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COMPREHENSIVE REVIEW

Uncovering the In Vivo Source of Adult Neural Crest Stem Cells

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Some late embryonic and adult postmigratory neural crest-derived cells (NCDCs) from diverse tissues were shown to grow as multipotent neurospheres. Neural crest stem cells (NCSCs) contained in these spheres were found to give rise not only to neuroectodermal derivatives but also to some of the progeny of the other embryonic germ layers. In this review, evidences regarding the in vivo properties of NCDCs contributing to NCSCs are discussed. Even though in many cases the final proof for the phenotype identity of in vivo cells generating NCSCs is lacking, some evidences suggest that such postmigratory NCDCs would differ from neural crest cells. The streamline of this review follows a historical perspective that helps understanding the advancements in knowledge of this field of research and highlighting its importance, in an appropriate context. Finally, the potential for regenerative medicine purpose of NCDCs and more specifically of tissues that can be a source of peripheral glia progenitors in the adult is underlined.

Keywords: neural crest, Schwann cell precursors, lineage tracing, in vivo, regenerative medicine

Introduction

The neural crest cells: their specification and properties

The neural crest is a population of cells common to all vertebrates, aroused at the boundary in between the prospective neural and superficial ectoderm. Neural crest cells (NCCs) undergo epithelial-to-mesenchymal transition and, once delaminated, they are induced to migrate throughout the body following stereotyped pathways. Gene regulatory network specifying NCCs, as well as establishing their main properties, have being reviewed elsewhere [1–3].

Among relevant NCC genes, Forkhead Box D3 (FOXD3) was highlighted as a key transcriptional factor linked to their stem cell-like properties, such as multipotency and self-renewal [4], and together with SRY-Box 10 (SOX10) is known to orchestrate neural crest identity, survival, and migration [2]. FOXD3 was found coexpressed with SRY-Box 9 (SOX9) and Snail2 (Slug) in premigratory NCCs within the dorsal neural tube, in a region that is increasingly narrowed with time [5]. It is worth noting that FOXD3, SOX10, and Snail2 are able to induce each other, thus reinforcing neural crest specification, with the involvement of histone demethylases such as JMJD2A as well as other chromatin remodelers [2]. In addition, FOXD3 and AP-2α are also expressed in Schwann cell precursors (SCPs) and the latter is downregulated in immature Schwann cells (SCs) [2].

New insights into cranial/facial versus trunk premigratory and migratory NCCs and their developmental potential

It has been recently shown in mouse that NCCs are largely composed of multipotent premigratory as well as migrating cells [6]. When multipotent NCCs reach their target tissues and become postmigratory, they can differentiate into an unexpectedly broad diversity of lineages and contribute to virtually all organs and tissues, according to extrinsic signals they face in the migratory path and/or at destiny [7].

In the trunk, presumptive NCCs reside in the dorsal quadrant of the neural tube. Interestingly, some of these cells located closer to the dorsal midline delaminate from the neural epithelium in a stochastic manner and thereafter the remaining presumptive NCCs are ventral-to-dorsally relocated and intermix with other cells [5,8]. Thus spatial segregation of presumptive NCCs might, but not necessary, bias them to contribute to specific tissues/organs [5,8].

In the head, a first wave of cranial/facial NCCs, likely originated in the nonneural ectoderm [9], in regions of the neural tube devoid of Hox genes expression [10], has the capacity to originate skeletal bones in vivo. Interestingly, in amniotes, some trunk NCCs are also able to originate mesenchymal-like cell types, although in limited cases such as endoneural fibroblasts and stromal cells of the bone marrow [2,11]; nevertheless, all trunk NCC derivatives seem to be originated in the neural ectoderm [9] and they are

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unable to give rise in vivo to bone or cartilage during development [10].

Role of NEDD9 in the migratory behavior of multipotent NCCs and postmigratory neural crest-derived cells

FOXD3 ectopic expression in NCCs was shown to result in SOX10, cadherin-7, and β1-integrin upregulation [1]. Interestingly, an increase in dermomyotome-derived retinoic acid levels during development was found to induce the expression of the β1-integrin signaling pathway scaffolding protein NEDD9 in premigratory multipotent NCCs, which likely result in their efficient migration [12].

As shown in Fig. 1, during embryonic stages, multipotent Nedd9^{+} NCCs migrate through the sclerotome and later on close to the border of dorsal root ganglia (DRG). Moreover, as soon as mouse and chicken NCCs become lineage restricted, they downregulate NEDD9, which result in the reduced migratory behavior of neuronal precursors, a feature likely involved in the emergence of the DRG [12]. In the same study, Nedd9 was never found expressed in postmigratory NCCs and their derivatives, as soon as they reached ventral levels of the neural tube or the growing dorsal rami of spinal nerves, and thereafter in development (Fig. 1). Contact of NCC with peripheral axons induces their differentiation into peripheral glia progenitors.

It is worth to note that FOXD3 downregulation was associated with differentiation of NCCs into the melanocytic lineage, likely due to lack of FOXD3-mediated repression of the microphthalmia-associated transcription factor promoter [13].

