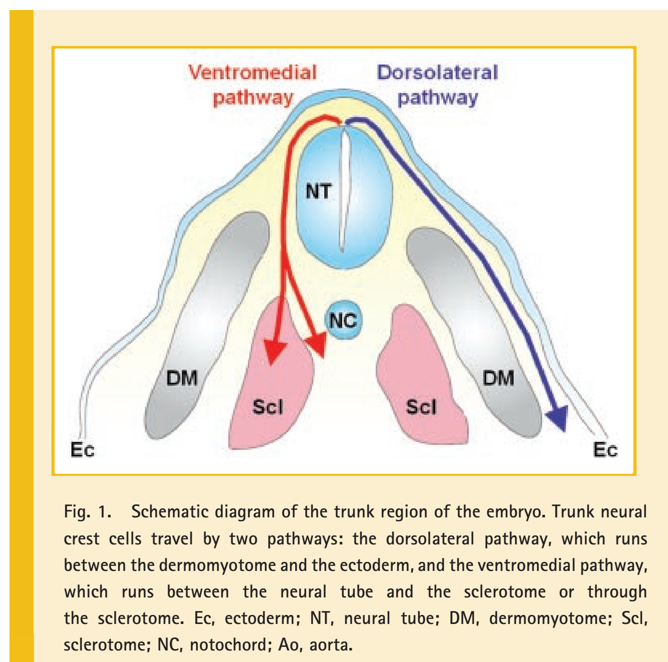

Fig. 1. Schematic diagram of the trunk region of the embryo. Trunk neural crest cells travel by two pathways: the dorsolateral pathway, which runs between the dermomyotome and the ectoderm, and the ventromedial pathway, which runs between the neural tube and the sclerotome or through the sclerotome. Ec, ectoderm; NT, neural tube; DM, dermomyotome; Scl, sclerotome; NC, notochord; Ao, aorta.

Kalcheim, 1999]. Neural crest cells that travel medially contribute to the sensory and sympathetic ganglia, and generate Schwann cells and chromaffin cells. Neural crest that migrates dorsolaterally generates melanocytes.

Recent work has identified the signaling mechanism for this migration [Kuriyama and Mayor, 2008]. Briefly, neural crest cells are known to undergo epithelial–mesenchymal transition when they delaminate from the dorsal neural tube, a process controlled by *cadherins*, *connexins*, *snail*, *twist*, and *matrix metalloproteases (MMPs)* genes. Once the neural crest cells delaminate from the dorsal neural tube, their well-regulated migration is controlled by some specific signals such as Eph/ephrins, semaphorins/neuropilins, and Slit/Robo, which play important roles as repulsive signals modulating the migration of neural crest cells into target areas of embryo [Kuriyama and Mayor, 2008].

The migratory routes and destinations chosen by transplanted NCSCs that were isolated from adult rodent heart were examined in the chick embryo. These NCSCs migrated to the dorsal root ganglia (DRG) and ventral spinal nerve by the medial route, and to the outflow tract and conotruncus of the developing heart by the lateral route [Tomita et al., 2005]. Similarly, skin-derived NCSCs, also called skin-derived precursors (SKPs), that were harvested from rodent trunk skin and transplanted into the dorsal neural tube of the chick embryo, migrated back to the skin by the lateral route and to the DRG and spinal nerve by the medial route [Fernandes et al., 2004]. These studies showed that transplanted NCSCs could migrate like other neural crest cells and contribute to a variety of neural crest-derived tissues when reintroduced into the embryonic environment.

Recently, our group reported that NCSCs also migrate into the aorta-gonad-mesonephros (AGM) region in embryonic mice [Nagoshi et al., 2008]. The AGM region is a transient embryonic tissue in which the first adult-type hematopoietic stem cells (HSCs) are generated. Late in embryogenesis, the HSCs migrate within the

