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Neural Stem Cells: Progenitors or Panacea?

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Abstract
Are neural stem cells (NSCs) maintained as totipotent precursors by the specialized environment within the stem cell niche or are they simply progenitors, which, while retaining their ability to proliferate, are parcellated and restricted along with their postmitotic brethren? In this review, we focus on what has been learned in recent years about endogenous populations of NSCs in the embryonic and adult brain. We compare the data garnered from in vitro analysis to what has been learned from the transplantation of NSCs into the developing, adult or lesioned brain.

Introduction

The differentiation potential of neural stem cells (NSCs) and their prospective use as therapeutic tools has fascinated the scientific community as well as the general public.

Central to the fervor surrounding NSCs is the question of their multipotency. During normal development, stem cells are thought to become progressively restricted in their differentiation potential. If left unperturbed, these stem cells are thought to generate only the cell types present in the tissue where they reside. While during normal development, embryonic stem (ES) cells in the inner cell mass give rise to the embryo in its entirety, NSCs in the brain appear to produce a limited range of glia and interneurons. Nonetheless, hints exist that adult stem cells from the specialized niches throughout the mature organism can be persuaded to adopt a far broader spectrum of fates [Clarke et al., 2000, Krause et al., 2001]. Despite their in vivo constraints, various investigators have argued that if transplanted, these same populations can contribute to tissues throughout the body.

Neurogenesis to NSCs

The existence of stem cells in the adult was first described in the hematopoietic system [McCulloch et al., 1965]. Until relatively recently, the situation in the blood has been considered the exception rather than the rule. However, convergent data from numerous studies over the past decade have shown that stem cell niches exist in

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the skin, gut and brain [Gordon et al., 1992; Lois and Alvarez-Buylla, 1993; Gage et al., 1995b; Doetsch et al., 1999; Spradling et al., 2001; Alonso and Fuchs, 2003; Machold et al., 2003]. Among these, the discovery of neurogenesis within the brain was the most antithetical, as neurogenesis in the central nervous system (CNS) was considered to be complete shortly after birth [Rakic, 1985].

Despite the recentness of the revelation that neurogenesis occurs in the CNS, considerable work has already been done to describe the specialized germinal regions of the adult brain: the subventricular zone (SVZ) in the ventral forebrain and the subgranular layer in the hippocampus. Furthermore, the developmental progression by which these niches are established is now beginning to be appreciated [Gage, 2000; Alvarez-Buylla et al., 2001; Doetsch, 2003].

To understand how the adult stem cell niches in the brain arise, the earliest events in brain development should be considered. The brain begins as a single layer of pseudostratified neuroepithelial cells surrounding a fluid-filled ventricle. The pseudostratified organization of this germinal zone is maintained throughout development but subsequent to the onset of neurogenesis is referred to as the ventricular zone (VZ). As development proceeds, the VZ gives rise to a second germinal layer immediately above it, the SVZ. The mode of cell division that allows this to occur is notable. Prior to neurogenesis, neuroepithelial cells are thought to divide symmetrically to expand the pool of neuroepithelial cells. With the onset of neurogenesis, the asymmetric division of cells in the VZ gives rise to both the first postmitotic cells, as well as the SVZ [Caviness et al., 1995; Huttner and Brand, 1997]. The onset of asymmetric cell division in the VZ is coincident with the transition of neural progenitors from neuroepithelium to radial glia. Elegant time-lapse studies from the Kriegstein laboratory have shown that radial glia cells in the VZ of the cortex give rise to neurons by dividing asymmetrically [Noctor et al., 2001]. Hence, it would appear that the radial glia, not the neuroepithelial cells are the true embryonic NSCs and are responsible for both neurogenesis as well as the population of the SVZ.