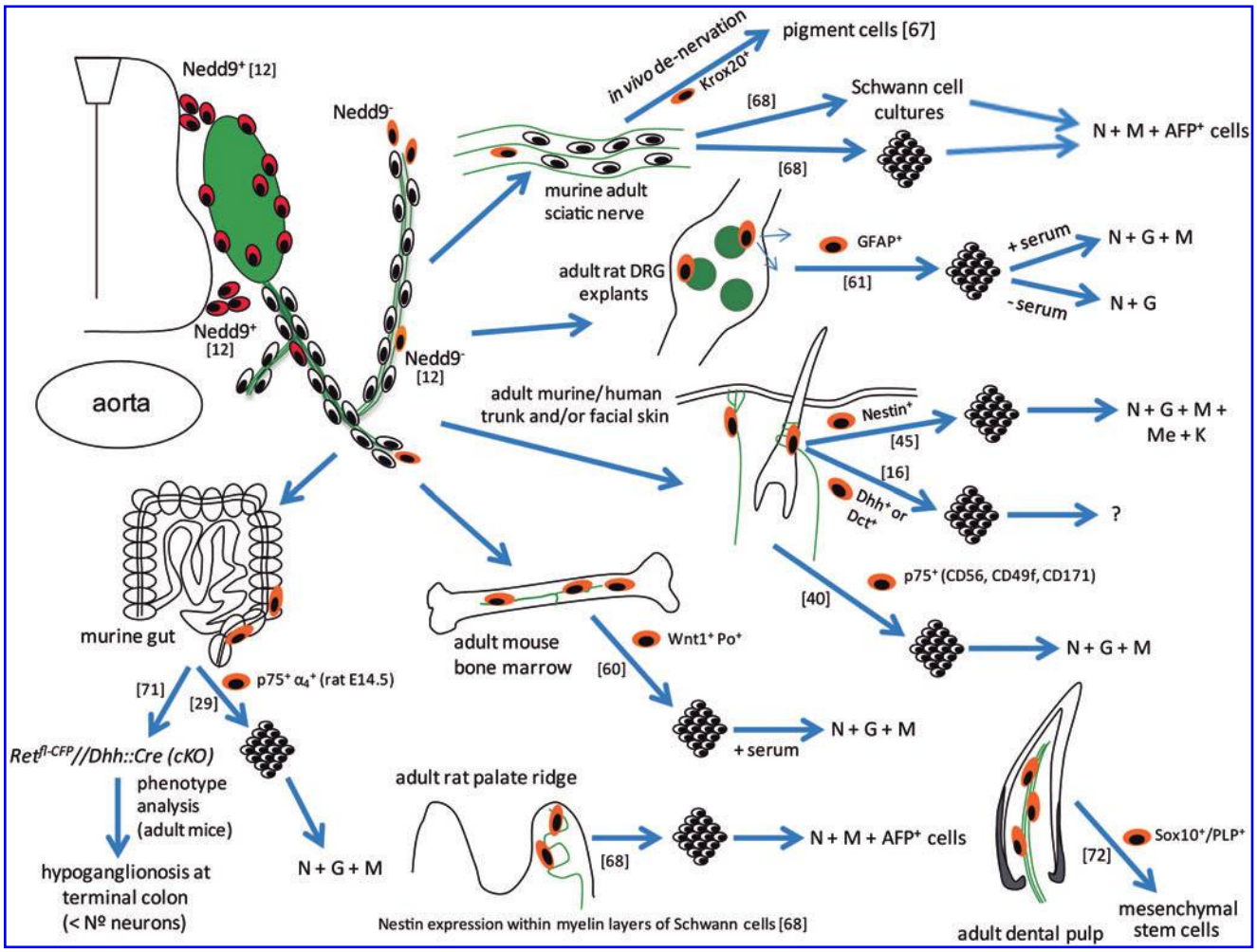

FIG. 1. The in vivo phenotype of cells originating NCSCs. This schematic illustration shows some of the main evidences regarding the in vivo phenotype of adult cells with multipotent properties or able to grow as neurospheres. Please note that multipotent Nedd9^{+} NCCs are depicted with cell symbols in *red*, and postmigratory Nedd9^{-} NCDCs in *orange*. First-order *blue arrows* from the *upper left*, point to tissues in which the in vivo phenotype of cells was analyzed. Second-order *blue arrows*: specific publications are indicated by the reference list number, in between *brackets*, and abbreviation of markers specifically expressed by them in vivo is shown besides cell symbols in *orange*. The capacity of cells to grow as neurospheres is depicted as a cluster of cells, of rhomboidal shape. N, neurons; G, glia; M, myofibroblasts; Me, melanocytes; K: keratinocytes; AFP, alpha fetoprotein. When it is not indicated, differentiation medium for NCSCs was devoid of serum. With regard to publication [40], p75^{+} cells of the human foreskin were enriched in terminal nerve markers (indicated in between *parenthesis*). NCDCs, neural crest-derived cells; NCSCs, neural crest stem cells; NCCs, neural crest cells. Color images available online at www.liebertpub.com/scd

Mouse genetic models for tracing NCC derivatives

Genetic tracing made possible to confirm and expand our knowledge on NCC derivatives. As previously highlighted [7], none of available mouse lines seems to mark all NCC lineages. One of NCC earlier markers with available Cre recombinase mouse lines is Wnt1, although this protein is also expressed by non-NCCs at the dorsal neural tube. SOX10 is also expressed in premigratory NCCs and is induced shortly after Wnt1 [7]. Myelin protein zero (P_0) is found in NCCs after their delamination from the neural tube [7]. Other mouse strains were used to trace NCC derivatives such as transgenic Cre recombinase lines specific for the 1.6-kb proximal paired box 3 upstream region [14], expressed in the dorsal neural tube and NCCs, but not in the somite, or human tissue plasminogen activator [15,16].