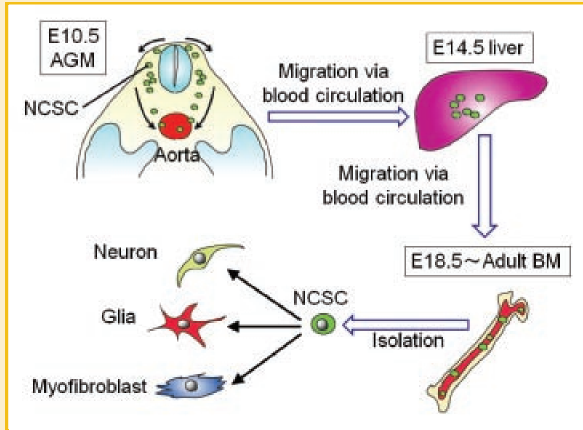

Fig. 2. Simplified cartoon of the NCSC migratory stream from the dorsal neural tube to the BM. During embryogenesis, some NCSCs migrate into the AGM region, enter the blood circulation, pass through the fetal liver, and reach the BM just before birth. The migrated NCSCs persist in the BM throughout life, and maintain the potential to differentiate into various types of neural-crest lineage cells.

bloodstream, from which they pass through fetal liver; the HSCs then enter the bone marrow (BM) just before the mice are born [Dzierzak and Speck, 2008]. We found that, like the HSCs, NCSCs migrate from the AGM region through the bloodstream, and the fetal liver to the BM (Fig. 2) [Nagoshi et al., 2008]. The timing of the NCSC migration coincides with that of the HSCs, implying that some undefined relationship exists between the NCSCs and hematopoiesis.

NCSCs IN ADULT TISSUES

NCSCs have been found in diverse adult mammalian tissues. Because the methods for harvesting and analyzing the NCSCs differ among reports, as do the NCSCs' reported characteristics, in the following text, these findings are systematically compared and summarized (Table I).

BONE MARROW

We demonstrated the existence of NCSCs in the BM of adult rodents [Nagoshi et al., 2008] using the Cre-lox system-mediated lineage analysis and sphere culture technique. Transgenic mice expressing Cre recombinase under control of the P0 promoter (P0-Cre) [Yamauchi et al., 1999] or Wnt1 promoter/enhancer (Wnt1-Cre) [Danielian et al., 1998] were mated with EGFP reporter mice (CAG-CAT$^{loxP/loxP}$-EGFP) [Kawamoto et al., 2000] to obtain P0-Cre/CAG-EGFP or Wnt1-Cre/CAG-EGFP double-transgenic mice [Nagoshi et al., 2008]. P0 promoter expresses genes after differentiation of neural crest cells from the dorsal neural tube [Yamauchi et al., 1999], and Wnt1 promoter/enhancer expresses genes in the dorsal neural tube and roof-plate from the onset of neural crest migration [Danielian et al., 1998]. In these transgenic mice, the transient activation of the P0 promoter and Wnt1 promoter/enhancer induces Cre-mediated recombination in premigratory neural crest cells, indelibly tagging neural crest-derived cells with EGFP expression. Prospectively isolated EGFP$^+$ cells from the BM of P0 and Wnt1-Cre/CAG-EGFP adult mice proliferated in vitro to form clonal spheres, showed self-renewal activity, and differentiated into neurons, glial cells, and myofibroblasts [Nagoshi et al., 2008]. The presence of NCSCs in the BM is also supported by a recent report using the same P0-Cre/CAG-EGFP reporter mice to demonstrate that a portion of mesenchymal stem cells (MSCs) in the BM of the lower extremities are of neural-crest lineage [Takashima et al., 2007]. In addition, we recently showed that neural crest-derived cells contribute to MSCs, which can give rise to adipocytes, chondrocytes, and osteocytes [Morikawa et al., 2009]. Considering that a part of MSCs are derived from neural crest, NCSCs might play a role as an HSC niche by controlling HSC maintenance, proliferation, differentiation, and recruitment in the BM [Uccelli et al., 2008].

