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Neural Crest As the Source of Adult Stem Cells

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Abstract

Recent studies suggest that adult stem cells can cross germ layer boundaries. For example, bone marrow-derived stem cells appear to differentiate into neurons and glial cells, as well as other types of cells. How can stem cells from bone marrow, pancreas, skin, or fat become neurons and glia; in other words, what molecular and cellular events direct mesodermal cells to a neural fate? Transdifferentiation, dedifferentiation, and fusion of donor adult stem cells with fully differentiated host cells have been proposed to explain the plasticity of adult stem cells. Here we review the origin of select adult stem cell populations and propose a unifying hypothesis to explain adult stem cell plasticity. In addition, we outline specific experiments to test our hypothesis. We propose that peripheral, tissue-derived, or adult stem cells are all progeny of the neural crest.

INTRODUCTION

Cells arising from a given embryonic germ layer (ectoderm, mesoderm, or endoderm) are committed to germ layer-specific developmental fates (1). Recent studies have called this dogma into question and given rise to the possibility that adult stem cells can cross germ layer boundaries. For example, under certain conditions, bone marrow-derived stem cells appear to differentiate into neurons and glial cells, as well as other types of cells (2,3). How can stem cells from bone marrow, pancreas, skin, or fat become neurons and glia (3–6)? What processes bring mesodermal cells to a neural fate? Transdifferentiation, dedifferentiation, and fusion of donor adult stem cells with fully differentiated host cells have been invoked to explain plasticity of adult stem cells, as observed in culture and after transplantation into host mammals (7,8). Here we review the current literature on the origin of adult stem cells and propose a unifying hypothesis that may resolve much of the controversy surrounding adult stem cell plasticity. Also, we outline specific experiments to test our hypothesis. We propose that peripheral, tissue-derived, or adult stem cells are all progeny of the neural crest (NC).

What are NC cells?

NC forms during the neural plate stage in the developing vertebrate embryo at the junction between epidermal ectoderm and presumptive neural ectoderm. Therefore, in the early vertebrate embryo, NC cells can first be found at the dorsal lip of the developing neuroectoderm. As development progresses, they migrate from the lateral margins of the neural tube and contribute to virtually every organ of the vertebrate body (Fig. 1) (9–12,47).

NC cells are pluripotent (10,11), and it has been proposed that this pluripotency is modified during migration to their final destination (13). Our hypothesis suggests that plasticity

exhibited by NC derivatives and the widespread distribution of their progeny in the vertebrate body implicate NC as the source for adult stem or progenitor cells.

Are all peripheral, adult stem, or progenitor cells NC progeny?

An analysis of several known adult stem or progenitor cell populations provides support for a NC origin. Table 1 compares published results with the stated hypothesis for several adult stem and progenitor cell populations.

Perhaps the system that best addresses these questions and has the most complete evidence in support of our hypothesis is the skin-derived progenitor cells, or SKPs (14). The SKPs can be isolated from hair follicles, where they are found in a unique stem cell niche (4). NC cells with β -galactosidase expression driven by the *Wnt-1* promoter were used in a lineage study to demonstrate a NC origin for SKPs (15). The conclusion that SKPs are derived from NC is also supported by their expression of NC markers, including, Sox-9, Pax-3, Slug, Snail, Twist, and Nestin (4). Consistent with these results, NCs populate a dermal niche (15) and can differentiate into melanoblasts, smooth muscle cells, neurons, Schwann cells, and chondrocytes (15,16). Therefore, the origin of SKPs can be traced to NC, they express a complement of NC markers, they are in an area populated by derivatives of the NC, and they are capable of what we refer to here as retained alternative fates (see below).

Lineage studies show a NC contribution to the heart (17), and adult cardiac muscle progenitors are derived from NC. In addition, expression of Pax-3 accompanies migration of these NC cells to the cardiac outflow tract (18). In fact, an outflow tract progenitor cell population may contribute to the cells found throughout heart (19), and recently a population of cardiac progenitors was found in the heart that expresses the NC markers Nestin, Musashi-1, and p75. These cells can contribute to smooth muscle of blood vessels and the peripheral nervous system (PNS). Also, when transplanted into the chick neural tube, they migrate along the NC dorsolateral pathway to the cardiac outflow tract (20).

