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Copernican Stem Cells: Regulatory Constellations in Adult Hippocampal Neurogenesis

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Abstract In the adult, neurogenesis occurs where constellations of signaling molecules are correctly orchestrated and where competent cells are present to interpret these signals. As the instruments used to observe adult neurogenesis become more sophisticated, the concept of a discrete competent ''stem cell'' has become less concrete. Neural progenitor cells once thought committed to a single lineage can be influenced to become multipotent and somatic tissues appear to yield cells capable of tremendous peripheral and central lineage potential. The variety of cell types that appear competent to generate neurons suggests that the ''Hilios'' of adult neurogenesis may not necessarily be a single cellular entity but rather the sum of signals that dictate, ''Make a new neuron here.'' These signals may not be limited to the recruitment of preexisting neural stem cells but may also, in some subtle way, reprogram local precursors to create ''stem-like cells,'' where needed. J. Cell. Biochem. 88: 41–50, 2003.

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The central nervous system (CNS) is generated by a vigorous progression of cellular specialization that occurs under tightly regulated spatial and temporal cues [Anderson, 2001]. At the beginning of this dynamic universe of activity, multipotent neurectodermal stem cells are signaled to divide. Some of the resulting daughter cells retain stem cell attributes (self-renewal). Other daughters generate committed glial and neuronal progenitors. The committed progenitors undergo further expansion and, ultimately, exit the cell cycle to become fully differentiated and highly specialized neurons, astrocytes, and oligodendrocytes of the newborn brain.

After birth and throughout adulthood, proliferative neural stem/precursor cells (NSCs) remain widespread in the CNS.[1] Although the vast majority of these cells are destined to generate glia, neurogenesis does continue in the hippocampus and olfactory bulb [Cameron and McKay, 1998]. Other areas, such as neocortex [Gould et al., 1999b], also generate neurons but at such low levels that the phenomenon is quite naturally debated [Kornack and Rakic, 2001]. There is growing evidence that adult NSCs in these silent regions could be induced generate neurons if the correct local cues were supplied.

Injury alone may be sufficient to activate local neurogenesis but neurogenic responses are still highly region-dependent [Magavi et al., 2000; Arvidsson et al., 2002]. In many instances, injury is not adequate to stimulate neurogenesis. The application of growth factors or transplants of competent cells as well as an alteration in local signaling [Lim et al., 2000; Nakatomi et al., 2002] may be needed to stimulate neurogenesis. Despite the adult brain's reputation as an

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[1]Note: In its common use, the term "precursor" is synonymous with "progenitor," but many use this term within the neural stem cell field to describe populations of cells that behave similarly to stem cells (e.g., produce neurons and glia but have not been formally evaluated for the presence of multipotent self-renewing stem cells).

immutable structure, it is becoming clear that the adult CNS can add or replace neurons using tightly regulated and, perhaps, regionally specific intrinsic programs.

How then does the brain specify where and when to generate new neurons? Brain regions that naturally generate abundant neurons are strikingly distinct from areas that make few or no neurons. This dichotomy provides a unique opportunity to speculate on the signals and cellular substrates necessary to generate a newborn neuron within the adult brain.

STEM CELLS AND PROGENITORS: THE ELUSIVE HELIOS OF ADULT NEUROGENESIS

What is a neural stem cell? Evidence of "self-renewal" and "multilineage potential" within a clonal population of cells is currently accepted as functional criteria for stem cell identity, i.e., post hoc evidence that a single cell was able to generate neurons, astroctyes, and oligodendrocytes, as well as additional stem-like cells. There is no shortage of evidence for stem-like cells in primary cultures from fetal, postnatal, and adult brain [Reynolds and Weiss, 1992; Palmer et al., 1999; Uchida et al., 2000]; however, a lack of unique stem cell markers has made it difficult to directly identify these cells in vivo.