Main aim of the review

I will herein focus on reviewing the current knowledge regarding the multipotency and/or plasticity of some neural crest-derived cells (NCDCs) in late embryonic development and up to adulthood. Special attention will be placed on characterizing the in vivo phenotype of the postmigratory NCDCs able to expand for long term and originate all neural stem cell lineages when cultured in vitro, which was not until now the subject of any review [7].

Multipotency and/or Plasticity of Some NCDCs is Maintained into Late Development and the Adulthood

Although NCCs are thought to progressively lose their multipotent properties and/or developmental plasticity when they become postmigratory, many studies suggest that some subpopulations with high plasticity and sphere-forming capacity reside in diverse tissues at late embryonic and postnatal stages and even in the adult. Some of these features have been the subject of review articles [7,10,17–20].

First reports, in quail embryos

To my knowledge, Duff et al. published in 1991 the first article reporting in vitro self-renewal and multipotency of some postmigratory NCCs [21]. In this work, DRG and paravertebral sympathetic ganglia (SG) were dissected from embryonic day 4.75 (E4.75; Hamburger Hamilton -HH-stage 26) to E8 (HH stage 34) Japanese quail embryos, and from them, single cell suspensions were seeded on collagen and cultured in a basal medium supplemented with 10% chick embryo extract (CEE), 15% horse serum, and 25 ng/mL nerve growth factor (NGF). Under these conditions, cells were able to grow as colonies. In addition, for developmental potential studies, the authors performed clonal analyses, following procedures previously described [22].

Early DRG were found to produce clones containing cells able to differentiate into unpigmented, pigmented-only, and mixed cells. On E8, the proportion of unpigmented clones from DRG was increased from 70% (at E4.75) to 99% (at E8), while a significant part of SG clones were able to produce pigmented cells. On E8, most DRG or SG cells were not able to form neurons anymore, but instead gave rise to glia and other cells.

A similar approach was followed shortly after by Sextier-Sainte-Claire Deville et al. [23], also using DRG from Japanese quail embryos (E6–E14). They cultured a cell subpopulation, with sizes smaller than neurons and lacking from processes, into growth-inhibited 3T3 feeder layer. Clonal efficiency of these cells (19%) was similar to that of $HNK1^+$ migratory NCCs obtained from E3 sclerotome. Formulation of propagation culture composition included 10% fetal calf serum (FCS) and 2% CEE and epidermal growth factor (EGF). In parallel, some reports were thereafter able to produce multipotent clones from the skin and gut of quails, by using calf/horse serum and CEE with/without EGF [24,25].

Embryonic rat sciatic nerves

In year 1999, a milestone study published by Morrison et al. [26] has established the concept of neural crest stem cells (NCSCs) and introduced a whole new approach to analyze properties of sphere-producing NCDCs. From E14.5 to E17.5 rat sciatic nerves and by using fluorescence-activated cell sorting (FACS) techniques, the authors were able to select NCDC fractions expressing neurotrophin receptor p75 ($p75_{NTR}$ or p75; expressed both in glia and in the SC lineage [27]) and/or P_0. From this article on, in most publications using NCSCs, the serum was avoided in cell culture propagation media composition and it was replaced by specific growth factors, most frequently basic fibroblast growth factor (bFGF) and/or EGF, and in few cases adding CEE as supplement.

It is worth noting that this was the first study in which different culture medium compositions were used for NCSC propagation and differentiation purposes. Moreover, some differentiation medium components were changed to more efficiently generate specific NCC derivatives. Indeed, from the same subpopulation of rat E14.5 sciatic nerve NCDCs, the authors were able to generate highly enriched neuronal or glia populations in vitro when cells were challenged with BMP2 or neuregulin-1β (NRG1), respectively.

They found that only the $p75^+$ cell fraction was able to produce multipotent clones (with the capacity to generate neurons, glia, and myofibroblasts) when plated on poly-D-lysine/laminin. In addition, they showed that the greatest proportion of multipotent cells in the embryonic rat sciatic nerves was found within the $p75^+$ P_0^- cell fraction. Moreover, $p75^+$ P_0^- cells freshly isolated from the sciatic nerve generated both neurons and glia after direct transplantation into the NCC ventral–lateral migratory pathway of chick embryos. The authors also reported that the multipotency of clones drops drastically in between E14.5 and E16.5, due to restriction to SC and myofibroblast lineages. Finally, evidences of in vivo proliferation of $p75^+$ cells by analysis of 5-bromo-2′-deoxyuridine incorporation were also provided.

Embryonic rat DRG

Shortly after, Hagedorn et al. also used p75 as marker for FACS isolation of NCDCs from E14 and E16 OFA rat DRG [28]. Since >80% of the nonneuronal population of E14 DRG and E12–E16 sciatic nerve was positive for P_0 and peripheral myelin protein 22 (PMP22), they concluded that multipotent cells would coexpress P_0 and PMP22. However,

no prospective lineage-tracing analyses of $P0^+$ $PMP22^+$ cells were therein performed and it could not be ruled out whether some rare multipotent NCCs could eventually generate the NCSCs instead.

