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CONCISE REVIEW

STEM CELLS

On the road again: Establishment and maintenance of stemness in the neural crest from embryo to adulthood

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Abstract

Unique to vertebrates, the neural crest (NC) is an embryonic stem cell population that contributes to a greatly expanding list of derivatives ranging from neurons and glia of the peripheral nervous system, facial cartilage and bone, pigment cells of the skin to secretory cells of the endocrine system. Here, we focus on what is specifically known about establishment and maintenance of NC stemness and ultimate fate commitment mechanisms, which could help explain its exceptionally high stem cell potential that exceeds the "rules set during gastrulation." In fact, recent discoveries have shed light on the existence of NC cells that coexpress commonly accepted pluripotency factors like Nanog, Oct4/PouV, and Klf4. The coexpression of pluripotency factors together with the exceptional array of diverse NC derivatives encouraged us to propose a new term "*pleistopotent*" (Greek for *abundant, a substantial amount*) to be used to reflect the uniqueness of the NC as compared to other post-gastrulation stem cell populations in the vertebrate body, and to differentiate them from multipotent lineage restricted stem cells. We also discuss studies related to the maintenance of NC stemness within the challenging context of being a transient and thus a constantly changing population of stem cells without a permanent niche. The discovery of the stem cell potential of Schwann cell precursors as well as multiple adult NC-derived stem cell reservoirs during the past decade has greatly increased our understanding of how NC cells contribute to tissues formed after its initial migration stage in young embryos.

KEYWORDS

neural crest, neural crest derivatives, pleistopotent, stem cell maintenance, stemness

1 | INTRODUCTION

Stem cells are unique in their capabilities to self-renew and differentiate into various cell types. In humans, the first three cell divisions following fertilization results in totipotent stem cells capable of giving rise to a whole organism (i.e., all the embryonic and extra-embryonic cells). According to traditional developmental dogma, these totipotent stem cells differentiate over time to pluripotent stem cells (capable of forming all cell types of the embryo) that will go through the gastrulation process and give rise to multipotent stem cells, unipotent stem cells, and finally mature differentiated cells.

The neural crest (NC) is a population of stem cells that arises from the ectodermal germ layer and gives rise to a broad range of derivatives. Although multipotency is usually described as the ability to give rise to all cell types within a particular lineage, the NC gives rise to cells of several lineages, spanning from cells of the nervous system to cartilage and bone to endocrine cells, representing ectodermal as well as mesodermal-like and endodermal-like cell types. According to the rules set during

gastrulation, certain types of derivatives are specific to each germ layer. The diversity of NC derivatives contradicts this concept and has raised discussions on how the NC possesses such exceptionally high stem cell potential—higher than any other cell type post-gastrulation and is sometimes referred to as the fourth germ layer.[1] It has been suggested, based on similarities on gene expression profiles of certain genes and gene families in the blastula and the NC, that NC stemness is preserved from pregastrula stages.[2] Recent studies have revealed coexpression of generally accepted pluripotency factor genes such as *Nanog*, *Klf4*, and *Oct4/PouV* within a subpopulation of the premigratory NC, a temporary niche, which supports the idea of a substantially higher molecular stem cell signature of the NC than previously anticipated,[3] although the function of these genes in NC cells remains largely unknown. The formation of this temporary NC stem cell niche is poorly understood and further proof is required to determine whether NC formation is enabled via escaping from the earliest fate determining cues provided during gastrulation, or if the high stem cell capacity is gained de novo in the ectodermal germ layer.

Here, we discuss what is currently known about the developmental potential of the NC, the regulation of its stem cell characteristics and how data from decades ago fits with new discoveries published within the recent years. We also discuss how the existing stem cell nomenclature is inadequate to describe the NC stem cell population. Due to the lack of precise terminology, the NC is commonly referred to as anything between pluripotent stem cells to multipotent progenitors, which from a stem cell perspective represent two very different things (not to mention the problematic usage of the word "progenitor" in this context that as such refers to a cell with restricted proliferation capacity that can differentiate to a few cell types within a single tissue or organ, and would typically represent an oligopotent cell). Finally, we address the maintenance of NC stemness during embryogenesis and adulthood in the form of different NC-derived stem cell populations and briefly discuss how the implications of these findings relate to the regenerative potential of these cells.

Significance statement

The neural crest is a remarkable embryonic stem cell population that contributes to a wide variety of derivatives ranging from neurons, glia, cartilage, bone, pigment cells, and endocrine cells. As the list of neural crest derivatives has greatly expanded since its identification over 150 years ago, the clinical significance of the neural crest has become more evident, whereas some central questions regarding stemness formation and maintenance remain poorly understood. This concise review discusses studies related to how this post-gastrulation cell population maintains its exceptional stem cell potential from embryonic to adult stages.

2 | DEVELOPMENTAL POTENTIAL OF NC CELLS

2.1 | Brief overview of development of the NC and its derivatives

The NC, a transient population unique to vertebrates, arises in a temporal, anterior to posterior progressing fashion, from the dorsal aspects of almost the entire axial length of the neural tube during the formation of the nervous system. After their initial induction at the neural plate border, the cells are fully specified into *bona fide* NC stem cells as the neural folds rise and meet each other in the dorsal neural tube. Soon after, these NC stem cells undergo epithelial to mesenchymal transition (EMT) and emigrate from different axial levels of the neural tube to migrate throughout the developing embryo giving rise to a wide array of derivatives, as shown in Figure 1 and Table 1. Despite their transient nature, NC cells are able to self-renew in order to multiply and give rise to the correct size of the stem cell pool at the premigratory stage[46,47] before emigration and differentiation into ectodermal, mesodermal, and endodermal derivatives, making them the most astonishing stem cell population post-gastrulation. New derivatives and contributions of NC cells, such as phagocytotic activity of the migratory NC cells,[48] are identified even now, over 150 years after the NC was first discovered.

There are some differences in the developmental potential of the NC cells originating along the various axial levels that are broadly subdivided to cranial, vagal, trunk, and lumbosacral NC cells based on the segments of origin.[49] Despite this variance along the axial levels, NC cells at all levels give rise to neurons and glia of the peripheral nervous system and melanocytes (reviewed in Reference 50). Unique to the axial level (as presented in Figure 1 and Table 1), the cranial NC (from forebrain to hindbrain) gives rise to several mesenchymal derivatives of the face and neck such as smooth muscle, odontoblasts, chondrocytes, osteocytes, connective tissue, pericytes, meninges, facial dermis, and adipocytes.[4,6-8] The main contribution of the vagal NC (at the level of somites 1-7), in comparison, is to the neurons and glia of the enteric nervous system[32] and several derivatives in the cardiac system.[34-38] In addition to giving rise to neurons and glia, the trunk NC (somites 8-24 in chick and to 8-27 in mouse) gives rise to adrenal chromaffin cells.[33] Finally, the sacral NC (posterior to the trunk NC) gives rise to a small portion of the enteric nervous system along with the vagal NC.[43-45]

Taken together, the NC gives rise to more than 30 different cell types, although many derivatives are specific to a certain axial level (Figure 1; Table 1). The trunk NC has commonly been considered to lack the capacity to form bone and cartilage[49] at least in the more intensely studied model systems for NC development such as the chicken, frog, mouse, and zebrafish. However, contribution of trunk NC cells to some skeletal derivatives has been reported in zebrafish[51] (although contradicting with another report[22]), turtle[52,53] and skate.[54] A recent molecular level study in the chick elegantly showed that lack of skeletogenic potential in trunk NC cells is due to differences in the gene regulatory network at the premigratory stage, which is reversible by genetic manipulation by activating a set of cranial genes in the trunk.[55] In line with this, a recent spatiotemporal transcriptomics study suggests that, in the