Current models of adult neurogenesis begin with an assumption that uniquely identifiable stem cells are actively recruited to divide and that local cues dictate whether progeny adopt neuronal or glial fates. The proliferative areas of the subventricular zone or hippocampus are often used as stem cell source tissues. Some cell surface epitopes provide a significant level of enrichment for stem cells when initiating cultures from brain tissues; e.g., CD133 for fetal human neural precursors [Kumihashi et al., 2001], Notch expression by ciliated cells [Johansson et al., 1999], glial fibrillary acidic protein (GFAP) expression [Doetsch et al., 1999], or the absence of heat-stable antigen/peanut agglutinin-binding for rodent precursors [Rietze et al., 2001]. Generic physical attributes have also been used to isolate stem-like cells from tissues. These include low buoyant density [Palmer et al., 1999] or the spontaneous formation of neurospheres from within a mixed population of cells [Seaberg and van der Kooy, 2002]. Unfortunately, none of these attributes translate well for stem cell identification in brain tissues and it has been quite difficult to rigorously and convincingly perform clonal analyses in vivo [Walsh and Cepko, 1992]. In spite of numerous inferences [Doetsch et al., 1999; Johansson et al., 1999], direct evidence for self-renewal and multilineage potential in vivo remains to be demonstrated.

It is obvious that definitive markers are sorely needed, but the assumption that the adult neural stem cell is a discrete entity may not be well justified. Neurogenesis and gliogenesis are developmentally modeled as linear progression that starts from a uniform lamina of neurectodermal stem cells. Once development is complete, the resulting adult brain is somewhat more complex and there is increasing evidence that self-renewal and multilineage potential may belong to cell populations distinct from the developmentally known neurectodermal stem cells. It is also possible that these adult cells may be capable of varying their outward display of markers.

Culture alone may alter marker expression and this provides a curious uncertainty principle where the method used to document a unique marker may itself alter the outward identity of the cell. For example, white matter contains an abundant population of proliferating glial progenitor cells that display specific markers at each stage of differentiation. Cells from white matter can be sorted on the basis of these markers to form pure glial-restricted progenitor populations [Kondo and Raff, 2000]. When propagated under conditions optimized for glial progenitors, these cells retain their glial programming in vitro and display distinct and reproducible marker profiles that correlate with differentiation status. However, minor alteration in the culture paradigm stimulates these same cultures to yield cells that are indistinguishable from a multipotent stem cell (i.e., the addition of basic fibroblast growth factor, FGF-2) [Palmer et al., 1999; Kondo and Raff, 2000]. This may be due to the recruitment of a cryptic stem cell population but the stringent sorting for glial phenotypes by Kondo and Raff does suggest that cell surface phenotype is dynamic and that the local signaling environment can induce alterations in cell phenotype and behavior.

To add another nick to the edge of Occam's razor, it is widely assumed that proliferative activity in the adult brain may indicate the presence of stem cells. For this very reason, the mitotically active ventricular zone is frequently

targeted for stem cell isolation. The hippocamal formation with its proliferative zone at the margin of the granule cell layer (GCL) yields a lesser, yet still abundant, population of stem-like cells and non-proliferative areas such as neocortex yield low but detectable numbers of stem cells. In this context, white matter displays an intermediate proliferative status and produces a moderately abundant population of stem-like cells in culture when stimulated with FGF-2 [Palmer et al., 1999; Kondo and Raff, 2000]. There is one interesting exception. Seaberg and van der Kooy [2002] show that careful removal of ventricular margins from a hippocampal tissue preparation depletes the resulting cell population of "neurosphere"-forming cells. The implication is that anchorage independent growth of cells in spheres, as an indicator of stem cells, is not one of the attributes exhibited by the proliferative population of the GCL. What then are the rapidly dividing cells of the dentate GCL and can a closer look at these cells and their environment provide insight into adult neurogenesis?

Within the adult hippocampus, neurogenesis involves the replication of precursors at the margin of the hilus and GCL. The dividing cells subsequently exit the cell cycle, differentiate, and mature into functional granule layer neurons [Shors et al., 2001; van Praag et al., 2002]. The astrocyte marker, GFAP may be expressed by a significant fraction of the dividing cells but the relative number GFAP-positive cells detected is highly dependant on the specific antibody used [contrast Palmer et al., 2000; Seri et al., 2001].The precursors that are actively dividing can be chemically ablated and quiescent precursors are subsequently activated to repopulate the system [Seri et al., 2001; Shors et al., 2001]. This allows one to evaluate the phenotypes of the earliest repopulating cells. Seri and colleagues use retroviral marking to show that an astrocyte-like GFAP-positive cell is the first dividing cell to express the viral marker gene during this restoration process. Unless extraordinary measures are taken to account for cells that do not express the marker gene (i.e., the cells responsible for later neurogenesis might not have been detected [Walsh and Cepko, 1992]), it seems probable that "glia" play a direct role in producing neurons in the adult as well as in development [Doetsch et al., 1999; Noctor et al., 2001]. In vitro characterization of stem/precursor cells from the adult hippocampus indicates that the uncommitted multipotent precursor cell [Palmer et al., 2000] does not express GFAP.