Embryonic and postnatal rat gut

In year 2002, Bixby et al. [29] showed that a fraction enriched in $p75^+$ $\alpha4$ $integrin^+$ cells from the gut of E14.5 Sprague-Dawley rats were able to self-renew and were multipotent in vitro. In addition, they were able to generate peripheral neurons and glia when injected into hindlimb bud somites of stage 17–18 chick embryos [29]. Interestingly, $p75^+$ $\alpha4$ $integrin^+$ gut cells were more sensitive to neurogenic factors and less sensitive to gliogenic factors than $p75^+$ P_0^- sciatic nerve cells. Hence, when incorporated into the developing chick sciatic nerve, they gave rise to both neurons and glia, while $p75^+$ P_0^- sciatic nerve cells only produced glial cells. In the same issue of Neuron, the group of Morrison also showed that the two kinds of plexuses at the postnatal gut contain multipotent NCDCs, which were enriched in the $p75^+$ fraction [30]. Interestingly, they found that postnatal multipotent cells were more responsive to gliogenic factors when compared to embryonic counterparts and that before postnatal day 15 (P15), gut multipotent cells likely lose the capacity to produce TH^+ serotoninergic neurons in vitro.

Adult teeth

Different adult tooth structures were found to provide with progenitor cells of cranial NCC origin, as it was recently reviewed [19]. Some of them were grown as adherent or floating colonies, resulting in different gene expression profiles as well as physiologic properties [31–36]. Such culture conditions might select diverse in vivo cellular populations and/or act on the same set of cells to induce different genetic programs.

Adult facial versus trunk skin

In addition, multipotent spheres with different properties were derived from the skin. Considering that skin samples can be obtained with minimally invasive procedures from the same patients, this source of cells is of interest with regard to their potential use in clinics. Nevertheless, caution should be taken since according to the region of sampling, these spheres were found to contain cells of different lineages, including in some cases a predominant mesodermal origin. In vivo lineage tracing analyses of mouse trunk skin showed that NCDCs are located at the follicle papillae and occasionally at hair matrix, bulge region, and within the interfollicular epidermis, as well as in nerves of the dermis [37]. Dermal cells surrounding adult whisker follicles were also found to be of NCC origin in mouse [16].

In year 2001, Toma et al. were able to generate skin precursors (SKPs) from dorsal and ventral skin pieces of juvenile and adult transgenic mice and from adult human scalp [38]. After three passages in vitro, >60% of mouse cells were $nestin^+$ $fibronectin^+$; nevertheless and surprisingly by this time, the majority of cells in skin spheres lacked p75 and polysialylated-neural cell adhesion molecule (PSA-NCAM) expression (other studies showed similar results in most SKPs from mice [37,39,40] and humans [39,40]). When differentiated without growth factors and with low levels of fetal bovine serum (FBS), clonally expanded SKPs gave rise to glia and neurons; in contrast, when incubated in 10% FBS, SKPs were able to produce smooth muscle cells and adipocytes [38].

By using different mouse strains to identify the origin of SKPs in the neural crest (Wnt1-Cre) or the somites (Myf5-Cre), Jinno et al. [41] showed that most cells in early-passaged SKPs were of somitic origin, while SKPs obtained from facial skin were neural crest derived (NCD). Nevertheless and somehow contradictory to such results, at long-term in vitro (>20 passages), the majority of mouse trunk skin-derived SKPs were double positive for p75 and Sox10, which might suggest an NCD phenotype [16]. Interestingly, Etxaniz et al. [40] have reported evidences in support of some neurogenic properties being related to a subpopulation of mouse dorsal skin and human foreskin-derived spheres expressing $p75^+$ $CD56$ $(PSA\text{-}NCAM)^+$. They found that $\sim$3% of cells in primary human dermal sphere cultures were $p75^+$ $Nestin^+$ $CD34^-$ and 75% of these cells coexpressed Sox2; therefore, this rare cellular subpopulation would likely overlap with the dermis $Sox2^+$ fraction described in mouse by Biernaskie et al. [42]. Furthermore, Sox2 knockdown in $p75^+$ $CD56^+$ cells was shown to abolish the neurogenic capacity of these skin progenitors [40].

In year 2004, Sieber-Blum et al. isolated whisker follicles from Wnt1Cre-R26lacZ mice, with the aim of tracing NCDCs [43]. They found that some cells emigrated from the bulge region of these follicles and were thereafter named as epidermal neural crest stem cells (eNCSCs). The eNCSCs showed high rate of proliferation in a medium supplemented with 5% CEE and 10% FCS, and gave rise to neurons, glia, smooth muscle cells, and chondrocytes. Human epithelial (hEPI) NCSCs were also derived from female pubis skin hairs using a medium supplemented with bFGF and EGF, and they were shown to be able to differentiate into neurons ($Tuj1^+$, TH^+), $GFAP^+$ glia, SMA^+ myofibroblasts, early melanocytes, chondrocytes, and, in adherent cultures, osteocytes [44]. hEPI-NCSCs expressed typical NCSC markers (Sox10, nestin, SNAI2, TWIST1, Musashi, and p75) as well as pluripotent markers (Sox2, cMYC, KLF4, LIN28, POU5F1/OCT4, and NANOG); nonetheless, only cMyc was found at levels similar to H9 human embryonic stem cells (hESCs). In vivo nestin-driven GFP^+ cells from the vibrissa follicular bulge area of mice were also $CD34^+$ $CK15^-$ and were found to also originate keratinocytes [45].