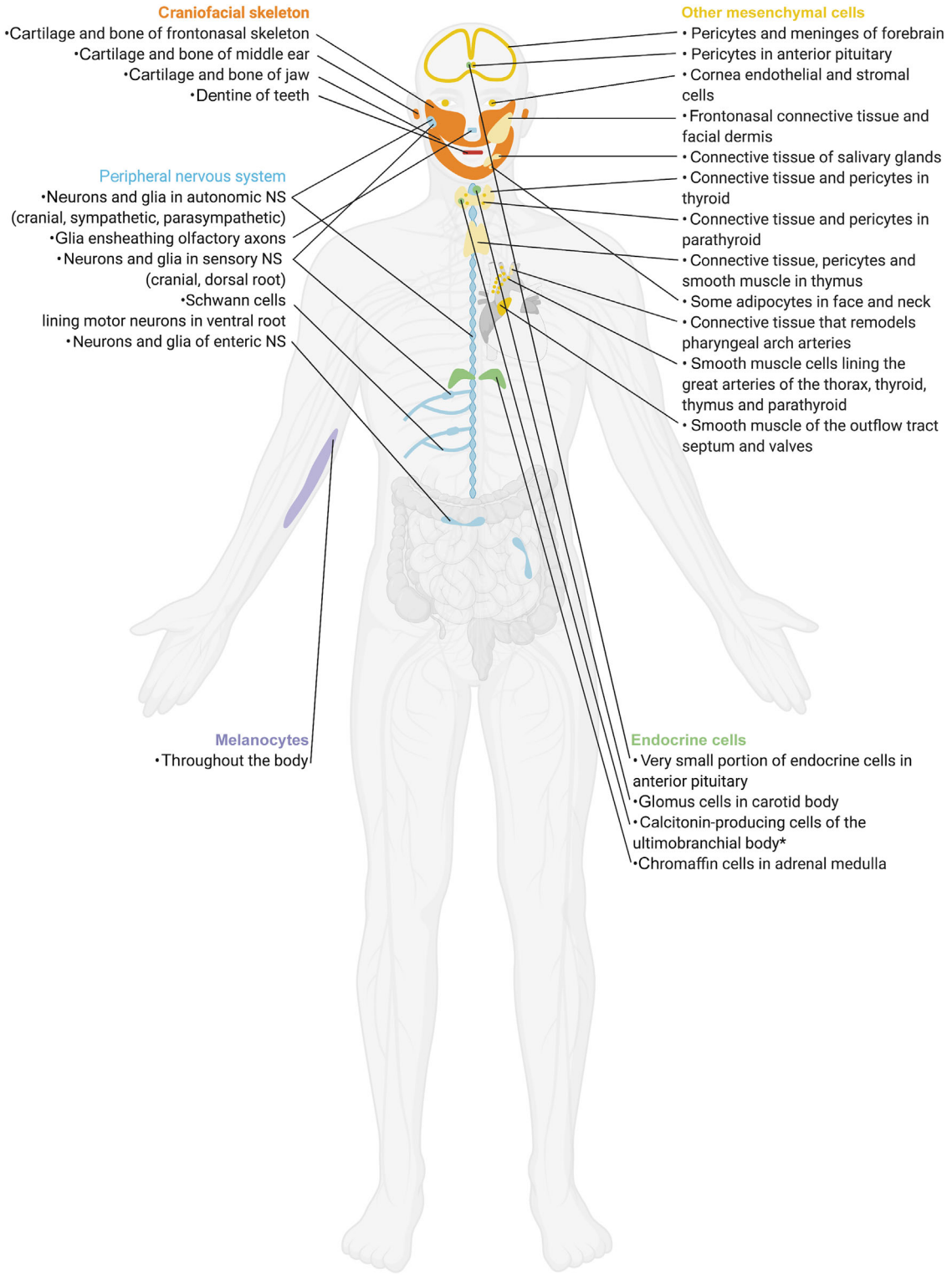

FIGURE 1 Neural crest cell populations contribute to a diverse range of cells in adult tissues and organs. Schematic of an adult human showing the tissues and organs derived from the neural crest based on studies in different animal species (chick, mouse, frog, and zebrafish). These neural crest derivatives can be broadly categorized to five main cell groups: craniofacial skeleton (mesenchymal cells), other mesenchymal cells, cells of the peripheral nervous system, endocrine cells, and melanocytes. Please see Table 1 for a detailed overview of these derivatives. Asterisk denotes contradictory evidence between species. Figure created with BioRender

TABLE 1 The diverse range of derivatives to which the neural crest gives rise, based on studies of different animal species: chick, mouse, frog, and zebrafish.

Axial level	Cell types	References
Cranial	Cranial ganglionic neurons, satellite cells in ganglia, Schwann cells in peripheral nerves	4
Cranial	Olfactory ensheathing glia that envelop bundles of olfactory receptor neuron axons (derived from the olfactory placode)	5
Cranial	Ectomesenchymal derivatives of the face: Some smooth muscle Dentine of the teeth All cartilage and bone of the facial skeleton (frontonasal skeleton and jaw) Bones of the middle ear Hyoid bone and thyroid cartilage Facial mesenchyme (connective tissue) Facial dermis	4,6-8
Cranial	Mesenchyme (connective tissue) of the salivary glands	9
Cranial	Glomus cells of the carotid body	6,10,11
Cranial	Pericytes and meninges associated with the forebrain.	4,12-15
Cranial	Pericytes and possibly also a very small portion (approximately 3.3%) of hormone producing cells (growth hormone, adrenocorticotropic hormone, thyroid-stimulating hormone, luteinizing hormone) in the anterior pituitary	4,16,17
Cranial	Adipocytes around the salivary glands and ears	6,18
Cranial	Corneal endothelium and stroma, iris stroma, ciliary body stroma and muscles, trabecular meshwork of the eye, and periocular mesenchyme	6,19-21
Cranial	Mesenchyme (connective tissue) of lobes, pericytes, and smooth muscle in thymus	6,22-27
Cranial	Mesenchyme (connective tissue) and pericytes in thyroid and parathyroid	6,23,28
Cranial	Calcitonin-producing cells of the ultimobranchial body (in chick, although lineage tracing in mouse has shown that these cells are derived from the endoderm)	29-31
Vagal	Neurons and glia of the enteric nervous system	32
Vagal	Neurons and glia in autonomic ganglia	33
Vagal	Smooth muscle of the outflow tract septum and valves; smooth muscle cells lining the great arteries of the thorax, thyroid, thymus, and parathyroid; mesenchyme that remodels pharyngeal arch arteries and cardiac ganglion	34-38
Vagal	Some cardiomyocytes of the trabecular myocardium of the ventricles	39-42
Trunk	Neurons and glia in dorsal root and autonomic ganglia, Schwann cells that line CNS-derived motor neurons in the ventral roots	33
Trunk	Chromaffin cells in the adrenal gland	33
Sacral	Small portion of the posterior enteric nervous system	32,43-45

mouse, the transcription factor Twist plays a critical role in the determination of the mesenchymal fate in the cranial NC, and overexpressing this single factor in the trunk NC results in mesenchymal potential in the trunk NC.[56] However, many open questions remain on the developmental potential of NC cells, how much of these differences are determined by the axial level at the stem cell stage and, on the other hand, by the microenvironment during the migration period toward the respective target tissues.

2.2 | Stem cell potency of the NC cells

2.2.1 | Abundant evidence for multipotency accompanied with some contradicting reports

As briefly discussed above, the NC is a unique population of stem cells able to generate a great variety of cell and tissue types that include representatives of various different major lineages. NC cells have, thus, on several occasions been proven to fulfill the criterion of multipotent stem cells. First in vivo evidence for multipotency comes from lineage tracing studies in the chick embryo in which single premigratory trunk NC cells were labeled by intracellular injection of a vital dye decades ago.[57] Clonal analysis of a majority of these labeled trunk NC cells showed descendants in as many as four NC derivatives (i.e., sensory and sympathetic neurons, Schwann cells, melanocytes, and adrenomedullary cells) in the chicken embryo.[57,58] Follow-up work with more sophisticated molecular biology tools has since confirmed the original results. Targeted photoconversion in single cells of the dorsal neural tube and time-lapse imaging of NC cells in the chick embryo showed that NC cells could generate multiple differentiated cell types in distinct structures, regardless of their location within the neural tube or time of emigration.[59] Furthermore, recent in vivo mouse work using genetic multicolor single cell tracing in the confetti system combined with lineage differentiation analysis revealed the broad developmental potential of NC cells in a mammalian system. The majority of trunk premigratory and, interestingly, also migratory NC cells were found to be able to generate sensory and autonomic neurons, glia and melanocytes.[60] More recently, advanced profiling of the molecular signature of NC cells using single cell sequencing and spatial transcriptomics has shown that fate biasing of NC is already detectable when these cells delaminate from the neural tube.[56] However, since premigratory and migratory NC cells have been shown to be multipotent,[60] future studies would need to address if the fate biases observed based on the molecular signature, particularly at the very early stages, reflect actual fate choices of the cells. Isolating these particular biased cells and performing lineage analysis in vivo and/or in vitro would help us understand if the core multipotency network is still maintained in these biased cells.

