Cell Stem Cell
Minireview

Home at Last: Neural Stem Cell Niches Defined

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DOI 10.1016/j.stem.2009.05.008

Adult neural stem cells (NSCs) are involved in regulating mammalian behavior and are controlled by diverse external stimuli. Improved understanding of the physical location of NSCs and the microenvironmental cues that regulate their behavior, which combine to define the NSC "home," or niche, may reveal how to control their function.

Although the idea that the adult nervous system contains stem cells was viewed as a radical one in the not-so-distant past, we now know that the adult brain contains NSCs that can and do generate neurons and glial cells on an ongoing basis. These adult NSCs, which are generated from the precursors that build the nervous system during development (reviewed in Kokovay et al., 2008), are maintained into adulthood in at least two niches, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) in the hippocampus, although there is lively discussion concerning the possibility that NSCs are more widely scattered throughout the adult brain (see Gould, 2007). What are the functions of adult NSCs? Though this topic has recently been reviewed elsewhere (Zhao et al., 2008), their most important function is to generate neurons. NSCs in the SVZ generate neuroblasts that migrate a significant distance via the rostral migratory stream (RMS) to the olfactory bulb, where they generate interneurons essential for maintenance of the olfactory bulb. In contrast, neurons produced in the hippocampal SGZ integrate into the immediately adjacent granule cell layer, where they are important for learning and memory. Adult NSCs are also involved in gliogenesis, with those in the SVZ generating oligodendrocytes (Jackson et al., 2006) and those in the SGZ generating astrocytes (Suh et al., 2007). Finally, although the brain is notoriously bad at repairing itself, adult NSCs do respond to neural injury with an attempt at repair, a finding that has led to therapeutic strategies aimed at recruiting and improving this endogenous ability (Kokovay et al., 2008; Zhao et al., 2008).

So what instructions must be provided by the adult NSC niche to support these functions? First, the niche must maintain adult NSCs in a quiescent, undifferentiated state, particularly because NSCs are not immortal and can be depleted, for example, by aging. Second, the niche must provide a neurogenic environment because we know that NSCs transplanted into the brain outside of these niches largely differentiate into glial cells. Third, the niche must be structured so that both the number and type of differentiated progeny can be modulated in response to a diverse array of physiological cues, some of which derive at a significant distance (Kokovay et al., 2008; Zhao et al., 2008). Here, we will review recent progress delineating how NSC niches accomplish these various functions.

The Adult NSC Niche: From Fractones to Pinwheels

Though the adult NSCs that reside within the SVZ and SGZ are both primarily involved in generating neurons, these two populations occupy very different niches. In the SVZ, three populations of precursors, including adult NSCs, lie adjacent to a layer of ependymal cells lining the lateral ventricle wall (Figures 1A and 1B). The NSCs (called B cells) are relatively quiescent cells that express markers reminiscent of embryonic radial precursors, as well as the astrocyte protein GFAP. B cells give rise to transit-amplifying cells (called C cells), a more rapidly dividing population that is GFAP negative but positive for EGF receptor and the transcription factor Dlx2. The third population is the neuroblasts (called A cells) that express markers of newborn neurons such as doublecortin and PSA-NCAM. A cells migrate in glial tubes to the olfactory bulb and generate neurons that integrate into the neural circuitry. Though earlier studies established the lineage and anatomical relationships among these populations (Kokovay et al., 2008), a trio of recent papers has greatly expanded our understanding of the structure of the adult SVZ niche (Figure 1B) (Shen et al., 2008; Tavazoie et al., 2008; Mirzadeh et al., 2008). The first important finding from these studies was the elucidation of an extensive blood vessel network that spans the entire SVZ just beneath the ependymal layer and is closely associated with NSCs and their progeny. Second, these studies showed that many GFAP-positive NSCs are intercalated into the ependymal layer and have a short apical process with a single primary cilium contacting the ventricular wall and a second long basal process contacting a blood vessel, a morphology reminiscent of their radial precursor parents (Figure 1A). Intriguingly, at sites where neural precursors contact blood vessels, astrocytic end feet are absent, thereby modifying the blood brain barrier and exposing cells within the SVZ to blood-born molecules. Finally, by looking at the ventricular wall, Mirzadeh et al. (2008) showed a remarkable "pinwheel" organization, with the core of the pinwheel formed by apical processes of the NSCs and the pinwheel itself composed of two anatomically distinct types of ependymal cells.