The cardiac outflow tract is linked to another important stem cell population, bone marrow-derived stem cells. Hematopoietic stem cells (HSCs) originate within the dorsal aorta and the surrounding mesenchyme of the mouse embryonic day-11 aorta-gonad-mesonephros (AGM) (21), an area that receives a major complement of NC cells. Also, other cells of the bone marrow can be linked to the NC, such as a circulating mesenchymal stem cell (MSC) of NC origin (22–24). Bone marrow-derived HSCs and MSCs are capable of differentiation into neural fates (25,26). In fact, a recent report demonstrates that HSCs can produce neural stem cells (27).

Some skeletal muscles arise from NC during embryonic development, and others may harbor stem or progenitor cell populations with NC origin into adulthood. In the craniofacial region, muscle can be found derived directly from NC cells (11). Adult stem or progenitor cell populations for skeletal muscle found throughout the body may be derived from NC, including satellite cells and nonsatellite cell myogenic progenitors. First, satellite cells, defined positionally as sublaminal cells and functionally as myogenic progenitors, can be isolated routinely from muscle fibers (28,29). Marker expression most often linked with satellite cells includes the NC marker Pax 3/7 (28,30). Satellite cells exhibit myogenic, osteogenic, and adipogenic differentiation (31), and nonsatellite cell myogenic progenitors differentiate into myofibers, endothelial cells, neurons, and Schwann cells (32,33).

Although controversy exists regarding the developmental source of stem or progenitor cells in the pancreas, we propose that the evidence supports a NC origin. An analysis of a heterogeneous population of cells isolated from pancreatic islets suggests a link to NC based first on marker expression (34). Furthermore, these cells can be driven toward fates

consistent with a neuroectodermal origin. However, van der Kooy and colleagues (5) describe a clonal population of pancreatic multipotent precursors and conclude they are not progeny of NC because the precursors do not express the known NC markers Pax-3, Twist, Wnt-1, and Sox-10. Nonetheless, these cells express a subset of NC markers, including Snail, Slug, and p75 and can generate neurons (5). We maintain that persistent expression of subsets of NC markers is suggestive of a NC origin. The expression of Snail and Slug is consistent with the known transition from an epithelial to mesenchymal cell-type that precedes NC cell migration.

There are numerous examples that support a neural link for adult stem or progenitor cells such as progenitor populations from adipose tissue (35), umbilical cord blood (36), Wharton's jelly (37), and deciduous teeth (38). All of these stem or progenitor cells appear able to differentiate into neurons. Moreover, support for a common origin of many adult stem or progenitor cells can be found in cases where systemic effects are monitored following changes in gene expression that may be related to NC. For example, Slug knockout mice show impaired hematopoiesis and pigmentation deficiencies (39). Also, some cases of human piebaldism, characterized by congenital depigmented patches of skin, are associated with mutations of Slug (40), and it is suggested (40) that piebaldism in these cases results from a deficiency in Slug function during NC development.

Neural stem or progenitor cell populations found in the central nervous system (CNS) (41) may be related to the cell populations described here. However, due to the overlap in timing during early development of primordial brain and NC, a method of identification of a unique stem cell population for CNS neural stem cells is not addressed by our current hypothesis.

Testing our hypothesis

To assert that identified adult stem or progenitor cell populations originate in NC, one should define criteria for the proof of principle, and each cell population should be tested in a reproducible manner using these criteria.

We propose the following conditions to establish a NC origin for adult stem or progenitor cells.

1. If adult stem or progenitor cells are of NC origin, they can be traced with an appropriate lineage tracking system. A direct lineage trace between the NC and adult stem or progenitor cells in a particular tissue is the most definitive method for testing our hypothesis. In modern lineage studies, promoter-specific, fate-independent marker expression can be used to trace the developmental outcome of any cell. One such method specific for NC is the Wnt-1-Cre/R26R transgenic system, which links early *Wnt-1* promoter activity, considered to be specific to NC, with persistent ROSA26 expression (42). Upon activation of Cre recombinase by early Wnt-1 expression, persistent β -galactosidase activity is maintained in all progeny. Theoretically, this makes it possible to achieve an accurate lineage trace of all NC descendants.
2. Adult stem or progenitor cells of NC origin will retain a 'marker history'. A partial list of NC-related markers includes Slug, Snail, Twist, Wnt-1, Musashi-1, Pax-3, Pax-7, Sox-9, Sox-10, Nestin, and p75 (4,5,11,20,34). Expression of these markers may denote NC origin. However, by no means should marker expression alone be left as proof of NC origin, nor should absence of a given marker be taken as evidence to exclude a cell population as being of NC derivation. For example, the transcription factors Slug and Snail may be down-regulated at the end of NC migration and entry into an adult stem cell niche.