In contrast to these studies that agree on the multipotency of NC cells, contradicting evidence has been reported. A study by Krispin and colleagues that labeled premigratory trunk NC cells by either vital dye microinjection or plasmid electroporation in the chick embryo suggests that single NC cells are restricted in their differentiation potential and are segregated inside the dorsal neural tube in a dorsoventral and spatiotemporal manner that determines the order of colonization beginning with sympathetic ganglia, Schwann cells, sensory ganglia, and finally melanocytes, and the restriction/specification to neural fates is independent of the migratory environment and is likely to occur prior to cell emigration from the neural tube.[61] Some of this discrepancy may be explained by a slightly later timing of the analysis as previous studies have also found melanocytes to be the last population to emigrate from the neural tube in chick embryos and that this later emigration time point is a prerequisite for the melanocytic fate.[62,63] Along similar lines, a study by Weston and colleagues identified an ectomesenchymal (double positive for ectodermal E-Cadherin as well as the mesenchymal marker PDGFR) domain lateral to the "traditional" neuro-epithelial NC domain. The follow-up study shows that the NC driver Wnt1Cre is activated in some of these cells that possibly represent a separate, additional source for mesenchymal NC derivatives.[64,65] Finally, the most recent paper from the group points out that the more lateral portions of the ectoderm form the upper midline portion of NC cells that emigrate first, whereas Sox1-positive neuroepithelial cells were shown to emigrate during a later wave and mostly, but not exclusively, give rise to ganglia,[22] although an earlier study performed by using transplantation techniques suggests early- and late-migrating cranial NC cell populations are not lineage restricted and have equivalent potential.[66] Future work is required to determine the differences in the transcriptome and developmental potential of these cranial domains, to what extent they exhibit overlap, and if there are species specific features that explain some of the reported differences.

Even though a majority of the premigratory NC cells were found to be multipotent in the study by Baggiolini and colleagues, they reported differences in the developmental potential of clones, from some giving rise to all possible fates to some only producing two possible fates, suggesting heterogeneity of the stem cell potential of individual cells within the NC population.[60] This is consistent with in vitro clonal analyses, which have shown that NC cells are multipotent and able to self-renew[46,67,68] but, as seen in the in vivo studies of multipotency, show differences in developmental potential (the number of derivatives to which it gives rise).[67-69] However, it is important to keep in mind that what a NC cell becomes is not necessarily synonymous with its potential, although a recent study does show that overexpression of the stem cell gene *Lin28* significantly increases the existence of multipotent clones in vitro.[70] Further molecular profiling is needed to confirm the differences between NC cells that give rise to different numbers of the possible fates.

2.2.2 | Exceptionally high stem cell potential—pleistopotent?

NC cells have a higher stem cell potential than what is typically understood as multipotent, which is defined by having the ability to differentiate into all cell types within one particular lineage (e.g., hematopoietic

stem cells that give rise to a myriad of blood cells—stem cells that do not coexpress pluripotency factors). However, so far there is no evidence of a NC cell having the capacity to form a whole new embryo with all its cell types, either, which would be considered as true pluripotency. Thus, as noticed within the NC stem cell field for decades, the existing stem cell nomenclature lacks an intermediate version of these two states, which would adequately describe the potency of NC cells being higher than "just" multipotent but lower than pluripotent, which is different from any other stem cell population described to date, except for neuromesodermal progenitors that could fall into a similar category.[71-76]

Although the diverse outcomes of what the NC cells ultimately become have been known for decades, only recent work has provided molecular level proof that may explain the exceptionally high stem cell potential. A recent theory based on observations in the frog embryo suggests a shared regulatory program between the blastula and the NC.[2] The study hypothesizes that, unlike all other cells in the vertebrate body that go through gastrulation, the high stemness of the NC may be preserved from pre-gastrula stages by a yet unknown protection mechanism in the particular ectodermal domain.[2] The hypothesis is based on data which shows several NC regulatory transcription factors such as *Id3*, *Sox5*, *TF-AP2*, *Ets1*, *FoxD3*, and *Snail1* expressed in the same region with core pluripotency factors, or their paralogues (*Oct24*, *Oct60*, *Vent2*, *Myc*, *Sox2*, and *Sox3*) in blastula cells in *Xenopus*. Although the study shows that independent blocking of some of these factors (Snail1 and Sox5) resulted in the loss of the pluripotency network components and other factors linked to NC state and the ability to respond to endogenous inducing signals,[2] the data still leave room for an alternative possibility of the NC re-gaining its blastula-like transcriptional signature post gastrulation due to later reprogramming events in the ectodermal domain. In fact, a recent single-cell transcriptomic study in zebrafish found that NC cells and epiblast cells do not express the same pluripotency factors, rather they express paralogous pluripotency factors,[77] although of course species-specific differences cannot be completely discounted. However, a recent study in frog embryos shows that post-gastrulation lateral ectoderm can be reprogrammed to form NC by ectopic expression of neural plate border inducers together with Ventx2,[78] although primary determination of a NC domain in the epiblast prior to gastrulation has also been recently suggested.[79] Lineage tracing follow-up studies combined with single cell transcriptomics will be needed to elucidate the differences and similarities as well as the steps in between these two developmental stages. In line with the hypothesis, however, a recent study identified a transcriptionally and spatially distinct subdomain of premigratory cranial NC cells in the chicken embryo that indeed coexpress the generally accepted pluripotency factors *Nanog*, *Oct4/PouV*, and *Klf4*, which forms the core of a temporary NC stem cell niche discussed in more detail in the next section.[3] Since then, Oct4/PouV expression has also been shown in the NC in frog together with Ventx2, and in zebrafish.[71,72] Although, currently, there is not enough evidence to fully specify how similar the potency of NC cells is to pluripotent blastula cells, the existing gene expression and lineage tracing data certainly verifies NC stem cell potential to be far beyond what we understand by multipotency and necessitates a reconsideration of traditionally used terms to describe the potency of these unique NC cells (Table 2). For this, we contacted a linguistics expert in Latin and Greek who suggested NC potency to be referred to as pleistopotent (*pleisto* is Greek for "abundant, a substantial amount"), which we suggest to refer to stem cells that produce progeny that display features of more than one germ layer. Neuromesodermal progenitors that have been shown, during the past decade, in multiple species as a population of Sox2 (neural)/Brachuyry (mesodermal)-double positive stem cells surrounding the node in the posterior axial levels with potential to form both neural and mesodermal derivatives[74-79] can also be classified in the pleistopotent category.

2.3 | Heterogeneity of NC population within a single axial level

For the longest time, the NC within a single axial level was considered as a single, homogenous population of cells. This has only recently been contradicted as studies using single cell level accuracy have emerged clearly showing differential gene expression in neighboring NC cells during induction and specification stages.[3,46,80] Along these lines, in vivo lineage studies examining stem cell potency of NC cells suggest asymmetric division of NC cells at the premigratory stage that results in a "resident stem or precursor cell population" in the dorsal midline in addition to cells that migrate to target sites and give rise to NC derivatives, although the study did not pinpoint a molecular signature that would separate these functionally distinct cell types.[57,58,60] A recent study, however, reveals compartmentalization of the chick dorsal neural tube based on transcriptional differences. The multiplex analysis of the expression of 35 genes at single cell resolution revealed five spatially and transcriptionally distinct subpopulations shortly after neural tube closure at the onset of NC migration.[3] These domains include a distinct NC stem cell niche consisting of NC cells that coexpress true pluripotency factors (*Nanog*, *Oct4/PouV*, and *Klf4*) that centers around the dorsal midline, and is surrounded by NC cells that lack the high stemness signature.[3] Notably, these two populations are indistinguishable from each other based on their traditional NC marker expression (e.g., *SoxE*, *FoxD3*, *Pax7*, and *Snail2*), suggesting that none of these NC genes per se account for the high stemness. Interestingly, the cells with the pluripotency signature, but not the surrounding NC cells, also expressed marker genes of all the different NC lineages (melanocytes, cartilage, glial, and neural). This was interpreted as presence of a more widely open chromatin in the stem cells to maintain all options "open". Importantly, none of the NC cells expressed only a certain lineage marker supporting the comprehension of fate choices not being made at the premigratory stem cell stage. Also, some newly migrating cells still expressed the pluripotency factors. These results do not provide straightforward suggestions on which of the two NC populations, or perhaps both of them, contain the cells preparing to emigrate from the neural tube.