Mouse boundary cap cells and their in vitro differentiation into SCs

In year 2005, Hjerling-Leffler et al. derived NCSCs from NCDCs located at the boundary in between the dorsal (and ventral) roots and the spinal cord, named as boundary cap NCCs (bNCCs) [46]. During embryonic development, bNCCs keep proliferating and migrating into the DRG and contribute to cells of spinal cord roots. In addition, boundary cap NCDCs (bNCDCs) were recently shown to likely migrate through nerves to the skin [47]. Boundary cap NCSCs (bNCSCs) can be propagated for long term and differentiated into several modalities of sensory neurons as well as into SCs [46,48]. In the latter case and to produce mature SCs, bNCSCs were cultured for several days in a medium supplemented with B27, laminin, forskolin, neurotrophic

factors, and β-heregulin (NRG1). By using specific SC lineage-specific markers, Aquino et al. [48] were able to compare the in vivo differentiation of NCCs into SCs with that of bNCSCs in vitro. After 5.5 days of incubation in an SC differentiation medium, bNCSCs grew forming parallel arrays of bipolar $S100^+$ $GFAP^{+/-}$ cells, characteristic of SCPs. Consistent with in vivo data, bNCSC-derived SCPs were found to express S100 before GFAP. By day 8, concentration levels of NRG1 were reduced in culture medium composition (from 125 to 50 ng/mL) and NGF was increased (from 10 to 30 ng/mL), and 3 days later medium was further supplemented with ascorbid acid. By day 30 in vitro, $Sox10^+$ $Sox2^-$ $S100^+$ $GFAP^+$ $Krox20^{+/-}$ mature SCs were obtained. These cells were able to myelinate axons both in vitro and in vivo.

In a parallel study, McKenzie et al. were able to derive SCs from SKPs by also adding NRG1 and forskolin to culture medium composition [49]. Conditions required to produce SCs are less complex when SKPs were used instead of bNCSCs [48,49], which might suggest that the phenotype of some cells contributing to skin spheres could be of peripheral glia progenitors rather than NCCs. Interestingly, bNCDCs have been isolated from the E12.5 meninges of $Krox20^{Cre/+}$:$R26R^{YFP/+}$ mice [50] and spheres derived from this cellular fraction were found to likely originate only central nervous system derivatives, consisting mainly in glia. When these sphere cells were grafted into the forebrain subventricular zone of newborn Shiverer (MBP^+ deficient) mouse, they produced MBP^+ myelin, but did not express P_0.

Murine postnatal heart

Also, in year 2005, floating spheres with characteristics of NCSCs were obtained from the side population (SP) fraction of cells collected from the heart of 1-day-old neonatal ICR (CD-1; Institute for Cancer Research, Caesarean Derived-1) mice, Wistar rats, and P0-Cre:CAG-CAT-EGFP double transgenic mice [51]. SP cells were $myosin^-$ $CD11b^-$ $CD13^-$ $CD45^-$ $Ter119^-$ $CD29^+$ $CD44^+$ $CD34^{+/-}$ $c\text{-}Kit^{+/-}$ $Flk\text{-}1^{+/-}$ $Sca\text{-}1^{+/-}$. SP-derived sphere cells were able to differentiate in vitro into $bMHC^+$ cardiomyocytes (0.3%), $peripherin^+/MAP2^+$ neurons (0.4%), $GFAP^+/P0^+/p75^{+/-}$ glia (68%), and smooth muscle cells (43%). Cardiospheres were $GATA4^+$ $Nkx2.5^-$ $MEF2C^-$ $MAP2^-$ $GFAP^-$ MHC^- and only few of them were SMA^+. In 2-day-old mice, SP cells were found to contribute with 3.5% of heart tissue cells, but this proportion decreased rapidly by postnatal day 7, and at 6 weeks of age, they only contributed with 0.02% of cells. Interestingly, some $actinin^+$ cardiomyocytes in vivo were traced in double-transgenic mice, thus suggesting an NCC origin; nevertheless, the frequency of this event was not reported and therefore it might be eventually explained by ectopic activation of the P0 promoter.

Also, in the heart, Nakamura et al. showed that some NCDCs in the developing valves and the cardiac conduction system maintained the expression of immature markers [52]. For instance, some cells positive for brain lipid-binding protein (BLBP; also known as fatty acid binding protein 7), a marker of SCPs and immature SCs [53,54], and glutamate aspartate transporter were found to coexpress neuronal markers (NF160 and Tuj1) in the His bundle and bundle branches. El-Helou et al. described $nestin^+$ cells within the myocardium, intercalated in between cardiac muscle cells, of possible NCC origin [55]. The authors were able to generate neurospheres, with neurogenic capacity, from scar-derived cells obtained from the infarct region at 1 week after injury.

Mouse cornea and iris

In year 2006, Yoshida et al. derived NCSCs from the adult cornea of Wnt1-Cre:CAG-CAT-EGFP and P_0-Cre:CAG-CAT-EGFP mice [56]. These cranially derived NCSCs were expanded as clones and differentiated into keratinocytes, fibroblasts (with addition of serum), adipocytes (with insulin supplementation), and myofibroblasts (induced by transforming growth factor-β3 stimulation). In poly/L-ornithine-coated slides, these progenitors originated $Tuj1^+$ neurons (33%) and $GFAP^+$ glia (37%). By in vivo lineage tracing, the authors showed that corneal stromal cells and corneal endothelium were of NCC origin. NCSCs expressed nestin, Musashi1, Notch1, CD34, Sca-1, Twist, Slug, Snail, and Sox9 and were negative for CD133, c-kit, and CD45. Interestingly, a subset of these cells exhibited the SP phenotype.

A similar kind of cells was also isolated from the adult iris of P_0-Cre:CAG-CAT-EGFP mice [57]. Traced cells were able to differentiate into $Tuj1^+$ neurons (11%), $GFAP^+$ glia (29%), and SMA^+ myofibroblasts, and when grown as pellets, they originated chondrocytes. Brandl et al. were able to derive NCSCs with different properties, the mouse corneal cells, from juvenile mouse cornea [58]. These cells were only obtained when the limbo area was included in the tissue sample and not after postnatal day 8. Sphere cells were positive for NCSC markers and also for CD133, vimentin, Pecam1, connective tissue, and corneal stromal keratocyte markers, as well as α-smooth muscle actin. They were found to give rise to neural and mesenchymal lineages with the exception of chondrocytes.