Stem cell identity becomes even more vague when CNS evidence is joined by a growing body of literature suggesting that somatic tissues can generate neuron-competent stem/precursor cells [Brazelton et al., 2000; Mezey et al., 2000] and the distinct impression remains that precursor phenotypes and behaviors may be highly context-specific. A single observation or context may not necessarily represent the full repertoire available to precursors in the adult brain or soma. How then does the adult brain orchestrate neurogenesis and what cell types represent competent substrates for these regulatory cues? The answers to these questions do not yet exist but the hippocampus, with its abundant neurogenesis and clear functional role in learning and memory, makes an attractive platform to explore both the mechanisms and functional outcomes of adding new neurons under unperturbed conditions.

HIPPOCAMPUS AS A MODEL NEUROGENIC ANATOMY: A UNIQUE COSMOS OF CELLULAR INTERACTIONS

Within the hippocampus, the neural precursors divide in a discrete lamina that has several unique attributes. The subgranule zone is an area of dense axonal projections from the adjacent granule layer neurons to area CA3. It is also an area where astroglia naturally express GFAP in the absence of injury. Interestingly, the density of small capillaries is unusually high relative to other areas of the brain [Palmer et al., 2000]. Closer evaluation of the hippocampal SGZ shows that proliferative clusters are frequently found in contact with small capillaries. Cells within the neighboring vascular wall also divide suggesting that neurogenesis is accompanied by a highly localized endothelial response. Recent work by Levinson and colleagues demonstrate that angiogenesis is an intrinsic element of adult avian neurogenesis [Louissaint et al., 2002].

The proximity of the precursors to blood vessels and the response of mammalian neural precursors to endothelial brain-derived neurotrophic factor (BDNF) [Leventhal et al., 1999] suggest that precursors communicate intimately with cells of the vasculature. However, there is no evidence that these small capillaries

lack a blood-brain barrier formed by pericytes and astroglial endfeet. This suggests that vascular effects may be "translated" by other cell types within the neurogenic niche. Not withstanding their proposed role as stem cells [Seri et al., 2001], astrocytes are more commonly known for their functional role in forming the blood brain barrier. Astrocytic endfeet envelop the capillary bed and selectively transport proteins and metabolites to and from the brain parenchyma. Astroctyes are a prime candidate for mediating vascular cues and recent work demonstrates that cultured astrocytes support the proliferation and survival of developing neurons and can stimulate the production of neurons from subventricular zone (SVZ) precursors [Lim and Alvarez-Buylla, 1999]. Furthermore, astrocytes isolated from the hippocampus (but not spinal cord) are able to induce maturation of neural progenitors to functional neurons in vitro [Song et al., 2002] and are known to regulate synapse formation and synaptic transmission [Haydon, 2001].

Pericytes also reside at the abluminal side of the microvasculature niche. Pericytes send out cellular projections encircling the endothelial cells and are thought to provide vasodynamic regulation and structural support to the microvasculature as well as mechanisms for intimate communication between the vasculature and brain parenchyma. Pericytes are particularly interesting in the context of neurogenesis because they play a key role in endothelial activation and integrin upregulation during angiogeneis. Interestingly, pericytes respond to and produce many of those factors which play a role in both angiogenesis and neurogenesis such as IGF-1, VEGF, PDGF, and FGF-2 [Rucker et al., 2000].

There is considerable evidence suggesting that neurogenesis and angiogenesis would coincide simply due to the extensive overlap in mitogen response. Endothelium and neural precursors respond similarly to FGF-2, IGF-1, VEGF, EGF, and TGF-alpha. Direct administration of FGF-2, IGF-1, and VEGF in vivo robustly upregulates hippocampal neurogenesis [Wagner et al., 1999; Aberg et al., 2000; Jin et al., 2002]. In addition, many stimuli that increase angiogenesis in the CNS also stimulate hippocampal neurogenesis. Physical activity (running) is particularly effective at stimulating adult neurogenesis [van Praag et al., 1999b] and has previously been shown to trigger widespread angiogenesis in brain regions controlling motor activity [Isaacs et al., 1992]. Combined, the anatomical observations suggest the unique neurogenic microenvironment of the hippocampus may involve neurons and astrocytes, as well as cells of the microvasculature (Fig. 1).