TABLE 2 Stem cell nomenclature for different stem cell potential. Current, existing stem cell nomenclature is missing a category that would adequately represent the neural crest cells, which have higher stem cell potential than multipotent cells but are not pluripotent. For this reason, we suggest the addition of an intermediate stage, pleistopotent (in bold), which describes stem cells that give rise to cell types that display features of "more than one germ layer," and thus would adequately describe the potential of neural crest cells and also neuromesodermal progenitors.

Term	Meaning	Example	Biological description	Coexpression of pluripotency factors (Oct4/Nanog/Klf4)
Totipotent	Latin, "ability for all things"	Zygote	All embryonic and extraembryonic cell types	Yes
Pluripotent	Latin, "ability for many things"	Epiblast/inner cell mass	All embryonic cell types	Yes
Pleistopotent	**Greek, "abundant, a substantial amount"**	**Neural crest, neuromesodermal progenitors**	**Broad range of very different cell types that display features of progeny of more than one germ layer**	**Yes**
Multipotent	Latin, "many"	For example, hematopoietic stem cells	Typically, broad range of cell types within a certain lineage	No
Oligopotent	Greek, "a few"	For example, lymphoid progenitors	Restricted amount of cell types within a sublineage	No
Unipotent	Latin, "one"	For example, hepatoblasts	Nondifferentiated but fully committed to a single cell type	No

However, expression of proliferation markers was highest in the stem cell population suggesting it may serve as the "resident stem cell population" for both NC and the remaining central nervous system cells that rebuild the dorsal neural tube. Further studies are required to understand the functional roles of the individual pluripotency factors as well as the dynamics of the niche.[3]

Finally, the multiplex analysis also identified a region of cells just lateral to the NC domain expressing neural markers together with differentiation and pluripotency genes, revealing the existence of a novel neural stem cell domain and providing proof for an overall higher plasticity of the neural tube than previously anticipated.[3]

3 | HOW IS STEM CELL POTENTIAL ESTABLISHED AND MAINTAINED IN THE EMBRYONIC NC?

There are many open questions regarding how the exceptionally high stemness is established and maintained in the early embryonic NC. Numerous studies have suggested that NC development involves BMP/TGFβ, Wnt, FGF, and Notch signaling molecules (as reviewed elsewhere[80,81]). With advances in molecular biology, numerous studies have also identified many transcription factors (e.g., *Tfap2A/B*, *Pax3/7*, *Msx1/2*, *Axud*, etc.) important for NC induction at the neural plate border, which in turn are required for the expression of NC specifier genes (e.g., *FoxD3*, *Sox10*, *Sox9*, *Snail/Slug*, etc.) that emerge in the dorsal neural tube as the NC starts to prepare for emigration.[82-86] Substantial amount of work has also been invested in revealing regulatory links between these transcription factors.[87,88]

In comparison, signaling mechanisms and molecules required for establishing and controlling self-renewal and/or developmental potency of NC cells is still poorly understood. Although there are a multitude of studies on how individual NC genes and growth factors regulate NC formation, maintenance, and differentiation, it is not clear, however, how all this information fits together. The fundamental question is whether some of these genes per se function to promote stemness or, alternatively, are some of them expressed in order to maintain a certain lineage option, which, when expressed together makes the cell highly multipotent/pleistopotent? In summary, as discussed in this section, current knowledge on the roles of individual NC markers suggests that NC stemness is built by coexpression of a combination of factors that (a) have roles in receiving signals from growth factors to activate the NC specification network, (b) have roles to regulate the size of the stem cell pool to ensure that the correct amount of NC cells will be produced, and (c) have roles to ensure that promoter regions of genes are widely accessible and the cell is thus maintained poised for a circuitry that promotes a particular lineage before instructions for a definitive fate choice take over. As different transcription factors bias the cell for different lineages, the cell is maintained in a highly multipotent/pleistopotent stage as long as all these lineage promoting genes are actively coexpressed (Figure 2).

Signaling pathways and molecules identified to have a role in promoting a differentiated cell fate have also been identified to have a role in maintaining stem cell potency in NC cells. For example, synergistically, Wnt and BMP signaling suppresses differentiation of NC stem cells and maintains NC stem cell potency whereas BMP signaling alone promotes autonomic neurogenesis and the development of smooth muscle cells and Wnt signaling alone promotes sensory neurogenesis.[89-93] Culture conditions with FGF, IGF, and retinoic acid were found to maintain chick and human NC cells in a self-renewing state that reflects their premigratory character[46] and addition of BMP4 was found to enhance expression of some NC marker genes to maintain trunk NC cells as self-

renewing entities.[94] BMP4 has been identified as a pluripotency factor that promotes self-renewal in mouse embryonic stem cells (ESCs),[95] in human periodontal ligament cells[96] and adipose derived stem cells.[97]

Although several other signaling molecules also undoubtedly contribute to the stem cell properties of NC cells, we are yet to uncover the mechanisms of action and the downstream and upstream players involved. Here, we discuss in detail some transcription and other factors already suggested to have a role in NC stemness maintenance and fate determination at the time when the NC is first established in the early embryo: SoxE, Sox2, Foxd3, Snail1/Snail2, Sox5, Lin28, cMyc, Pax3/Pax7, and Nanog. Most of these factors are also important for stem cell function in other cell populations suggesting they are part of a global network maintaining stem cell characteristics.[98-102] We realize there are many more studies that could be included into this section and we apologize for not being able to mention all of them.

3.1 | Sox family of transcription factors

3.1.1 | Sox10 (promotes glial and melanocytic fate)

Sox10, a member of the SoxE family, has been identified as a key transcriptional regulator of NC development[103-105] that maintains glial, neurogenic and melanocytic potential,[106-108] and loss of Sox10 has resulted in defects in the formation of peripheral glia, melanocytes, and the sympathetic and the enteric nervous systems.[109-114] Gain-of-function experiments by Kim and colleagues suggest the role of Sox10 may also be dose and timing dependent: although Sox10 maintains both glial and neurogenic potential, constitutive expression inhibits differentiation of NC stem cells into neurons to allow for gliogenesis.[108] This complements in vivo studies showing that in dorsal root ganglia, in the absence of *Sox10*, sensory neurons are generated (though they later degenerate) but no Schwann cells or satellite cells are formed.[112] Although Sox10 is crucial for the differentiation of peripheral glia, a high level of Sox10 expression is later needed for the differentiation of NC-derived peripheral neurons[103,108] and melanocytes.[115]

3.1.2 | Sox9 (promotes chondrogenesis, mesenchymal fate, and cell survival and delamination)

Sox9, another member of the SoxE family, is turned on before *Sox10* expression during specification of NC cells[116,117] and *Sox9* expression largely overlaps with the pattern of *FoxD3* during NC specification in the frog.[116] In line with this, a recent multiplex spatial genomics study revealed unbiased hierarchical clustering of *Sox9*, *FoxD3*, and *Snail2* genes in premigatory NC cells in chicken embryos suggesting a high probability of coexpression and thus possibility of the three acting as co-binding factors in the gene regulatory network.[3] In comparison, *Sox10* and *Sox8* clustered further away from this core cluster