Since the revelation that radial glia are the embryonic NSCs [Noctor et al., 2001], much attention has been given to whether radial glia represent a homogeneous or heterogeneous population. It has been suggested that regional differences exist in the ability of radial glia to give rise to neurons versus glia [Gaiano et al., 2000; Hartfuss et al., 2001; Malatesta et al., 2003]. However, recent data suggest that these regional differences can be attributed to the gradient of neurogenesis in the brain rather than true regional differences [Anthony et al., 2004]. Nonetheless, there do appear to be regional variations in the way radial glia give rise to neurons in pallial versus subpallial regions of the telencephalon. While radial glia in the cortex appear to directly give rise to neurons, in subcortical regions radial glia appear to populate the SVZ, which in turn divide symmetrically to produce two daughter neurons [Malatesta et al., 2003; Noctor et al., 2004]. Beyond broadening our understanding of embryonic neurogenesis, recent work suggests that radial glia are directly lineally related to the adult stem cell populations in the SVZ and hippocampal gyrus [Alvarez-Buylla et al., 2001]. Indeed, the transition of radial glia to the B cell astrocytes that function as the adult NSCs has recently been documented in the SVZ and dentate gyrus [Doetsch et al., 1999; Seri et al., 2001; Doetsch, 2003].

In the adult mammalian brain, while the extent of neurogenesis is quite extensive, the repertoire of cell types generated is limited. From the adult SVZ, a continuous stream of nascent neurons is produced throughout life. These neurons migrate from the SVZ anteriorly to the olfactory bulb, where they develop into interneurons [Alvarez-Buylla and Garcia-Verdugo, 2002]. In the adult hippocampus, neurogenesis occurs in the subgranular zone, a thin layer of cells between the hilus and the granular cell layer of the dentate gyrus. The newly born neurons migrate a short distance to the granule layer and differentiate into granule cells that project into the CA3 region of the hippocampus. Recently, it has been shown that these cells contribute directly to brain circuitry. Van Praag et al. [2002] were able to demonstrate that these cells are capable of functionally integrating into the hippocampus. Evidence has also suggested that neurogenesis in adult rodents is influenced by behavior. For instance, spatial navigation learning, a hippocampus-depending task, enhances neurogenesis in the rat hippocampus, whereas tasks, that do not involve hippocampal learning [Gould et al., 1999] did not change neurogenesis in the hippocampus. Similarly, enriched environments and antidepressants increase neurogenesis in the hippocampus [Kempermann et al., 1997, 1998; Gould et al., 1999; van Praag et al., 1999, 2000; Malberg et al., 2000], while stress, chronic administration of heroin, morphine [Eisch et al., 2000] or alcohol [Gould and Tanapat, 1999; Herrera et al., 2003] decrease neurogenesis in this region. Furthermore, behavioral correlates to neurogenesis have been observed. Specifically, blocking neurogenesis in the hippocampus can abrogate the effects of antidepressants [Santarelli et al., 2003].

Taken together, the case for both neurogenesis in the adult brain and the evidence supporting the idea that newborn cells contribute to adult brain function is quite compelling. Despite this, the range of neurons generated through this process and their integration into brain circuitry appears to be extremely specific. Hence, while the existence of adult NSCs would appear to be irrefutable, the notion that these cells have a more general role in brain maintenance and repair is at present unproven.

What Are NSCs? A Top-Down Approach

The general acceptance that NSCs exist in the CNS came from the demonstration by Reynolds and Weiss [1992] that single cells from the adult brain can be expanded and passaged in the presence of epidermal growth factor (EGF) and fibroblast growth factor (FGF) while remaining multipotent. This approach termed the neurosphere assay has been widely adopted and extensively used by many laboratories. Despite its glaringly unphysiological nature, this assay has proved quite useful for its ability to access the three basic characteristics of NSCs, namely (1) proliferation, (2) self-renewal and (3) multipotency [Reynolds and Weiss, 1992]. The general means to implement this approach begins with the dissection and dissociation of the tissue of interest into a single cell suspension. The resulting cells are then plated at clonal density in the presence of growth factors such as EGF or/and basic FGF (FGF-2) on a nonadhesive environment. After several rounds of cell division, the cells form clusters that are sphere-like in organization. The ability of the cells within these clusters to self-renew can be tested by dissociating these cellular spheres and replating them in the presence of growth factors. The differentiation potential of these cells can be examined by withdrawing the growth factors and presentation of an adhesive substrate, followed by immunostaining of the resulting differentiated cells with markers specific for astrocytes, oligodendrocytes and neurons.