REGULATION OF HIPPOCAMPAL NEUROGENESIS: CONSTELLATIONS OF ENVIRONMENTAL INFLUENCE

Just as the wobble in a distant star tells tales of unseen planets for the prepared observer, the perturbation of neural precursor behavior can predict the presence of likely regulatory systems. Neurogenesis may be regulated at multiple levels including the migration of precursors, signals that may modify a local precursor's intrinsic potential, and the local array of regulatory cues that recruit stem cells, direct adoption of neuronal fate, amplification of committed neuroblasts, and/or progression of neuroblasts toward a fully functional neuron. This provides the casual observer with a delightful array of variables to consider. What physiologies impact neurogenesis and how might they impinge on this progression of events that leads from stem cell to functional neuron?

Stress and Depression

Neurogenesis is downregulated by chronic stress or depression [Kempermann, 2002, review]. Stress and depression are accompanied by elevated cortisol levels and the suppressive effects of stress can be mimicked by administering glucocorticoids (GCs). However, the intracellular GC and mineralcorticoid receptors that moderate the effects of GCs are not detected in precursors in vivo [Cameron et al., 1998]. This suggests that GCs must act via indirect

Fig. 1. The vascular niche. Neurogenesis is easily detected within the mouse hippocampus. The schema in (**A**) stylizes the cellular relationships that are likely to instruct precursor cells in vivo (**B**). New neurons are produced in a discrete lamina between the hilus and GCL termed the subgranule zone (SGZ). The proliferative precursors can be identified on the basis of nuclear bromodeoxy uridine incorporation (white). Precursors rapidly differentiate into immature neurons that express double cortin (green) when they reside near microcapillaries (red, glucose transporter, Glut-1). Instructive or selective cues may be provided by many cells within this unique microenvironment including astrocytes, endothelium, pericytes, and mature granule layer neurons.