Rat adult palate

Multipotent cells of cranial NCC origin were also obtained from the adult rat palate [59]. In this study, in vivo $nestin^+$ cells were seen in association with neural structures and Merkel cell-neurite complexes within palate ridges. Moreover, in this work, the highest expression levels of CD133, nestin, Sox2, Klf4, Oct3/4, and c-Myc were found within papilla incisiva and processus alveolaris. The fact that saliva contains increased levels of bFGF was therein suggested to play a role in the maintenance of multipotent properties in some NCDCs within the palate.

Mouse adult bone marrow

In a study published in year 2008, Nagoshi et al. traced NCDCs in the adult bone marrow of lower extremities as well as DRG and whisker pad by using P0-Cre/Floxed-EGFP and Wnt1-Cre/Floxed-EGFP mice [60]. NCDCs from all these sources were able to originate neurospheres with different NCSC efficiencies: thus, the proportion of multipotent spheres formed in clonal density cultures was of 74.6% in the DRG, 7.3% in the whisker pad (with 92% of spheres consisting more restricted progenitors originating neurons and myofibroblasts), and 3.3% in the bone marrow (with 65% of sphere cells giving rise only to SMA^+ cells).

These latter results might be influenced by culture media supplementation with 10% FBS in the context of differentiation analysis. The authors also reported incidence of $p75^+$ NCDCs in the blood stream of embryos in between E12.5 and E15.5 and suggested that these cells are likely recruited through systemic circulation to the liver and subsequently to the bone marrow during normal development, following a path similar to hematopoietic stem/progenitor cells. In between P2 and after 13 weeks of age, reporter gene expression was found to decrease in all tissues analyzed.

The ex vivo identity of multipotent sphere-forming DRG cells and the expression of neural crest-like markers in the adult

Li et al. by performing explant assays showed that emigrating $nestin^+$ $p75^+$ cells from adult DRG cells were able to form multipotent neurospheres [61]. Proliferating DRG cells were $nestin^+$ $GFAP^+$ and were found surrounding neuronal somas. It is worth noting that, as previously discussed, nestin expression was reported in cells associated with terminal axons surrounding adult hair follicle bulge—also expressing Sox2— [45], papilla of the adult palate ridges [59], and stromal cells of the bone marrow [11,62], and are at least partially of NCC origin [40,42]. Therefore, as it could be expected, dermal papilla-derived SKPs were found to be able to originate $Sox10^+$ $Krox20^+$ $p75^+$ $S100^+$ $GFAP^+$ MBP^+ $P0^+$ SCs [63]. Nonetheless, the in vitro acquisition of neural crest-like properties by cells of mesodermal origin could not be disregarded [41].

Adult human turbinate

Moreover, results from Hauser et al., published in 2012, also suggest multipotent/plastic properties of cranial NCDCs in adult human turbinate samples [64]. They found strong $nestin^+$ signal close to nerves at the lamina propria of the respiratory mucosa. Tissue samples and inferior turbinate progenitors (ITSCs), a kind of NCSCs, were seen to express nestin, p75, S100, Slug, Sox10, ABCG2, CD133, and low levels of pluripotency markers except from WDR5, KLF4, and c-MYC, which were similar to levels described in hESCs. Tissue sample-selected $p75^+$ ITSCs (with a purity of 62%) formed larger neurospheres and proliferated faster than the negative fraction, and expressed higher levels of Oct4 and KLF4. ITSCs were found to originate $Tuj1^+$, $NF\text{-}H^+$ neurons (and specifically $AMPA1^+$ and $NMDA^+$ glutamatergic neurons), as well as SMA^+ myofibroblasts, adipocytes, osteocytes, and chondrocytes.

The Multipotent Peripheral Glia: An Expanding Field with Interest for Regenerative Medicine Purposes

Recent literature suggests that in some cases, in vivo cells contributing with NCSCs from late embryonic and adult tissues could be of the glial lineage. Nataf and Le Douarin [65] were able to originate pigment cells from SC myelin protein $(SMP)^+$ SCPs from E10 to E15 Japanese quail sciatic nerves by culturing them on collagen and in medium supplemented with tetradecanoylphorbol 13-acetate (TPA; a factor triggering c-kit expression) and stem cell factor (SCF). In such cultures, endothelin-3 was found to be able to expand the SMP^+ cell population and originate melanocytes, and TPA was shown to induce melanogenesis. It is worth noting that the effect of TPA treatment on melanogenic capacity of DRG cells was reported even after E5 stage.

Are there differential properties among cells within the sciatic nerve and those associated with terminal axons?

Interestingly, after Morrison et al. seminal study using embryonic sciatic nerves, [26] some scientists have tried with little or no success to produce NCSC spheres from the same tissue, but in the adult [16,38,43]. Takagi et al. [66] were able to obtain spheres from the distal stump of adult mouse crushed sciatic nerves. Spheres were shown to be derived from mature SCs and therefore were named as Schwann spheres. Cells in these spheres generated SCs, but not neurons or smooth muscle cells. The capacity of the adult sciatic nerve SCs to generate pigment cells was not analyzed in this study, but it has been reported in vivo [67].