3. Adult stem or progenitor cells of NC origin display “retained alternative fates.” Transdifferentiation describes a cell fate inconsistent with the germ layer of origin. If it is shown that adult stem or progenitor cells originate in NC, the ability to differentiate into neural and other cell types may be retained. Rather than transdifferentiation or dedifferentiation, we propose that adult stem or progenitor cell populations contain cells, derived from NC, and are capable of achieving alternative fates. For example, bone marrow cells can become neural cells. We propose that this is due to a neuroectodermal origin of the stem or progenitor cells contained within the bone marrow.

We propose that all peripheral adult stem or progenitor cells have a NC origin. Newly described and previously reported populations of stem or progenitor cells should be assessed for NC origin using the criteria described above.

What are the ramifications of this hypothesis?

First, it provides an explanation for the plasticity exhibited by adult stem or progenitor cells. Pluripotent cells of the NC escape the basal lamina, become mesenchymal, and migrate to peripheral tissue, where their progeny are found throughout the body in all adult vertebrates. The presence of NC-derived cells in so many tissues begs an explanation for the question: What selective advantage have NC cells bestowed upon the vertebrates? One proposal is that NC is the source of cells for tissue regeneration in vertebrates (43), and a goal of stem cell research is the regeneration of tissues lost to disease or injury. An understanding of the plasticity of potential donor stem or progenitor cells is important to determine the therapeutic value of a given cell type.

Second, if all adult stem or progenitor cell populations originate in the NC, then any accessible adult stem cell population might be able to be used for transplant needs. For example, a strong case can be made for bone marrow stem cell transplants to replace neural cells if, as we propose, they share a neuroectodermal origin. Recent studies indicate that in some examples of neural disorders, bone marrow stem cell implants are useful as a cell-based therapy (44,45). The use of easily accessible and apparently pluripotent adult stem cells may eventually supplant the need for embryonic or fetal cells in clinical applications.

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Germ Layer of Origin	<u>Ectoderm</u>	<u>Mesoderm</u>	<u>Endoderm</u>
Organs (partial list)	CNS PNS Epidermis Sweat Glands Sebaceous Glands Mammary Glands Teeth	Bone Heart Skeletal Muscle Kidneys Blood/Blood Vessels Dermis Adipose	Liver Pancreas Thyroid Thymus Lungs Bladder Lining of Alimentary Canal

FIG. 1.

Cells from the NC contribute across germ layers to many organs of the vertebrate body. NC cells are found early in development migrating from the dorsal lip of the developing neuroectoderm, shown here at the neural-fold stage. During development, NC cells populate a host of organs in the body derived from ectoderm, mesoderm, or endoderm. The diverse migration of NC cells provides an opportunity for them to contribute to adult stem cell populations found in many of these organs.

Abbreviations: CNS, central nervous system; PNS, peripheral nervous system.

This figure is adapted from Fig. 1 of Trainor et al. (11) and Fig. 1 (bottom) of Trainor and Krumlauf (47).

Table 1

Stem or Progenitor Cell Population

Criterion	SKPs	Cardiac progenitors	Hematopoietic progenitors	Skeletal muscle/satellite cells	PMPs	Islet enriched	Others (adipose/umbilicus/Wharton's jelly)
Lineage trace	X	X					
Markers							
Slug	X				X	X	
Snail	X				X	X	
Twist	X					X	
Wnt-1	X						
Musashi-1		X					
Pax-3	X	X		X			
Pax-7				X			
Sox-9	X						
Sox-10						X	
Nestin	X	X				X	
p75		X			X		
Retained alternative fates	X	X	X	X	X	X	X
NC contribution to organ	X	X	X	X	X	X	X
Citation	(4, 14–16)	(17–20)	(21–23)	(11, 28, 30–33)	(5)	(34)	(35–48, 40, 46)