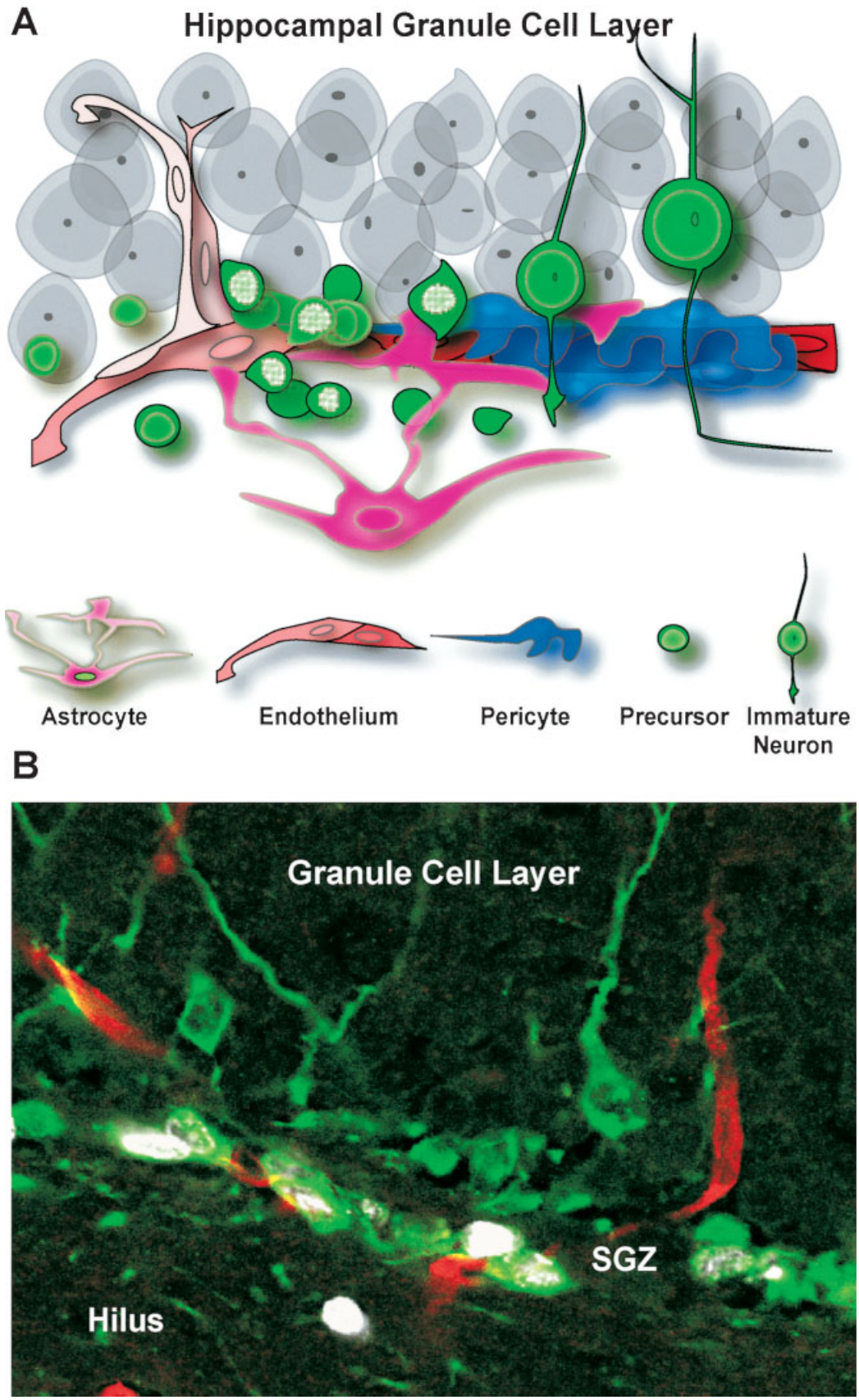
A
Hippocampal Granule Cell Layer
Astrocyte
Endothelium
Pericyte
Precursor
Immature Neuron
B
Granule Cell Layer
SGZ
Hilus

Fig. 1.

mechanisms. Since precursors reside within the dense axonal projections of the GCL and GCs are known to induce the subtle alterations in hippocampal synaptic activity that accompanies stress and depression, it is likely that neurogenesis is influenced by the activity of neighboring neurons.

Stress induces changes in hippocampal activity that are reflected in long-term potentiation (LTP), a physiological measure of synaptic strength that correlates with learning and memory function. LTP is strengthened following running (i.e., in concert with increased neurogenesis [van Praag et al., 1999a]) and these changes in synaptic activity are mediated by N–methyl–D–aspartate (NMDA) receptors. NMDA receptor blockade inhibits LTP and NMDA antagonists also stimulate neurogenesis in vivo, perhaps as a compensatory response to the decrease in synaptic function [Cameron et al., 1998]. Serotonin signaling also influences LTP by potentiating NMDA receptor action. Antidepressants that enhance serotonin availability by blocking serotonin reuptake also stimulate neurogenesis [Kempermann, 2002, review]. At face value, it would seem that either reduced or potentiated NMDA action can be accompanied by increased neurogenesis.

Stress and GCs also alter vascular dynamics and may exert their effects on neurogenesis by altering endothelial or smooth muscle status. For example, glucorticoids are well known for their ability to downregulate VEGF-receptors as well as decrease the expression of VEGF in models of CNS tumor angiognenesis and vascular cell interaction during angiogenesis [Heiss et al., 1996; Nauck et al., 1998]. Steroids are also potent downregulators of hepatic and serum IGF-1 levels, which could, in turn, reduce the IGF-1 component of neural precursor proliferation [Aberg et al., 2000]. However, not all physiological processes that elevate GCs result in decreased neurogenesis. Exercise, a potent stimulator of neurogenesis, is also accompanied by a pronounced increase in circulating GCs suggesting that the effects of corticosteroids may be entirely context specific.