Properties of some cells within terminal nerves in peripheral tissues seem to allow for generation of NCSCs. With this regard, in the previous section of this review, the expression of some markers on in vivo cells contributing to NCSCs, such as p75 and/or α4-integrin, was discussed [26,28–30]. Interestingly, those markers are expressed by both NCCs and SCPs [53]. Indeed, in the dermis, Etxaniz et al. found that SCs were the only cells coexpressing p75 and PSA-NCAM in vivo and some neurogenic activity of skin-derived spheres likely depends on this cellular fraction [40]. They also found that in vivo, the $p75^+$ fraction, as well as spheres produced from them, expressed higher levels of terminal nerve markers such as CD49f (α6-integrin) and CD171 (L1-CAM) when compared to the negative fraction. Consistently, the expression of some genes shared by embryonic NCSCs and SCPs (CDH2, a marker of terminal SCs), and others that are SCP specific (CDH19) or shared with immature SCs (PMP22, PLP1, and Dhh) were seen upregulated in the rare $p75^+$ $CD56^+$ cellular fraction of skin spheres [40].

Furthermore, Wong et al. [16] found that the reporter expression in the dorsal skin of Dhh-Cre:Rosa26R-EGFP was confined to the hair bulge region and in these animals, $p75^+$ $Sox10^+$ neurospheres were only obtained from the FACS-isolated $EGFP^+$ fraction. Finally, they have also found that only the EGFP fraction in Dct-Cre:Rosa26R-EGFP, being Dct expressed in early melanocytes, were able to produce $p75^+$ $Sox10^+$ neurospheres. These two findings together would argue that either some in vivo SCPs and early melanocytes, which share a common glia-melanocyte progenitor, or cells of these lineages with high developmental plasticity, which differ from NCCs, could regain in vitro some of the peripheral glia progenitor properties, allowing them to grow as neurospheres.

Could the capacity of in vivo cells able to grow multipotent neurospheres be linked to the glial lineage?

It is worth noting that despite the derivation of multipotent neurospheres directly from adult sciatic nerves remains unsuccessful, Widera et al. were able to grow them

from SC primary cultures obtained from this tissue [68]. This finding would suggest that some glial subpopulations with NCSC potential might be found in vivo. Otherwise, these controversies as well as other herein discussed could result from technical issues. In the same article, nestin expression within palatal ridges was found in association with β-III-tubulin-positive fibers reminiscent of Meissner corpuscles, but without a corpuscular structure. Interestingly, nestin immunoreactivity in palatal sections was confined to myelin layers of SCs.

Moreover, in the same study around 97% of nestin$^+$ cells were found to coexpress in vivo p75, while all nestin$^+$ cells coexpressed S100. Nevertheless, 66% of S100$^+$ cells did not express nestin. Finally, all p75$^+$ nestin$^+$ cells were also S100$^+$. Altogether, the expression profile analysis of nestin$^+$ cells within palatal ridges identified them as myelinating SCs. Finally, the authors showed that adult palatal NCSCs, adult sciatic nerve NCSCs, and adult sciatic nerve-derived SCs conventionally cultivated were able to originate neurons, SMA$^+$ myofibroblasts, and AFP$^+$ hepatocyte lineage-like cells.

Peripheral glia progenitors

In the neural crest field, one of the subjects showing a more spectacular growth in knowledge within the last decade is the in vivo multipotent/plastic properties of peripheral glia progenitors. Strong evidence supports that these cells, apart from originating myelinating and nonmyelinating SCs, during normal development can give rise to the following: endoneurial fibroblasts [69], melanocytes [67], parasympathetic neurons [54,70], postnatal enteric neurons [71], and mesenchymal stromal cells from dental pulp [72].

Interestingly, nerve cells able to originate parasympathetic neurons coexpress neural crest markers (Sox10, FOXD3, and p75) with SCP markers (ErbB3, Cadherin19, BLBP, and the myelin protein PLP) [54,70]. New studies would likely highlight the relevance for the developmental biology and the potential for regenerative medicine of peripheral glia progenitors, as a source of diverse NCDCs that are born after cessation of NCC migration. For instance, this could be the case of NCDCs found to differentiate into melanocytes within heart valves at postnatal stages [52].

The neurogenic glia of the carotid body

Another adult stem-like cell population derived from NCD glia is located in the carotid body [73]. It consists of GFAP$^+$ type II sustentacular cells that are able to self-renew and produce the two carotid body lineages: TH$^+$ neurons and SMA$^+$ cells.

Could the boundary cap be a source of NCDCs in the skin?

Finally and by using postnatal and adult skin samples from Prss56-Cre:Rosa26-Tomato double-transgenic mice, Gresset et al. showed that bNCDCs might be recruited to the skin through embryonic nerve development and would eventually give rise to glia of terminal nerves and of skin cells behaving in vitro as neurogenic stem cells [47]. Skin bNCDCs were found to produce SCs (mostly nonmyelinating ones) and endoneurial fibroblasts. In the dermis, traced cells were p75$^+$ and S100$^+$, but MBP$^-$ and some of them at lanceolate endings were seen to express nestin. Passage (P)2 and P5 bNCDCs in SKP cultures were shown to produce neurons and myofibroblasts and, with the addition of forskolin and NRG1, SCs [47]. Incubation of SKPs with SCF and endothelin-3 enhanced the generation of melanocytes, while treatment with ascorbic acid and BMP2 favored chondrogenesis. Moreover, inhibition of BMP2 and Shh signaling allowed the generation of OLIG-2$^+$ immature oligodendrocytes.