In contrast, the hippocampal SGZ niche has a more laminar structure and is the home for two types of putative NSCs, both of which express the precursor marker Sox2 (Figure 1C) (Suh et al., 2007; Zhao et al., 2008). One of these populations divides infrequently, expresses GFAP and Sox2, and has a radial process that spans the adjacent granule cell layer (radial NSCs or type 1 progenitors). The second population divides much more frequently, expresses Sox2 but not GFAP, and displays short processes (nonradial NSCs or type 2 progenitors). The lineage relationship between these two precursors is not yet clear. These Sox2-positive precursors

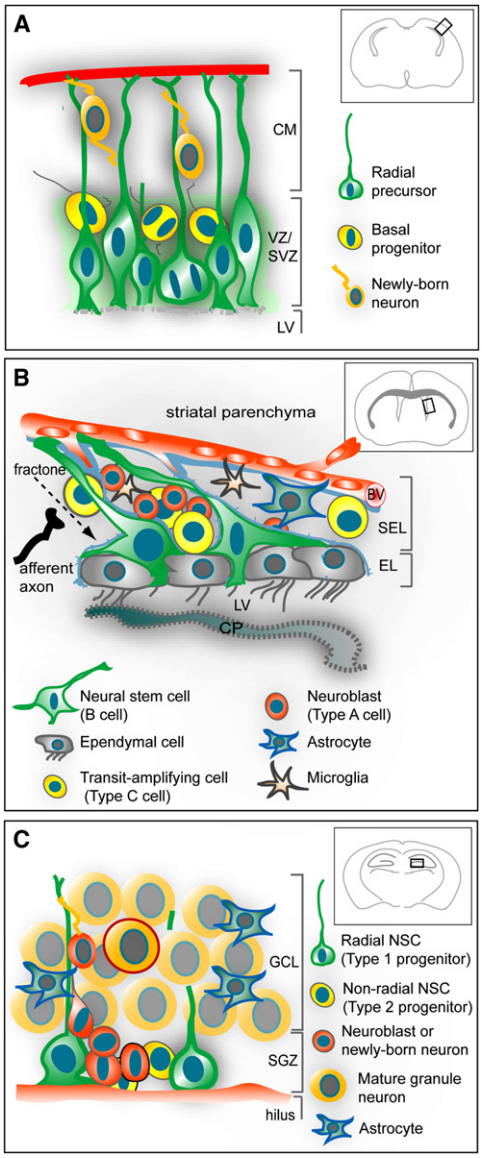

Figure 1. Different Neurogenic Niches in the Mammalian Brain

Schematics of coronal rodent brain sections depict (A) the VZ of the developing embryonic cortex, (B) the SVZ within adult cortex, and (C) the SGZ within adult dentate gyrus (see upper-right of each panel for section orientation).

(A) During embryonic cortical neurogenesis, radial precursors contacting the ventricular surface give rise to neurons directly or via an intermediate (basal progenitor) and facilitate the migration of newly born neurons along long radial processes.

(B) The adult SVZ niche is composed of three populations of lineage-related precursors: the relatively quiescent NSCs (B cells), mitotically active transit-amplifying cells (C cells), and neuroblasts (A cells) that lie immediately beneath a monolayer of ependymal cells lining the lateral ventricle. NSCs are intercalated into the ependymal layer and are also closely associated with the vasculature. NSCs within the SVZ are in contact with blood-born and CSF-born factors, with local endothelial cells, microglia, and astrocytes, as well as local or distal afferent-derived signals. The stem cells are also in contact with vascular basal laminae-derived fractones that are rich in extracellular matrix molecules.

(C) The adult SGZ niche is composed of three populations of precursors: the radial NSCs (type 1 progenitors), nonradial NSCs (type 2 progenitors), and neuroblasts. Neuroblasts migrate into the adjacent granule cell layer (GCL), where they mature into neurons.