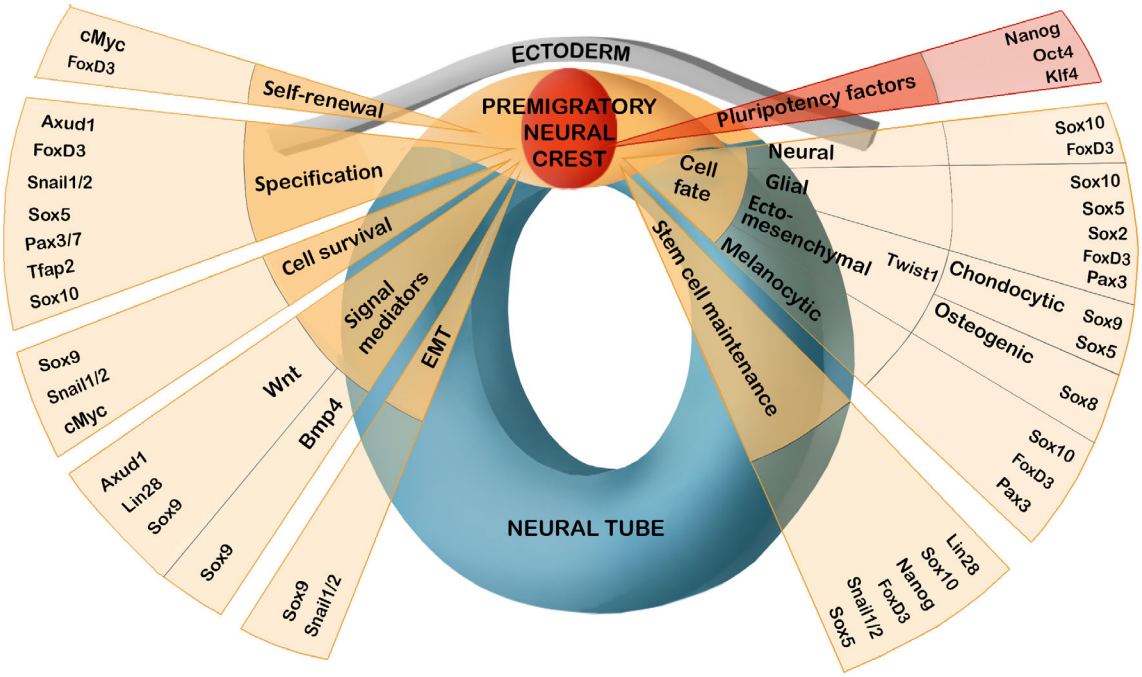

FIGURE 2 Neural crest stemness consists of multiple cellular functions driven by several genes in the dorsal neural tube. Schematic of a cross section through the neural tube showing the premigratory cranial neural crest containing a stem cell niche (dark orange region) where the pluripotency factor genes Nanog, Oct4, and Klf4 are coexpressed with neural crest markers. Current knowledge from different animal species suggests that several of the known neural crest specifier genes have different roles in maintaining neural crest stemness—whereas some regulate the size of the stem cell pool to ensure that the correct amount of neural crest cells will be produced in accordance with proper specification and preparation for emigration, some promote a particular cell fate such as neural, glial, chondrocytic, osteogenic, and/or melanocytic. As different transcription factors bias the cell for different lineages, the cell is maintained in a pleistopotent stage as long as all these lineage-promoting genes are actively coexpressed

suggesting that although there may be some redundancy in the SoxE genes in NC cells, there may also be some independent functions to each of the genes, and these roles may differ depending on the developmental stage of the NC.[3]

Sox9 has been reported to have multiple roles during NC development. It has been shown to promote cartilage formation by binding and activating the chondrocyte specific enhancer of collagen type II (Col2a1).[118,119] Loss of *Sox9* did not prevent mouse NC cells from normally migrating to the branchial arches but, instead, resulted in failure of cartilage differentiation causing a complete loss of endochondral skeletal elements and an increase in osteoblast markers.[120] Sox9 is also associated with cell survival in the trunk NC[121] and activation of anti-apoptotic factors like Bcl2 in mesenchymal stem cells.[122] Finally, phosphorylation of Sox9, activated by BMP and Wnt signaling, facilitates SUMOylation essential for NC delamination in the trunk.[96]

3.1.3 | Sox8 (cell survival and migration and possibly a negative regulator of the osteogenic lineage)

Sox8, the third member of the SoxE family, has also been shown to play a role in NC specification, although some differences in the spatiotemporal expression pattern occur between different species (reviewed in Reference 117). In frog, loss of Sox8 results in defects in cranial and trunk NC derivatives,[123] and in mouse Sox8 is required for the maintenance of the vagal NC population and is thought to have a role in cell survival.[124] Sox8 has also been identified to be a negative regulator of osteoblast differentiation, whereas there is no effect on chondrogenic differentiation.[125] Although this study did not particularly focus on NC-derived craniofacial bone development, Sox8 could potentially have a similar role in NC cells, negatively regulating the osteogenic lineage.

3.1.4 | Sox2 (promotes cell survival and glial fate)

Sox2, a member of the SoxB family, is highly expressed at the neural plate and well known for its role for maintaining the neural stem cell population required for proper formation of the central nervous system.[126,127] However, lower Sox2 levels are also expressed in the NC cells in the neighboring domain,[3,80,128] which may function to maintain glial lineage as it is known that Sox2 expression is upregulated in NC-derived cells that contribute to the peripheral nervous system and is restricted to Schwann cells and satellite glia later in development.[129]

3.1.5 | Sox5 (regulator of the size of NC domain in the early ectoderm; promoter of chondrogenesis and gliogenesis)

Sox5, a transcription factor of the SoxD family, has two isoforms in mammals, a short SSox5, only expressed in testis, and a long isoform LSox5, which in the mouse in conjunction with LSox6 and Sox9 drives activation of Col2a to promote chondrogenesis.[130,131] In the chicken embryo, LSox5 regulates the size of the NC domain in the dorsal neural tube.[132] The study also found that in addition to promoting chondrogenesis, Sox5 is expressed in the cranial glia, Schwann cells and satellite glia, and could promote formation of the glial lineage as well.[132] Similarly, Sox5 is linked to early ectodermal patterning in the early frog as well, where it was shown to be a DNA-binding cofactor essential for recruitment of Smad1/4 to BMP regulatory elements.[133] Additionally, as discussed in more detail in the Snail1/Snail2 section, Sox5 is required for maintaining the pluripotency network required for NC stemness and other coexpressed factors linked to NC state.[2]

3.2 | FoxD3 (promotes stemness and self-renewal; driver of neural fate, repressor of mesenchymal and melanocytic fates)

Several studies in chick, mouse, frog, and zebrafish have shown that FoxD3 is expressed in premigratory and early migratory NC cells at all axial levels.[134-142] The timing of FoxD3 reduction soon after the onset of migration[143] suggests a role in maintenance of stem cell characteristics. Indeed, a recent study that used single-cell RNA sequencing, ATAC-seq, and Chip-seq to characterize the transcriptional and epigenetic landscape of FoxD3-expressing NC cells in zebrafish shows molecular evidence for bimodal activity of FoxD3 in NC formation. First, it modulates the NC chromatin regulatory landscape by opening cis-regulatory elements and reshuffling nucleosomes to prime the onset of genes required for NC specification and to maintain stem cell programs.[71] After this, it changes into a repressor to modulate lineage choices during the early migratory stage.[71] These findings are similar to FoxD3 activity during transition of ESC to epiblast cells, which show that, by recruiting chromatin remodeling factors, it prepares cognate genes for future maximal expression by establishing and simultaneously repressing enhancer activity.[144] Through switching of target sites FoxD3 can thus modulate the developmental potential of pluripotent cells as they differentiate.[144]