Several groups have demonstrated the presence of stem or precursor cells in the BM that generate neurons. For example, bone marrow stromal cells (BMSCs) harvested from rat and human express Nestin and differentiate into neurons and glial cells in vitro [Sanchez-Ramos et al., 2000]. Another report demonstrated that rat and human BMSCs cultured with FBS expand as undifferentiated cells, and upon differentiation, they become neurons [Woodbury et al., 2000]. The differentiation of BMSCs into functional neurons is enhanced by Noggin [Kohyama et al., 2001]. However, the

TABLE I. Identification of NCSCs From Various Tissues

Refs.	Age	Place	Isolation	Marker	Animal	Genotype
Stemple and Anderson [1992]	E10.5	Neural tube	Retrospective	p75+	Rat	W/T
Morrison et al. [1999]	E14.5	Sciatic nerve	Prospective	p75+P0−	Rat	W/T
Bixby et al. [2002]	E14.5	Gut	Prospective	p75+α4+	Rat	W/T
Kruger et al. [2002]	Adult	Gut	Prospective	p75+	Rat	W/T
Hagedorn et al. [1999]	E14	DRG	Retrospective	p75+	Rat	W/T
Hjerling-Leffler et al. [2005]	E11.5	DRG	Retrospective		Mouse	W/T
Li et al. [2007]	Adult	DRG	Retrospective		Rat	W/T
Nagoshi et al. [2008]	Adult	DRG, whisker pad, bone marrow	Prospective	EGFP	Mouse	P0 and Wnt1-Cre/CAG-EGFP
Toma et al. [2001, 2005]	Juvenile and adult	Skin (face and back)	Retrospective		Mouse	W/T
	Adult	Skin (scalp)	Retrospective		Human	
Sieber-Blum et al. [2004]	Adult	Skin (bulge)	Retrospective		Mouse	Wnt1-Cre/ROSA-LacZ
Wong et al. [2006]	Adult	Skin (back)	Retrospective		Mouse	W/T
	Adult	Skin (back)	Prospective	EYFP	Mouse	Dhh and Dct-Cre/ROSA-EYFP
Toma et al. [2001, 2005]	Children	Foreskin	Retrospective		Human	
Tomita et al. [2005]	Juvenile and adult	Heart	Prospective	SP cells	Mouse	W/T, P0-Cre/CAG-EGFP
Yoshida et al. [2006]	Adult	Cornea	Retrospective		Mouse	W/T, P0, and Wnt1-Cre/CAG-EGFP
Pardal et al. [2007]	Adult	Carotid body	Retrospective		Rat	W/T
	Adult	Carotid body	Prospective	EGFP	Mouse	GFAP promoter-EGFP

developmental origin and differentiation potential of the BMSCs remain unclear, largely because it is difficult to understand how neurons, which are ectodermal in origin, are generated by the BM-derived cells. Our results demonstrating the presence of NCSCs in the BM suggest that this unusual differentiation potential is owing to NCSCs inhabiting the BM. It will be interesting to clarify the relationship between NCSCs and the BM-derived stem cells that are reported to generate neural cells.

DRG

Neural crest cells were first discovered in chick embryos as the precursors of the spinal sensory ganglia, the DRGs [His, 1868]. In recent years, detailed analyses of mammalian NCSCs in the DRG have been carried out. In one report, single cells dissociated from rat embryonic DRGs were labeled with p75 by live-cell staining, and the identified p75^{+} NCSCs were shown to give rise to neurons, glial cells, and smooth muscle-like cells in response to instructive extracellular cues, but their self-renewal activity was not assessed [Hagedorn et al., 1999]. A recent study showed that neural crest boundary cap cells, found in embryos, can generate neurons and satellite cells [Maro et al., 2004]. Boundary cap cells are neural crest derivatives that form clusters at the entry and exit points of peripheral nerve roots; they migrate to and colonize the DRG during embryogenesis. Boundary cap clusters contain multipotent NCSCs that self-renew, show multipotency, and differentiate into mature sensory neurons and Schwann cells under appropriate conditions [Hjerling-Leffler et al., 2005; Aquino et al., 2006]. These findings raised the possibility that NCSCs might persist in the DRGs throughout life, and this has now been demonstrated [Li et al., 2007]. Interestingly, the NCSCs probably originate from satellite cells [Li et al., 2007]. Given that some of the satellite cells are thought to derive from boundary cap cells [Zirlinger et al., 2002; Maro et al., 2004], these data indicate that the NCSCs, which form a subpopulation of the boundary cap cells and migrate into the DRG during embryogenesis, are maintained in an undifferentiated state throughout the life of the animal.