BV, blood vessel; CP, choroid plexus; CM, cortical mantle; VZ, ventricular zone; LV, lateral ventricle; SEL, subependymal layer; EL, ependymal layer.

give rise to neuroblasts that migrate into the adjacent granule cell layer, where they mature as neurons and integrate into the hippocampal circuitry. At least some of these Sox2-positive precursors also self-renew and generate both astrocytes and neurons in vivo, thereby fulfilling the criteria for stem cells (Suh et al., 2007). Interestingly, many of the proliferating hippocampal precursors are closely associated with the vasculature (Zhao et al., 2008). It is not known whether the vasculature is "leaky" at these points of interaction, as in the SVZ.

Collectively, these findings indicate that both neurogenic niches, though distinct in many ways, are built so that NSCs can receive, integrate, and respond to signals from the outside world (Figure 1). In both cases, NSCs are (1) closely associated with the vasculature, (2) adjacent to a variety of neighboring cells, including their own neuronal progeny, resident mature astrocytes and microglia, and blood vessel endothelial and smooth muscle cells, and (3) in close contact with basal lamina components. For example, SVZ precursors are closely associated with "fractones," slender projections of the vascular basal lamina that are rich in extracellular matrix components like laminin and that might sequester and concentrate growth factors (Kerever et al., 2007).

One of the most striking aspects of this shared architecture is that it allows NSCs to respond rapidly to signals that are generated far away. For example, a pregnancy-induced increase in olfactory bulb neurogenesis is necessary for appropriate maternal olfaction (Shingo et al., 2003). In this case, increased neurogenesis occurs in response to prolactin, a hormone that is secreted from the anterior pituitary and that likely arrives in the SVZ via the vasculature. Thus, an animal's physiological state, monitored and controlled in one neural location, can regulate behavior via a distant NSC intermediate.

Do these findings generalize to humans? Though adult human NSCs exist and neurogenesis occurs in this species (see references in Gould, 2007; Quinones-Hinojosa and Chaichana, 2007), the human niche structure differs from that of rodents. In humans, the SVZ niche has four layers: an ependymal cell layer adjacent to the lateral ventricle, a hypocellular gap, an "astrocyte ribbon" containing both astrocytes and adult NSCs, and a layer demarcating the niche from the adjacent parenchyma (Quinones-Hinojosa and Chaichana, 2007). As in rodents, the human NSCs within this niche live in close proximity to endothelial cells, microglia, ependymal cells, and neuronal processes. Moreover, human NSCs generate neuroblasts that migrate to the olfactory bulb (Curtis et al., 2007), although this model is still the subject of some debate. Thus, although differences exist between rodents and humans, the niches are apparently sufficiently similar to provide hope that lessons learned in rodents will be relevant to human therapeutics.

Ependymal Cells and NSCs: A Back-and-Forth Relationship

Within the forebrain SVZ, NSCs and ependymal cells share a close anatomical relationship. In fact, earlier studies suggested that ependymal cells were adult NSCs. However, though this identification was apparently mistaken (Kokovay et al., 2008), an intimate functional relationship between SVZ NSCs and ependymal cells is evidenced by the highly organized niche pinwheels comprised of these two cell types and by the finding that

ependymal cells synthesize molecules such as pigment epithelium-derived factor and noggin, which regulate the proliferation and differentiation of adult SVZ NSCs (Kokovay et al., 2008; Zhao et al., 2008). Recent work has revealed, in addition to this close anatomical relationship, an unexpected lineage relationship among the two cell types. During development, forebrain radial precursors generate ependymal cells that line the lateral ventricles, a capacity that is retained by adult NSCs (Luo et al., 2008). The converse is also true; although ependymal cells are normally quiescent, after an ischemic stroke, they move into the SVZ, where they re-enter the cell cycle and produce olfactory bulb neurons (Carlen et al., 2009). Thus, ependymal cells apparently "become" neuronal precursors, raising the intriguing possibility that they do so by being recruited into vacant neurogenic stem cell niches that arise following injury.