Learning, Environmental Enrichment, and Physical Exercise

Natural behaviors that relieve depression, such as environmental enrichment and physical exercise, are also known to stimulate neurogenesis. Mice living in an environment rich in toys, edible treats, running wheels, and numerous social cohorts generate more new hippocampal neurons than their normally housed counterparts [Kempermann et al., 1997]. Part of this increase in neurogenesis is likely due to learning since the acquisition of a spatial task during a Morris water maze test alone appears to enhance the retention of newly produced neurons [Gould et al., 1999a]. However, one of the most robust stimulators of neurogenesis is simple physical exercise [van Praag et al., 1999a]. Voluntary running on a running wheel increases the number of newborn neurons in mouse dentate gyrus by more than 60%. Not surprisingly, physical exercise is also one of the most potent natural moderators of depression but physical exercise also increases circulating levels of corticosteroids. The reason why physical exercise eliminates the suppressive effects of GC is unknown but it is attractive to speculate that the sharp increase in circulating growth, trophic, and angiogenic factors after physical exercise alter the stem cell vascular microenvironment and/or its response to corticosteroids.

Seizure, Hypoxia, and Neuronal Injury

In addition to natural moderators of neurogenesis, several injury or hypoxia paradigms are known to stimulate neurogenesis. Seizures induced by kainate stimulate a pathological increase in neurogenesis within the GCL and hilus [Parent et al., 1997; Scharfman et al., 2000]. Not only are neurons added to the GCL, but the stimulus is so robust that new granule neurons are inappropriately added to the hilus. These ectopic GCL neurons generate inappropriate connections and it is thought that abnormal neurogenesis may contribute to ongoing seizure activity in epilepsy [Scharfman et al., 2000].

Hypoxia or stroke stimulates neurogenesis, not only in the hippocampus but in other areas as well. As with seizures, global ischemia stimulates a robust neurogenesis within the hippocampal GCL [Liu et al., 1998]. Focal ischemia within the striatum and overlying cortex also induces neurogenesis in the GCL but new data also show that neurogenesis extends into the affected striatum [Arvidsson et al., 2002]. Interestingly, neurogenesis is not stimulated in the neighboring ischemic cortex. This is quite surprising given the fact that a more subtle injury targeted specifically to layer 3 pyramidal

neurons of the cortex can stimulate limited cortical neurogenesis [Magavi et al., 2000]. A picture of regional specification of precursor response to injury is beginning to emerge and several lines of evidence demonstrate how strikingly different a region's response to injury can be depending on the presence or absence of specific molecules within the local signaling network.

VASCULAR NICHE HYPOTHESIS: A NEW CENTER OF THE UNIVERSE FOR ADULT NEUROGENESIS?

It is overly simplistic to propose the vascular niche is necessary for neurogenesis, but the anatomy does provide insights into the types of signals that may be required to recruit competent cells. It is important to note that correct signaling alone is unlikely to be sufficient to stimulate neurogenesis in all areas. Neurogenesis is regulated in part by the distribution of competent precursors as well as positive and negative local cues that control stem/precursor cell activity and fate. For example, when competent precursors from the SVZ are physically relocated to the striatum, it appears that the striatum naturally provides dominant anti-neurogenic cues mediated through bone morphogen protein (BMP) signaling [Lim et al., 2000]. If one first expresses noggin, a potent BMP antagonist, then the incoming cells can mediate neurogenesis. However, noggin alone is not sufficient to recruit neurogenesis from local precursors. Neurogenesis only occurs following a cell translocation from SVZ to striatum and repression of BMP signaling. Cells of the vasculature also utilize a variety of signaling mechanisms mediated through the TGF-β/BMP superfamily of ligands. Although the array of candidate ligands elaborated during angiogenesis is large, the number of receptors mediating the signaling events are limited [Miyazono et al., 2001]. It seems likely that the vascular elaboration of BMP antagonists is one mechanism that may contribute to a permissive neurogenic niche.

The physiologies known to regulate hippocampal neurogenesis do have potent effects on vascular status. Physical exercise stimulates angiogenesis in the adult brain [Isaacs et al., 1992] in addition to triggering the release of circulating angiogenic factors such IGF-1, FGF-2, VEGF [Breen et al., 1996; Carro et al., 2000]. FGF-2, IGF-1, and VEGF are well known for their angiogenic activity and, not surprisingly, each is able to stimulate neurogenesis when administered exogenously [Wagner et al., 1999; Aberg et al., 2000; Jin et al., 2002]. FGF-2 is well recognized as a potent angiogenic factor as well as a direct mitogen for neural stem cells and FGF-2 activity is enhanced by CCg (glycosylated form of cystatin C), a autocrine/paracrine growth factor known to be expressed by vascular cells of the adult brain [Taupin et al., 2002]. IGF-1 is an autocrine/paracrine factor for the regulation of NSC proliferation [Arsenijevic et al., 2001] and injection of IGF-I mimics exercise induced increase in vascular BDNF expression in the hippocampus [Carro et al., 2000].