At later stages, FoxD3 is expressed only in a subset of NC derivatives—its expression is downregulated in cardiac and cranial NC-derived mesenchyme but maintained in cranial and dorsal root ganglia and the developing esophagus and trachea.[141] Conditional loss of *FoxD3* in NC cells in *FoxD3$^{flox/flox}$;Wnt1-Cre* mouse embryos causes a reduction in the number of NC-derived neurons in the diaphragm and absence of vagal NC cells in the esophagus and cardiac ganglia.[141] Defects in cranial ganglia and the development of NC-derived vascular smooth muscle cells in the aortic arch were also reported. In vitro experiments showed that *FoxD3* also promotes self-renewal and stem cell maintenance. In summary, the knockdown study suggests a model where FoxD3 functions to maintain stem cell potency in NC cells by promoting neural potential while repressing mesenchymal fates,[141] in line with other studs that additionally reports FoxD3 as a repressor of melanocytic lineage.[137,145,146]

3.3 | Snail1/Snail2 (promotes EMT, cell survival, and NC gene regulatory network)

Snail1 and Snail2/Slug have a well characterized role in promoting EMT during gastrulation, in the emigrating NC as well as in several cancers, by inducing machinery that breaks down cadherins to disrupt polarization of epithelial cells.[147-151] Snail1 and/or Snail2 also promote cell survival and NC specification in the neural folds and premigratory NC.[152-157] Additionally, a recent study by Buitrago-Delgado and colleagues showed that several NC regulatory transcription factors (*Id3*, *Sox5*, *AP2α*, *Ets1*, *Foxd3*, and *Snail1*) are coexpressed and thus shared with the pluripotency network in the blastula. To note, in mammals, coexpression of Oct4, Nanog and Sox2 constitute a core pluripotency network,[158] which in this *Xenopus* study was defined to translate to combined activity of *Oct24*, *Oct60*, *Vent2*, *Myc*, *Sox2*, and *Sox3* in the blastula.[2] This study identified Snail1 and Sox5 as linkers between the pluripotency network and the NC genes required for the stem cell maintenance in both of these cell types, and indeed, independently blocking either factor, respectively, in animal pole cells resulted in loss of pluripotency network components and other coexpressed factors linked to NC state.[2] However, although Snail may play an independent role in maintaining pluripotency, it is important to keep in mind that, since blastula cells also undergo EMT during gastrulation of the epiblast to germ layers, this may directly impact the success of NC formation. Future studies blocking Snail genes in NC cells will help clarify its specific role in maintaining stem cell potential.

3.4 | Lin28 (pluripotency/stem cell maintenance)

Lin28 is an established pluripotency factor that has been identified to have a role in maintaining stemness and drive reprogramming of somatic cells.[99,102,159] *Lin28* is expressed in chicken NC cells throughout their development, from specification stages at the neural plate border to late migration stages, with highest relative expression levels marked during the premigratory stage, which led Bhattacharya and colleagues to examine its possible role in maintaining stem cell potency. They show that Lin28a is directly activated by Wnt-signaling and operates via inhibition of *let-7* miRNA, and downregulation of Lin28a leads to silencing of the stem cell network before cell differentiation. Constitutive expression of *Lin28a* in migratory cells resulted in a delay of differentiation and maintenance of the early NC factors (*Pax7*, *FoxD3*, *Ets1*, *Myc*, and *Sox5*) even at stages when these genes would normally be downregulated. In contrast, knockdown of *Lin28a* resulted in a significant reduction in the number of *Sox10* and *FoxD3* positive cells and affected downstream NC derivative formation such as dispersed, abnormally condensed ganglia.[70] Furthermore, clonal analysis of cultured NC cells transfected with a *Lin28a* expression construct showed a sixfold increase in the number of highly multipotent cells compared to the control group, suggesting a specific role in maintenance of NC stem cell potential/pleistopotency and stem cell characteristics.[70]

3.5 | cMyc (self-renewal and cell survival)

Myc family members have been shown to play a role in the maintenance, expansion, and differentiation of stem cell populations.[101] Orthologs of cMyc are expressed in the NC cells from the neural plate border through migratory NC stages in several animal models.[47,143,160-163] Importantly, all functional studies on the role of cMyc in the NC indicate that it does not support maintenance of a certain lineage but is instead required for stem cell maintenance. Morpholino mediated cMyc knockdown in frog and conditional *cMyc* knockdown in NC cells in mouse show defects in multiple NC-derived cell types and tissues including facial skeleton (branchial arches), pigmentation, neurons, and glia.[161,162] Furthermore, a chicken embryo study showed that cMyc regulates the size of the premigratory NC stem cell pool, as loss of cMyc results in a decrease in the number of emigrating NC cells due to reduced self-renewal capacity, increased cell death, and shorter duration of the emigration process.[47] Similarly, overexpression of cMyc enhanced the production of NC cells in the dorsal neural tube and prolonged the duration of emigration resulting in an abnormally large amount of NC cells.[47] In the NC, cMyc operates via a noncanonical mechanism by binding to another transcription factor, Miz1, instead of using the more common pathway of binding to E-box enhancer elements.[47]

3.6 | Pax3/7 (NC stemness and specification, in the case of Pax3, maintenance of melanocytic and glial lineages)

The Pax genes Pax3 and Pax7 are both expressed in the neural plate border and are involved with activating the transcription of NC specification genes (reviewed in Reference 164). In addition to these early roles of the Pax genes, Pax3 has been specifically shown to maintain stem cell potential in NC cells. Pax3 together with Tgfβ2 regulate expression of Hes1, Neurog2, and Sox9 to maintain NC cells in an undifferentiated state.[165] Pax3 also has a role in maintaining the melanocytic and glial lineages via cooperation/activation of Sox10 to drive expression of the melanocytic lineage factor Mitf[166-168] and, in collaboration with histone deacetylases in Schwann cells, to drive expression of the early glial marker gene *Mpz*.[169]

3.7 | Nanog (maintenance of stemness)

As mentioned, a transcriptionally and spatially distinct subdomain of premigratory cranial NC cells in the chicken embryo coexpresses the generally accepted pluripotency factors *Nanog*, *Oct4/PouV*, and *Klf4*.[3] A recent study in amphibians also showed that *Nanog* paralogue *Ventx2* is broadly expressed in the neural plate border.[170] Knockdown of Ventx2 selectively affected expression of the NC specifiers *Snail2*, *Sox9*, *Sox10*, and *Twist1* whereas *Myc*, *Tfap2a*, and *Foxd3* levels were unaffected.[170] This altered expression profile resulted in reduced

migration to the pharyngeal arches and reduced craniofacial skeleton whereas the neuronal lineage was unaffected in vivo and in vitro (in comparison, the melanocytic lineage was affected in vitro but not in vivo).[170] Furthermore, ectopic NC cells that were not biased toward any certain fate were formed in the ectoderm lateral to the NC domain in a process that required Ventx2, thus suggesting a role for Ventx2 in enabling the NC with a broader potential than just being restricted to neural derivatives, which would be the default for an ectodermal cell population.[170]

4 | LINEAGE RESTRICTION OF NC CELLS

As NC development proceeds, stem cell mechanisms that support the highly multipotent/pleistopotent features at the premigratory stage need to be silenced or modified to allow for differentiation to take place. After the cells exit the dorsal neural tube, exposure to environmental cues, molecular as well as mechanical, likely play important roles in lineage restriction.[82,171-173] As discussed in section 2.2, current data suggest that fate determination does not occur before delamination,[3,56,59,60] even though differences in axial level specific transcriptional profiles are detectable at the premigratory stage.[55] The acquisition of a fully differentiated fate thus likely involves cell-intrinsic state and extrinsic signaling during NC migration. To note, the development of melanocytes derived from chicken trunk NC cells, the last population to emigrate from the dorsal neural tube, may represent an exception to this rule as some studies suggest they may become fate restricted before or shortly after delamination.[145,174,175]

After trunk NC cells delaminate from the dorsal neural tube, the ventrally migrating trunk NC cells follow a path between the somites and neural tube, and give rise to neurons, glia, and some endocrine cells, whereas the dorsolaterally migrating cells follow a path between the dermamyotome and ectoderm and become melanocytes in the skin.[174] By culturing single migratory NC cells isolated immediately after emergence from the neural tube and at intervals as development proceeds, it was observed that some isolated cells gave rise to mixtures of precursors (of neurons, glia, and melanocytes), whereas others gave rise to precursors of only one fate.[62] The results thus suggest the existence of fate restricted neurogenic and melanogenic sublineages and a broad time frame and heterogeneity in the ways the NC cells ultimately commit to their future fates.[62]