Is there any neural crest-like cell in the adult?

As it was previously discussed and shown in Table 1 and Fig. 1, some neural crest-like markers were found in certain cells associated with terminal neuronal circuits, such as the skin, the bone marrow, and the adult palate ridge. However, such markers were so far therein described in cells of the peripheral glia lineage. Moreover, some postnatal or adult cells of the peripheral glia lineage were shown to in vivo differentiate into pigment cells (when a fragment of the sciatic nerve was sectioned and left attached to the thigh muscle, without allowing for nerve regeneration), enteric ganglia neurons, and tooth mesenchymal stromal cells (Fig. 1). While the existence of rare adult neural crest-like cells exerting any prominent functions in adult organisms remains uncovered, these features seem likely a hidden capacity that reveals itself in vitro, while not so obvious inside of the body.

Summary

A summary of main findings and conclusions is shown in Table 1 and Fig. 1. In Fig. 1, first-order blue arrows from the upper left point to some tissues in which the phenotype of postmigratory NCDCs contributing to NCSCs or showing in vitro/in vivo multipotent properties have being analyzed. Second-order blue arrows often point to whether specific publications studied the capacity of cells to grow as neurospheres. In some of these studies, multipotent properties were analyzed in vivo and/or in vitro, that is, through the establishment of SC cultures. Third-order blue arrows point to the cellular derivatives obtained from NCDCs/NCSCs or to some of their markers. In between markers expressed by in vivo NCDCs contributing to NCSCs, being multipotent, or could regain multipotency, we could find some shared by NCCs and peripheral glia progenitors (p75, CD56, P_0, α_4 integrin, and Sox10, also found in melanocytes and throughout the glial lineage- and nestin-also expressed in other lineages) and others first expressed in the NCC lineage by peripheral glia progenitors (such as Dhh and PLP1), melanocyte lineage (known to be partially derived from peripheral glia progenitors; such as Dct), immature SCs (GFAP), and cells of the myelinating SC lineage (Krox20).

Final remarks and questions

It is not yet fully known whether and to which extent some already committed and perhaps fully differentiated peripheral glia could undergo reprogramming and regain plasticity and/or multipotency, although it seems clear that some tissues contain cells with properties allowing this process to take place more efficiently. In addition, for most tissues, it remains to be validated by lineage tracing using

Table 1. Multipotency/Plasticity of Neural Crest-Derived Cells with Peripheral Glia Progenitor Properties from Adult Stages

Study	*Tissue origin*	*In vivo properties of NCDCs contributing with NCSCs*	*Observations*
Gronthos et al. [31]	Normal human-impacted third molars from adults (19–29 years of age)	Nondetermined	Dental pulp stem cells (DPSCs) express nestin and GFAP (expressed by immature Schwann cells) DPSCs were grown in 20% FCS and had potential for bone and adipocyte lineages No lineage tracing studies
Wong et al. [16]	Adult skin of mice (whisker follicles and trunk)	$p75^+$ cells Dhh-Cre:R26R-EGFP and Dct-Cre:R26R-EGFP traced cells around the hair bulge	$P75^+$ cell fraction was enriched by flow cytometry Fifty-eight percent of all p75+ cells were multipotent (glia, neurons, myofibroblasts, and adipocytes), a ratio that is unexpectedly high if they were expected to be only derived from multipotent NCCs Lineage tracing: Only the EGFP+ fraction, in the Dhh-Cre:R26R-EGFP and in the Dct-Cre:R26R-EGFP transgenic mice, was able to grow $p75^+$ $Sox10^+$ neurospheres
Li et al. [61]	Adult lumbar four or five DRG from Sprague-Dawley rats	$Nestin^+$ $p75^+$ $GFAP^+$ $Tuj1^-$ emigrating cells	Ex vivo experiments. No lineage-tracing studies. Cells proliferating in DRG explants and subsequently forming neurospheres surround neuronal somas
Pardal et al. [73]	Adult carotid bodies from GFAP-Cre/floxed transgenic mice	$GFAP^+$ type-II glia	Lineage tracing: $Nestin^+$ glial cells proliferate and produce TH^+ neurons under hypoxic conditions
Widera et al. [68]	Adult palate of Wistar rats	$Nestin^+$ myelinating Schwann cells	Nestin was found confined to myelin layers of Schwann cells in palatal ridges Multipotent neurospheres were obtained from the palate and the sciatic nerve in the adult. Schwann cell cultures contained multipotent cells (neurons, myofibroblasts, and hepatocyte-like cells) No lineage-tracing studies
Etxaniz et al. [40]	Human foreskin (18–24 years of age) and adult mouse skin	$p75^+$ (enriched in CD56, CD49f, CD171 expression)	A rare $p75^+$ $CD56^+$ fraction in skin spheres was found to be neurogenic Only Schwann cells coexpressed both markers in skin tissue CDH2, CDH19, PMP22, PLP1, and Dhh, were found upregulated in human $p75^+$ $CD56^+$ sphere neurogenic fraction, suggesting properties of peripheral glia progenitors

NCDCs, neural crest-derived cells; NCSCs, neural crest stem cells; DRG, dorsal root ganglia; FCS, fetal calf serum; NCCs, neural crest cells.

for instance specific peripheral glia progenitor conditional Cre mouse strains, whether these cells could be a source of the NCSCs in late embryonic and adult tissues. Finally, in the next future, the spectrum of peripheral glia progenitor derivatives might expand to include other neural and nonneural-like cell types in late development, a knowledge that would likely be of potential interest to the regenerative medicine.

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