Our group also confirmed the existence of NCSCs in the DRG of adult mice [Nagoshi et al., 2008]. In this recent study, we compared the characteristics of NCSCs in various tissues of adult mice by examining the expression levels of the NCSC markers *sox10* [Paratore et al., 2001] and *p75* [Stemple and Anderson, 1992] and of markers for neural stem/progenitor cells, *nestin* [Lendahl et al., 1990] and *musashi1* [Sakakibara et al., 1996; Okano et al., 2002]. We found that these markers were expressed at higher levels in NCSCs from the DRG than in NCSCs from the whisker pad or BM. These results may reflect the self-renewal activity and multipotency of NCSCs, because the DRG-derived NCSCs displayed a greater ability to form secondary spheres and displayed a higher proportion of cells that maintained a multilineage differentiation potential [Nagoshi et al., 2008]. Although the methods for identifying NCSCs and the culture conditions were different in the reports cited above, the combined findings support the idea that the DRG contains a high proportion of NCSCs.

SKIN

One of the more surprising findings about NCSCs is their existence in the skin. Isolated cells from juvenile and adult rodent skin proliferate to form spheres and differentiate into several types of cells: neurons, glial cells, smooth muscle cells, and adipocytes [Toma et al., 2001]. Importantly, a single cell can also form a sphere that is self-renewed for at least 5 months of passaging and still shows the multilineage differentiation into cells of both neural and mesodermal origins. These cells are called SKPs [Toma et al., 2001]. Although the developmental origin of the SKPs was unclear when the report was published, the same group has since demonstrated that the SKPs in facial skin are derived from the neural crest [Fernandes et al., 2004].

Fernandes et al. [2004] used Wnt-Cre/ROSA-LacZ double-transgenic mice, a line used for neural-crest lineage tracing, and showed that whisker follicle dermal papillae are entirely neural crest-derived. In addition, SKP-derived spheres from the facial skin of the Wnt1-Cre/ROSA-LacZ mice were positive for β-galactosidase. The SKPs expressed the transcription factor genes *slug*, *snail*, *twist*, *Pax3*, and *Sox9*, which are also expressed in embryonic NCSCs [Fernandes et al., 2004].

Another group has demonstrated the existence of different NCSCs, "epidermal neural crest cells" (eNCSCs), in the adult mouse whisker follicle, using a different approach [Sieber-Blum et al., 2004]. This group used Wnt1-Cre reporter mice to show marker expression in the bulge region of the follicle. In explants of whisker follicle bulges, migrating eNCSCs were observed. An in vitro analysis of the emigrated eNCSCs revealed that they have self-renewal capacity and the potential to differentiate into neurons, Schwann cells, smooth muscle cells, and melanocytes, a finding that highlights the pluripotency of individual clones [Sieber-Blum et al., 2004]. The gene expression profile of eNCSCs was examined by longSAGE (long serial analysis of gene expression) [Hu et al., 2006]. The authors identified 19 genes expressed in common between eNCSCs and embryonic NCSCs. Although eNCSCs and the epidermal stem cells that generate keratinocytes share the bulge as their stem-cell niche, they are clearly distinguishable by their gene expression profiles. Interestingly, these authors also examined the expression of cell markers for SKPs in the eNCSCs, but the eNCSCs did not express any of them, showing that eNCSCs are very different from SKPs [Fernandes et al., 2004; Hu et al., 2006].

In addition to the whisker follicle of the facial skin, a recent study showed that a subpopulation of sphere-initiating cells from the murine trunk skin is also of neural crest origin [Wong et al., 2006]. Spheres derived from trunk skin contain cells that express the NCSC markers p75 and Sox10, display self-renewal capacity over more than 20 passages, and differentiate into neurons, glial cells, smooth muscle cells, chondrocytes, melanocytes, and adipocytes.

Wong et al. also used Desert Hedgehog (Dhh)-Cre/ROSA-LacZ mice, which express Cre recombinase in the peripheral glial lineage, and found that *LacZ*-positive cells in the bulge region of trunk skin were positive for p75 and Sox10, suggesting the existence of NCSCs which could give rise to glial lineage. Moreover, in Dct-Cre/ROSA-*LacZ* mice, which express Cre recombinase in melanocytes, *LacZ*-positive cells in the bulge region and hair follicle bulb were positive for p75 and Sox10, suggesting that NCSCs, which could give rise to melanocyte lineage, also exist in the bulge region and bulb [Wong et al., 2006].