Recent data based on genome-wide transcriptomic analysis derived pseudo-time trajectories in mouse embryos shows that newly migrating NC cells start to display a fate-biased transcriptional profile soon after delamination.[56] This is followed by lineage specification following a certain pattern in which future neurons and glial cells of the sensory lineage separate first from bipotent autonomic-mesenchymal progenitors, which then further divide into progenitors of the neurons and glia of the autonomic nervous system as well as mesenchymal cells.[56] However, a recent quantitative live imaging based study of trunk NC cells of the dorsoventral migration pathway in chick embryos suggests that the movement of individual cells is stochastic and unpredictable.[176] Although the overall direction of the movement is eventually toward the dorsal aorta, there was no correlation between the original position and total displacement of the individual cells, with some cells even moving backward.[176] The stochastic cell motion also led to sister cells ending up on different trajectories and final destinations with distinct fates, for example, sensory vs sympathoadrenal.[176] Finally, the confetti system was also applied to study cranial NC development during migratory stage.[177] Although the activation of the system is not early enough to evaluate stem cell potency at premigratory stages, the results fit with the theory of gradual decline of different lineage options during migration. At E12.5, none of the ectomesenchymal clones displayed any neuroglial progeny, but still gave rise to odontogenic, chondrogenic, osteogenic, and adipogenic cells.[177]

Although gene expression analyses provide insight to how the NC generates multiple cell types, future studies will need to examine how these relate to the migratory behavior of the NC and external signals they receive. In summary, it is not yet clear how, where, and when NC cells make their ultimate fate choices in order to shift from stem cells to a myriad of different derivatives around the vertebrate body.

5 | EXTENDED STEM CELL POTENCY OF NC-DERIVED CELLS THROUGH DEVELOPMENT TO ADULTHOOD

The NC starts to form in the ectodermal germ layer right after gastrulation, which in humans occurs during the third week of gestation and lasts until the cells have migrated to often distant sites in the periphery some weeks later. This, however, is substantially earlier than the development of many of the organs to which the NC contributes, which has left researchers puzzled for decades. Today we know, as discussed in this section, that although some NC cells directly migrate into their final destinations and start their differentiation process, some turn into Schwann cell precursors (SCPs) located along nerve fibers in the embryo that can later in embryonic development give rise to several NC derivatives[178] (Figure 3). SCPs can thus be considered as a relatively slow and fluent, although also transient, continuation of the developing NC populations that serves to populate late developing organs with NC derivatives.[178] In addition to these embryonic populations, NC-derived stem cell reservoirs have been identified in postnatal tissues. Although these NC-derived stem cell reservoirs maintain stem cell potential, we do not have a clear understanding to what extent these cells remain similar to the embryonic populations.

5.1 | Broad potential of embryonic NC-derived nerve-associated cells

5.1.1 | Schwann cell precursors (SCPs)

NC-derived SCPs, the earliest developmental stage of the Schwann cell lineage, have been discovered as a long-term supply of several NC

derivatives during embryonic development.[178,179] SCPs migrate along the sprouting nerves, using them as routes to reach destinations throughout the body. SCPs that remain attached to nerve fibers give rise to Schwann cells (their most common derivative), whereas SCPs that detach from nerve fibers give rise to melanocytes,[146,180-182] parasympathetic neurons,[179,183] sympathetic neurons,[184] neurons in the enteric nervous system,[185,186] and chromafin cells of the adrenal medulla[184,187] and Zuckerkandl organ.[184] SCPs also give rise to mesenchymal derivatives such as dental mesenchymal stem cells,[188] a subpopulation of bone marrow mesenchymal stem cells[189] and endoneurial fibroblasts.[190] Most recently, SCPs were identified to detach from nerve fibers to form a "small but significant fraction" of mesenchymal cells that differentiated to chondrocytes and osteocytes in both mouse and zebrafish.[191]

This broad developmental potential of SCPs raises questions about the difference between late migrating NC cells and SCPs. When NC cells enter developing nerves and associate with sensory axons, they differentiate into SCPs, but there is no clear molecular marker-based definition on the exact time point when the shift occurs. Differentiation of non-glial derivatives from SCPs is observed before SCPs start committing to immature Schwann cells, the more intermediate stage of the Schwann cell lineage[179,183,187,191] that finally differentiate to myelinating or nonmyelinating Schwann cells (reviewed in References 151 and 165). Gene array analysis of NC cells and SCPs show gradual changes in the gene signature between the two cell types.[192] Furthermore, other studies too have shown that although there is upregulation of early glial markers such as Fabp7 (fatty acid binding protein-7), Pmp22, Mpz, and other genes that are maintained throughout Schwann cell development, there is also maintenance of NC related genes such as FoxD3, Sox10, AP2α, α4 integrin, and N-cadherin (reviewed in References 193–195). FoxD3 is also expressed in a subset of glial cells, most likely a (sub) population of SCPs, as shown in a recent single cell transcriptomic study comparing different stages of mouse NC development.[56] Considering FoxD3 is linked to stem cell-like properties in the premigratory NC,[71,141] NC cells and SCPs may thus share similar stem cell potency networks. Comparison of the gene expression profile of NC cells and SCPs in more detail to identify which genes might be maintained in these populations could help elucidate the molecular mechanisms that maybe in common between the two populations