Despite general consensus as to the multipotency of NSCs grown using this assay, comparison of the results generated in different laboratories is not trivial. Although the central elements of the implementation of this approach are similar, there are major differences in the details in the methods used in different laboratories. For instance, some investigators only use FGF in the proliferative phase of the neurosphere assay, whereas others use FGF and EGF, or more complex growth factor cocktails or even serum [Parmar et al., 2003]. With regard to the culture conditions, some researchers have used nonadhesive aggregate cultures (neurosphere cultures) whereas others have grown NSC clonal cultures on an adhesive surface [Williams and Price, 1995; Gotz et al., 1998; Skogh et al., 2001]. Furthermore, different laboratories have utilized a variety of substrates and feeder layers and tissue culture media to promote the differentiation of NSC cultures.

In addition to variations in methodology, the analysis of NSC differentiation has by and large been superficial, often relying on a single marker to characterize specific cell classes. For instance, Tuj1/class III β-tubulin, a marker for newly born postmitotic neurons, does not reveal anything about specific neuronal cell types. Applying greater rigor has not proved straightforward, as even the characterization of NSCs using antibodies against specific neurotransmitters and neuropeptides, while indicating general classes, is insufficient to reveal neuronal subtypes. If future studies are to determine the extent to which in vitro expanded NSCs can be induced to acquire mature neuronal phenotypes, it will require the use of combinations of markers. Even this approach may prove insufficient as full maturation of neurons may require aspects of the in vivo milieu that can only be gauged by transplantation back into the embryo.

Transplantation of NSCs in vivo

As noted above, neural progenitors in the adult brain only give rise to specific neuronal subtypes. Do NSCs expanded in vitro have a broadened developmental potential? To assess this question Jonas Frisen's group [Clarke et al., 2000] analyzed the ability of adult NSCs by introducing them into stage 4 chicken embryos and into mouse blastocysts. Encouragingly, in these experiments NSC-derived cells were found in all 3 germ layers, including substantial contributions to the embryonic nervous system, heart, liver and intestine. Despite the apparent promise of these findings, the success rate of these experiments was very low and the degree of mosaicism varied between embryos. Moreover, the functional integration of NSC-derived cells in these tissues has yet to be demonstrated.

More recently D'Amour and Gage [2003], in an attempt to follow up on the experiments of the Frisen group, compared embryonic NSCs acutely dissected from the embryonic telencephalon with NSCs from the same region but subsequently cultured and expanded in vitro. To add an extra degree of stringency to their experiments,

they made use of the transcription factor Sox2 that is thought be a marker of neural progenitors [Collignon et al., 1996; Li et al., 1998; Zappone et al., 2000]. Acutely dissociated Sox2-expressing embryonic NSCs were injected into blastocysts, as well as taken into culture and subsequently injected into blastocysts. Only the acutely dissociated embryonic NSCs were able to contribute to embryonic tissues. No chimeric animals were obtained when cultured NSCs were injected. Nonetheless given the inefficiency of integration observed in the Frisen experiments, it remains possible that the failure to see integration of Sox2-positive NSCs in the study of D'Amour and Gage [2003] is a result of statistical variation. Alternatively, embryonic and adult NSCs may possess differing potential and thus behave differently when used in the generation of chimeras. That said the idea that adult NSCs expanded in vitro would have a broader potential than embryonic NSCs is quite provocative and inconsistent with the one study that has tested primary adult NSCs directly [Lim et al., 1997].

The more conservative approach of reintroducing in vitro derived NSCs back into the region of the brain they are derived from has proven more robust. The findings from experiments using this approach demonstrate that NSCs transplanted in this manner show a remarkable range of survival, migration and differentiation. Moreover, the specific behavior of the transplanted NSCs depends on their site of engraftment. When transplanted back into neurogenic regions of the neonatal or adult brain (e.g. SVZ and hippocampus), NSCs migrate along the routes usually taken by endogenous neural precursors and differentiate into area-appropriate neurons and glia cells [Sabate et al., 1995; Fricker et al., 1999; Rosser et al., 2000; Uchida et al., 2000; Englund et al., 2002c; Jain et al., 2003; Parmar et al., 2003]. For instance, NSCs obtained from the adult hippocampus or the SVZ can be expanded in vitro and transplanted back into the brain. Adult hippocampal stem cells give rise to neurons and astroglia cells when transplanted back into the hippocampus. However, when transplanted heterotopically into another neurogenic region (i.e. the rostral migratory stream) their behavior adapts appropriately to this new environment. Remarkably, in either case, the newly integrated cells are indistinguishable from the surrounding host tissue [Gage et al., 1995a; Suhonen et al., 1996].