When these authors prospectively isolated enhanced yellow fluorescent protein (EYFP)-positive cells from the trunk skin of Dhh-

Cre/ROSA-EYFP and Dct-Cre/ROSA-EYFP mice, the cells proliferated to form spheres, and the spheres contained cells positive for p75 and Sox10. The authors concluded that NCSCs or neural crest-derived progenitor cells that are restricted to the glial and melanocyte lineages also exist in the trunk skin of adult mice [Wong et al., 2006].

Stem cells from human skin have been identified as well. SKPs from the human scalp express Nestin by immunohistochemstry and differentiate into neurons [Toma et al., 2001]. The same group has also shown that SKPs exist in juvenile human foreskin that show self-renewal activity by several passages, differentiate into neurons, glial cells, smooth muscle cells, and adipocytes, and express the neural crest-specific markers *Pax3*, *Snail*, and *Slug* by RT-PCR [Toma et al., 2005]. Similar results were observed in the adult human dermis, which contains sphere-initiating cells [Joannides et al., 2004]. These spheres expressed Nestin and Musashi1, and differentiated into neurons, fibronectin, and smooth muscle cells. However, they did not express neural crest markers or differentiated glial cells [Joannides et al., 2004]. Since the sphere-initiating cells from human skin were retrospectively identified in all of these reports (Table I), it remains uncertain that these cells are derived from neural crest. Future studies will help identify novel neural crest markers to allow the prospective isolation of NCSCs and their selective enrichment from other sources.

OTHER TISSUES

NCSCs have been identified in adult rat gut as well as that of the embryo. A comparison of fetal and adult gut NCSCs showed that the adult NCSCs self-renew less efficiently and differentiate into a narrower range of neuronal subtypes [Bixby et al., 2002; Kruger et al., 2002]. Considering that the characteristics of these NCSCs are different both spatially and temporally [Bixby et al., 2002; Kruger et al., 2002; Nagoshi et al., 2008], it is not possible to categorize NCSCs as a homogenous population. It will be important to classify these populations according to their differentiation potential and self-renewal activity, and to elucidate the molecular mechanisms for the maintenance and lineage determination of NCSCs in both spatial and temporal aspects.

Another type of NCSC has been identified in the heart of adult mice [Tomita et al., 2005]. Cardiac side population (SP) cells contain a subpopulation of NCSCs, which can generate spheres and differentiate into neurons, glial cells, smooth muscle cells, and cardiomyocytes. By using P0-Cre/CAG-EGFP adult heart tissue for immunohistochemistry, Nestin-positive cells were identified among the EGFP-positive ones that proliferated to form spheres in vitro. These findings suggest that NCSCs that can differentiate into various cell types remain in the heart of adult mice.

NCSCs have also been identified in the adult mouse cornea [Yoshida et al., 2006]. Cornea-derived spheres express Nestin and Musashi1, self-renew over several passages, and differentiate into neural- and mesenchymal-lineage cells. The NCSCs in the cornea are also enriched in the SP cells, like the cardiac NCSCs [Tomita et al., 2005; Yoshida et al., 2006]. Cornea-derived cells from P0-Cre/CAG-EGFP and Wnt1-Cre/CAG-EGFP adult mice proliferate to form $EGFP^+$ spheres, indicating the existence of NCSCs in the adult cornea.

A recent study demonstrated the existence of NCSCs in the carotid body, an oxygen-sensing organ of the sympathoadrenal lineage that grows under conditions of hypoxemia [Pardal et al., 2007]. $GFAP^+$ cells in the rat carotid body incorporate BrdU in vivo, and proliferate in vitro to form spheres that differentiate into tyrosine hydroxylase (TH)-positive neurons and smooth muscle cells, suggesting that the $GFAP^+$ cells are the stem/progenitor cells that resemble NCSCs in some aspects. Although the $GFAP^+$ stem cells are reversibly converted to $Nestin^+$ progenitors in re-normoxia, the equilibrium is displaced toward the $Nestin^+$ progenitors, which give rise to TH^+ neurons under hypoxic conditions [Pardal et al., 2007].