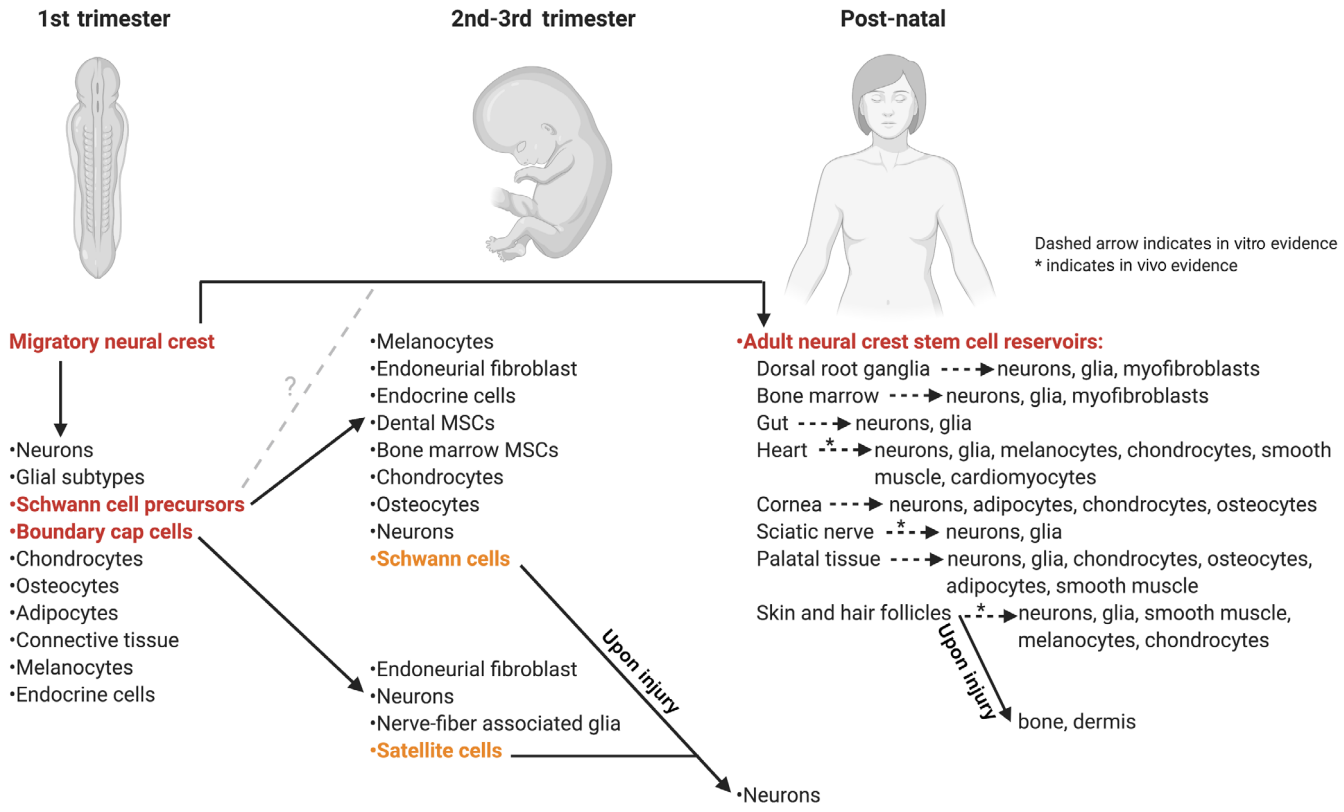

FIGURE 3 Neural crest cells maintain stemness throughout life by transforming to different stem cell reservoirs. The neural crest is a transient stem cell population that gives rise to several different derivatives. Although some neural crest cells, after leaving the initial premigratory stem cell niche in the developing dorsal neural tube, directly migrate to target sites and give rise to their derivatives, some differentiate into stem cell reservoirs such as Schwann cell precursors and boundary cap cells residing along peripheral nerves and likely populate late developing organs with neural crest derivatives. Neural crest-derived stem cell reservoirs have been identified in several postnatal tissues and organs as well, although the development and physiological roles of many of them are poorly understood. Although there is no direct indication that some of these postnatal stem cell reservoirs are derived from the Schwann cell prescursors, it cannot be ruled out either. The schematic is based on studies on different animal species. Figure created with BioRender

and their roles in maintaining stem cell potency. It could also help us understand any differences or similarities in the fate-specifying mechanism of differentiating NC derivatives directly from NC cells as opposed to through SCPs.

5.1.2 | Boundary cap cells and other SCP subpopulations

In addition to SCPs, other NC derivatives such as boundary cap cells have also shown to share common markers like Sox10 and FoxD3, and to perform similar supportive functions in the developing peripheral nervous system to circumvent the developmental constraints posed by the transience of NC cells.[178] Since SCPs are a heterogeneous group of cells, these may also be considered as SCP subtypes.[178] Boundary cap cells are derived from a subset of late migrating NC cells and found at embryonic nerve roots at the interface between the central and peripheral nervous systems (reviewed in Reference 196). They are also multipotent as in vivo lineage tracing studies have shown that boundary cap cells give rise to sensory neurons and satellite cells in the dorsal root ganglion, Schwann cells, and endoneurial fibroblasts in the dorsal and ventral nerve roots as well as myelinating and nonmyelinating Schwann cells and terminal glia in cutaneous nerves in the dermis.[196-198] In vitro studies also corroborated their stem cell nature by showing that isolated boundary cap cells from mouse embryos self-renew and give rise to neuronal and nonneuronal derivatives.[199,200]

As mentioned, comparison of the gene expression profile of NC cells, the different subpopulations of SCPs and boundary cap cells in more detail may help identify a common stem cell potency network in these cells.

5.2 | NC-derived stem cell reservoirs in neonatal and adult tissues

In addition to the broad developmental potential of the NC-derived nerve-associated cells during embryonic stages described above, in animal models NC-derived stem cells have been identified in developed, postnatal tissues such as dorsal root ganglia,[201] bone marrow,[189,201] gut,[202] heart,[203,204] cornea,[205,206] sciatic nerve,[207] carotid body,[10] skin and hair follicles,[208-210] and palatal tissue[211,212] (Figure 3). The human adult dental pulp also contains an odontoblast derived (and thus NC-derived) subpopulation of progenitors with a limited capacity to form dentin and pulp-like structures,[213] and has osteogenic potential[214] and possibly some neuroregenerative capacity as well.[215] Furthermore, NC-derived forebrain pericytes have also shown potential to differentiate to neural and nonneural cells following ischemia.[216,217] Although lineage tracing studies using P0 and/or Wnt1-cre recombination in mice have identified the NC origin of some of these neonatal and adult stem cell populations and/or the expression of common NC markers,[10,201,205,209,210] the mechanisms that maintain their stemness and the physiological roles of these cells are poorly understood. The NC-derived stem cells isolated from neonatal or adult tissues maintain high stem cell potency and can be differentiated into several of the NC derivatives such as neurons, glia, smooth muscle cells, melanocytes, bone cells, fibroblasts, and chondrocytes in vitro (reviewed in detail elsewhere[218,219]). Like SCPs during embryonic stages, these NC-derived stem cell reservoirs are likely in place to circumvent the transience of NC cells. However, although these neonatal/adult NC-derived stem cell reservoirs are still able to differentiate to derivatives of multiple lineages (eg, neuronal and mesenchymal), they are unlikely to retain as broad of a developmental potential as the embryonic NC stem cells nor have an identical gene expression pattern. Future work comparing the transcriptomes of embryonic NC stem cells (including the temporary embryonic niche expressing the pluripotency network Nanog, Oct4, and Klf4) and neonatal/adult NC-derived stem cell populations will help elucidate the molecular mechanisms that are maintained in these different populations.

The discovery of NC-derived stem cells in skin has been particularly exciting considering this could be an easily accessible source of the cells for autologous transplantation for treatment of disease conditions. Since this discovery, several studies have isolated NC-derived progenitor or stem cells from human skin.[220-223] A recent study also showed a high level of stem cell potency of NC-derived nerve associated adult mesenchymal stem cells, which upon tissue repair have the capacity to form bone and dermis.[224]

5.3 | Regenerative potential of NC cells

With the multitude of derivatives to which the NC gives rise, this stem cell population could have profound significance in regenerative medicine. Making a certain cell type via the correct differentiation pathway that mimics normal development as faithfully as possible is considered beneficial for obtaining the best possible outcome for therapeutic purposes. As such, for transplantation of NC derivatives into patients, it may be essential to first differentiate induced pluripotent stem cells (iPSCs) to NC cells before directing them to a particular NC derivative. However, before applying NC-derived stem cells for regenerative purposes, we need a better understanding of human NC development. Work related to human NC development has mostly been limited to in vitro studies, where human NC stem cells have been derived from ESCs and iPSCs during the past decade.[225-229] Human NC stem cells have also been derived from direct reprogramming of fibroblasts by introduction of transcription factors, such as Sox10[230] or FoxD3,[231] which both play roles in maintenance of stem cell properties in NC cells (section 3). More recently NC cells were derived from keratinocytes of the interfollicular epidermis (another easily accessible source) of neonatal and adult donors by using growth factors for reprogramming.[232,233] These derived human NC stem cells have a molecular profile compatible with NC cell identity in animal models, they express genes such as Sox10, FoxD3, and AP2α and give rise to several NC derivatives such as neurons, glia, melanocytes, cartilage, and bone.[46,225-233] Although future work will need to determine how similar the NC cells formed by these

various methods are to each other and how accurately they represent endogenous NC development, it is clear that these approaches hold great promise for regenerative therapy purposes.

6 | CONCLUSIVE REMARKS AND PERSPECTIVE

The data presented here bring together our current knowledge about the range of the diverse derivatives to which the NC gives rise, either directly from the initial embryonic NC cells or through an intermediate stem cell stage such as the SCPs and postnatal NC-derived stem cell reservoirs. Although the exceptionally high developmental potential (which we suggest to be referred to as pleistopotency) of NC cells is proven by several studies, there are several gaps in our understanding of how this potential is established and maintained in this unique post-gastrulation population of cells. Future studies combining transcriptomic data and lineage tracing will be imperative to clarify several current questions about the stem cell potential of NC cells, including how the NC obtains a pleistopotent network and how and when the NC becomes fate restricted from the time of specification. A better characterization of the pleistopotent network in NC cells and its progenitors will be crucial to uncover the molecular mechanisms that are responsible for the differences in potential and behavior observed between NC subpopulations. A thorough understanding of the establishment and maintenance of stem cell potential in NC cells will be important not only for proper understanding of embryonic development and neurocristopathies, but also for a broader understanding of stem cell biology, and for taking advantage of this knowledge for therapeutic approaches in regenerative medicine and cancer cell biology.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

S.N.P., L.K.: conceived overall review content and wrote the paper.

DATA AVAILABILITY STATEMENT

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