Adult NSC Maintenance: A Lifetime Endeavor

Ultimately, the maintenance of any tissue stem cell population is the result of a complex interplay between intrinsic mechanisms and extrinsic cues derived from the stem cell niche. In this regard, like other tissue stem cells, adult NSC maintenance requires the actions of proteins such as Bmi-1, Tlx, and Sox2 (Kokovay et al., 2008; Zhao et al., 2008). However, emerging evidence indicates that the adult NSC niche also provides an environment that ensures maintenance of NSCs for the lifetime of the animal. Though a thorough consideration of the relevant growth factors and signaling systems has been published elsewhere (Kokovay et al., 2008; Zhao et al., 2008), here we will consider distinct aspects of two particularly intriguing examples.

The first example highlights the importance of the interaction between NSCs and the vasculature. As discussed for prolactin-mediated regulation of neurogenesis during pregnancy, access to the blood itself provides a means by which systemic signals can regulate NSC biology. However, the cells that comprise the vasculature are also in a position to play a regulatory role. Evidence in support of this model comes from a study by Shen et al. (2004), who showed that endothelial cells secrete factors that promote renewal of SVZ NSCs. At the same time, these endothelial factors biased NSCs to generate more neurons, as would be predicted if they were to play a role within a neurogenic niche. Though the relevant factors are not yet defined, it will be interesting to determine whether they are present only on the vasculature or whether they are perhaps captured and presented on vasculature-derived fractones within the niche (Kerever et al., 2007).

The second example involves sonic hedgehog (Shh). Previous studies have shown that Shh serves to establish and maintain the adult NSC pool in both the SVZ and SGZ (reviewed in Kokovay et al., 2008; Zhao et al., 2008). More recently, Han et al. (2008) demonstrated that the primary cilium, the site of a Shh-signaling complex in nonneural cells, was necessary for hedgehog signaling within the SGZ. This finding is particularly intriguing in light of the potential sources of Shh within these neurogenic niches. In particular, in the SVZ, the primary cilia of many GFAP-positive NSCs project into the ventricular space (Mirzadeh et al., 2008) (Figure 1B), raising the possibility that the CSF is one relevant source of Shh. Recent evidence supports this idea; Shh is indeed present within the adult CSF (Angot et al., 2008), and the beating of ependymal cilia in the lateral ventricle moves CSF in a manner that can generate molecular concentration gradients within the SVZ (Sawamoto et al., 2006). Thus, both vasculature-derived and CSF-derived signals might play a key role in regulating adult NSC maintenance and self-renewal.

"Real" Astrocytes Direct Differentiation of Their Astrocyte-like Stem Cell Neighbors

The aforementioned examples demonstrate how different players within the NSC niche regulate stem cell maintenance. But the niche must also allow or even instruct appropriate differentiation. How does this happen? Rather than providing a review of potential mechanisms (see Kokovay et al., 2008; Zhao et al., 2008), we will instead focus and expand upon how one niche cell type, the astrocyte, locally regulates NSC differentiation.

Perhaps the best-characterized example of astrocytes regulating NSC differentiation is found in the SGZ. Within the hippocampus, the cell bodies of NSCs are immediately adjacent to the granule cell layer, and, for the radial NSCs, their processes extend throughout the entirety of the layer, meaning that these stem cells make direct contact with both mature granule neurons and local astrocytes (Figure 1C). The importance of these interactions was shown in an elegant series of papers from the Gage laboratory (Lie et al., 2005 and references therein); Wnt3a expressed by mature hippocampal astrocytes directly instructed SGZ NSCs to generate neurons, and inhibition of Wnt signaling in vivo decreased hippocampal neurogenesis by almost 8-fold. Thus, local astrocytes generate one of the key pro-neurogenic signals in the adult SGZ niche.