Although NCSCs from various adult tissues have been reported, it would be rash to conclude that all tissue-derived stem cells are NCSCs. For example, multipotent precursors that generate neural- and pancreatic-lineages have been identified in the adult mouse pancreas [Seaberg et al., 2004], and they did not express the neural crest markers *Pax3*, *Twist*, *Sox10*, or *Wnt1* by RT-PCR. The authors concluded that the precursors are not neural crest derivatives. However, these cells did express *slug*, *snail*, and *p75*, and therefore the possibility that they are derived from neural crest cannot be excluded, especially because the expression patterns of neural crest markers in NCSCs are quite different, depending on the tissue source [Nagoshi et al., 2008].

COMPARISON OF METHODS FOR IDENTIFYING NCSCs

So far, a perfect single marker has not been identified for the isolation of NCSCs, although several research groups have established their original methods for the identification of NCSCs (Table I). Sophisticated purification methods for NCSCs are expected to enhance the progress in this field. When comparing the respective protocols for identification, prospective rather than retrospective isolation seems much better for the purification with native condition. Retrospective isolation raises the possibility that there might be contamination by various non-NCSCs and that the characteristics of NCSCs will change during the cell culture procedure. In the rodent study, p75 is one of the good markers for the prospective isolation of NCSCs, and it has been widely used for purification by several groups [Morrison et al., 1999; Bixby et al., 2002; Kruger et al., 2002]. Considering that genetic lineage labeling techniques such as P0-Cre and/or Wnt1-Cre/CAG-EGFP are available for mice, until now, one of the best ways for NCSC purification has been the isolation of $p75^+$ $EGFP^+$ cells by flow cytometry. Although NCSC markers including p75 have been identified in rodents for prospective isolation, no such valuable markers have been established for human NCSCs. Needless to say, genetic lineage labeling techniques are not available for human. The possible identification of novel specific surface antigens for human NCSCs needs to be pursued further.

APPLICATION OF NCSCs TO REGENERATIVE MEDICINE

The NCSC is one of the most intriguing cells in the field of regenerative medicine, because it is easily harvested from accessible

peripheral tissues, which could make autologous transplantation possible. Autologous transplantation would avoid immunological complications as well as the ethical concerns associated with the use of embryonic stem cells. Of the various NCSCs, research on skin-derived NCSCs is the most advanced because of their accessibility. One of the critical questions for the application of NCSCs to regenerative medicine is whether cells that are differentiated from NCSCs are functional. Some evidence supports this. Cultured rodent and human SKPs generate Schwann cells when treated with neuregulins, and myelinate host axons after transplantation to an injured peripheral nerve [McKenzie et al., 2006]. These Schwann cells also myelinate axons in the CNS when transplanted into the brain. Furthermore, the SKP-derived Schwann cells were transplanted into the injured spinal cord of the rat, and improved locomotor function [Biernaskie et al., 2007]. This was the first report that NCSC-derived cells could contribute to the recovery of function following central nervous system injury, but these SKP-derived Schwann cells were harvested from neonatal murine trunk skin, not adult [Biernaskie et al., 2007]. The development of methods for the efficient collection of adult NCSCs that require only small tissue samples will be needed to accomplish the goal of using NCSCs clinically in autologous cell transplantation.

CONCLUSION

Numerous reports show that NCSCs survive in a wide range of tissues for the entire life of the animal, and other tissues may still harbor unidentified NCSCs. It is not altogether clear why the NCSCs persist in adult mammals. However, from the reports that some NCSCs maintain the potential to help tissue recover from damage, it is generally thought that the stem cells from various adult tissues retain the capacity for tissue repair; it is also likely that these cells have undiscovered biological roles that may be extremely helpful in the treatment of human disease. Even the currently known properties of adult NCSCs make them attractive for clinical application in regenerative therapies such as cell replacement therapy. NCSCs from different tissues have distinct characteristics, and further study of these NCSCs will hopefully lead to the culture and transplantation of NCSCs that are the most appropriate for treating specific lesions.

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