Brain injury and ischemia also trigger a strong vascular response. Upregulation of VEGF by Hypoxia-inducible factor 1 (HIF-1) in glia has been proposed as a primary angiogenic mediator in response to ischemia [Forsythe et al., 1996]. The induction of angiogenesis in cerebral hypoxia/ischemia may be a defense mechanism in which VEGF causes increased vascularization and compensates for the decreased oxygen tension in ischemic areas of the brain. As reported, the angiogenetic response is accompanied by neurogenesis in this injury environment [Liu et al., 1998; Arvidsson et al., 2002]. Interestingly, the vascular response to ischemia alone may not be sufficient to stimulate neurogenesis, since focal ischemia involving both cortex and striatum yields only striatal neurogenesis [Arvidsson et al., 2002].

Other hypoxia-induced factors may also play a role in vascular-mediated neurogenic signals. Erythryopoetin (EPO) expression is induced under hypoxic stress and a recent study suggests that EPO functions as an autocrine-paracrine factor for NSCs. Neural stem/precursor cells cultured under hypoxic conditions elaborate EPO and produce two- to threefold more neurons under low-oxygen conditions. EPO receptors are found in the SVZ and infusion of EPO to the lateral ventricles results in a decreased number of NSCs in the SVZ but an increase of neurons in the olfactory bulb suggesting that EPO promotes cell transit from a stem-cell compartment into a committed neuroblast pool [Shingo et al., 2001]. Given the vascular niche of the hippocampal precursor cells, one might predict that EPO is produced within the neuro-angiogenic clusters but this remains to be determined.

Although an attractive model for exploring neurogenic signaling, angiogenesis alone is clearly not sufficient to stimulate neurogenesis. Focal ischemia and brain injury create very strong angiogenic responses yet this response does not activate neurogenesis in all brain regions. An interesting dichotomy comes forth when contrasting global and focal ischemia. Both stimulate angiogenesis and robust GCL neurogenesis (i.e., in a location where both neurogenic niche and competent precursor cells preexist). However, reactive neurogenesis in other damaged areas is exceptionally limited, or absent (i.e., no cortical neurogenesis in focal ischemia and no neurogenesis within hippocampal area CA-1, the primary area of neuron loss following global ischemia). In cases where the natural recruitment process is not "turned on" the task of exogenously translocating cells and producing a permissive environment for brain repair is daunting. However a groundbreaking study by Nakatomi et al. [2002] shows that relatively elemental perturbations in signaling can trigger these complex global processes and lead to startling repair. The simple ventricular infusion of FGF-2 and EGF following global ischemia permits (or instructs) an anatomical and functional reconstruction of CA1. These data turn a new corner in the study of adult neurogenesis and clearly demonstrates that there is regional specificity in the brain's utilization of resident precursors and that this specificity is tightly controlled by the local microenvironment.

As in the birth of any new universe, the Big Bang of adult stem cell biology is coalescing into solar systems of ideas, some of which are bound to collide. The concept of the adult neural stem cell will undoubtedly undergo several revisionist periods as more information is gathered on the phenotypic identity and behavior of cells that mediate de novo cell production in the adult brain. The presence of "self-renewing multipotent stem cells" in vitro is well accepted and the identification of methods or markers useful for enriching cell populations for stem cell-like function is well justified. In fact, legally defensible methods or cell descriptions are a fundamental requirement for the rapid development and commercialization of new therapeutic technologies involving stem cells. However, the assumption that the neural "stem cell" is a single definable entity may not be well justified and the intellectual pursuit of stem cell biology requires an open mind. Though evidence for a direct stem cell role in adult neurogenesis is lacking, careful observation may resolve the issues of stem cell phenotype and function in the adult brain. As our colleagues who vigilantly observe the skies would agree that the absence of data is not proof of absence.

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