Somewhat paradoxically, astrocytes within the SVZ niche may also promote oligodendrocyte differentiation. In particular, Jackson et al. (2006) recently showed that SVZ NSCs (B cells) express PDGFRα and that these NSCs generate both neurons and oligodendrocytes. Interestingly, genesis of oligodendrocytes, but not neurons, was dependent upon PDGF within the niche. Given that other studies have shown that PDGFA is produced by astrocytes, these findings suggest that niche astrocytes might promote neurogenesis via one ligand, Wnt3a, and oligodendrogenesis via another, PDGFA. Therefore, this model raises a central question in stem cell biology: if a stem cell is exposed to different extrinsic cues within the niche environment and each cue leads to a different outcome, then how does the cell "sort them out" to enact the appropriate biological response? Answering this key question will require more delineation of the complex interactions present within any stem cell niche.

All Wired Up in the Stem Cell Niche

A common regulatory strategy utilized to maintain tissue homeostasis while also avoiding stem cell depletion is to establish a negative feedback loop from differentiated progeny back to the stem cells. In the case of adult NSCs, the primary progeny are neurons, and so one might predict that a feedback loop would engage neural circuitry. Recent evidence indicates that this model is in effect in the brain and that a number of mechanisms have evolved to transmit the negative feedback.

The first of these mechanisms is based upon the remarkable finding that adult neural precursors express receptors for neurotransmitters and that they are "innervated" in that they are in close contact with the transmitter-containing axons of mature

neurons and/or with neuronal/neuroblast cell bodies (Figure 1). Perhaps the best-characterized example of this model involves the neurotransmitter GABA. NSCs in both the SVZ and SGZ express GABA receptors, and GABA has been shown to depolarize both NSC populations and thereby inhibit their proliferation (reviewed in Zhao et al., 2008). The relevant source of GABA in the SVZ is newly born neuroblasts (type A cells), which likely secrete GABA spontaneously, thereby providing a direct, local negative feedback loop from differentiated progeny to their stem cell parents. In contrast, in the SGZ, the primary source of GABA is the mature granule cells, and GABA release in this case would be a direct reflection of neural circuit activity. In a second example, the axons of midbrain neurons provide dopaminergic input to the EGFR-positive transit-amplifying cells (C cells) in the SVZ. Dopamine promotes C cell proliferation, and lesioning the dopaminergic input decreases precursor proliferation in both the SVZ and the SGZ (Höglinger et al., 2004). In this case, the normal physiological rationale is somewhat unclear, but the disease implications are obvious; the same study shows a reduction in the number of proliferating precursors in the brain of Parkinson's disease patients.

By contrast, a very recent report describes a second way that neural circuit activity can regulate adult NSC biology. Specifically, Ma et al. (2009) showed that activity-dependent stimuli, such as physical exercise, enhance hippocampal neurogenesis via a mechanism involving induction of Gadd45b expression in mature hippocampal neurons. Gadd45b then caused demethylation of a number of genes, including the gene encoding BDNF, a growth factor that regulates hippocampal neurogenesis. Thus, neural activity epigenetically regulates the biology of neurons in the vicinity of the niche, and these neurons then regulate NSC-mediated neurogenesis. This finding has clear implications for NSCs in the SGZ, but its importance for those in the SVZ is less clear because their mature neuronal progeny are far away, within the olfactory bulb. Nonetheless, these findings uncover a completely distinct way by which neural activity can regulate NSC biology, reinforcing the concept that there are multiple, parallel mechanisms at work (reviewed in Zhao et al., 2008). Whether or not there are additional activity-dependent mechanisms that allow neural circuitry to control neurogenesis is a key remaining question.

Conclusions

The idea that the adult nervous system contains NSCs that generate all of the major neural cell types is a relatively recent one. Nonetheless, research has now produced an intriguing view of the locations where these stem cells reside, and how cues encountered within these sites regulate stem cell behavior and thus have defined functional NSC niches. Together, these studies have described how the environment regulates NSCs and have shown that one way experiences modify behavior is via NSC-mediated neurogenesis. Hopefully the results will also ultimately lay the foundation for successful therapeutic strategies that recruit and direct endogenous NSCs.

ACKNOWLEDGMENTS

We would like to acknowledge those primary publications that we could not quote due to reference limitations. F.D.M. is a Canada Research Chair and an H.H.M.I. International Research Scholar. We would like to thank David Kaplan and Derek van der Kooy for their helpful input.

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