By contrast with the transplantation of adult NSCs, only very few studies have transplanted embryonic NSCs back into the developing brain [Arnhold et al., 2000; Rosser et al., 2000]. Nonetheless, what has been done in this regard shows considerable promise. For instance, Auerbach et al. [2000] dissected NSCs from the developing rat cortex. They expanded and labeled the cells in vitro and injected them unilaterally into the developing hippocampus. Postnatal analysis of the brains of the injected animals showed that the transplanted cells integrate into the host hippocampus and received functional synaptic input from host neurons. When electrophysiologically stimulated, the transplanted cells possess endogenous neuronal currents, although their response displays features of more immature cells compared to the host tissue.

Embryonic Stem Cells: The Bottom-Up Approach

Although much has been written concerning the potential of both embryonic and adult NSCs (table 1), only in the case of ES cells has their totipotency been rigorously tested through germline transmission [Evans and Kaufman, 1981; Martin, 1981]. ES cell lines are derived from pluripotent cells in the early embryonic cell mass [Rossant, 2001]. The therapeutic use of ES cells as a means of cell therapy has attracted much public and political attention. Similarly, scientists have been examining the ability of ES cells to contribute to the nervous system for almost a decade. In the earliest studies, embryoid bodies [Yanai et al., 1995; Benninger et al., 2000] were transplanted into the adult rodent striatum. In the study by Yanai et al. [1995], the transplanted embryoid bodies formed fast-growing teratomas within the host brain. Moreover, Yanai and colleagues did not find any graft-derived cells in the host brain. By contrast, Benninger et al. [2000] did not report the formation of any tumors. Instead they found neurons, astrocytes and oligodendrocytes within the graft of the transplanted cells. The differences of the outcome in these studies are perhaps explained by the very different in vitro protocols to obtain embryoid bodies and ES-cell-derived NSCs as well as by the use of different mouse ES cell lines.

More consistent results have been generated when the ES cells were first pushed to a neuronal fate. Investigators using mouse ES cells showed that after being pushed to an NSC fate in vitro the transplanted cells were able to form Thy-1-expressing neurons, as well as astrocytes [Arnhold et al., 2000]. Parallel efforts by two independent groups to test the fate of human ES cells have led to similar results [Reubinoff et al., 2001; Zhang et al., 2001]. One however should take note that so-called human ES stem cells, while derived from the inner cell mass, have never had their totipotency validated by germline transmission for obvious ethical reasons.

Table 1. In vitro and in vivo potential of stem cells in the CNS

Source	In vitro expansion/factors	In vitro differentiation	Host region and age	In vivo differentiation	Reference
ES cells					
Mouse embryos, embryoid bodies, ES cells		n.a.	Adult mouse brain	Teratoma formation	Yanai et al. [1995]
Mouse embryoid bodies	Fibroblast feeder layer, LIF, later serum or synthetic serum	n.a.	Adult mouse striatum	Neurons (GAP43+, MAP2+), astrocytes, oligodendrocytes	Benninger et al. [2000]
Mouse ES cells	Recapitulation of in vivo induction	Motor neurons	Embryonic chicken spinal cord	Motor neurons	Wichterle et al. [2002]
Mouse ES cell line	n.a.	n.a.	Embryonic rats, telencephalon	Neurons (Map2+), astrocytes, oligodendrocytes	Brustle et al. [1997]
Mouse ES cell line	4–/4+ protocol	n.a.	Adult rat: spinal cord lesion	Neurons (Thy-1+, NeuN+), astrocytes, oligodendrocytes	McDonald et al. [1999]
Mouse ES cell line	LIF, 15% fetal bovine serum, astrocyte conditioned medium	Neurons, astrocytes, oligodendrocytes n.a.	Adult rat striatum	Neurons (Thy-1+) and astrocytes	Arnhold et al. [2000]
Mouse ES cell line	LIF, 15% fetal bovine serum, astrocyte conditioned medium	Neurons, oligodendrocytes, astrocytes	Adult rat striatum	Neurons (Thy-1+, MAP2+), astrocytes, oligodendrocytes n.a.	Andressen et al. [2001]
Mouse ES cell line	LIF	n.a.	6-OHDA-lesioned adult rat striatum	neurons (TH+, NeuN+, DAT), astrocytes, oligodendrocytes n.a.: teratoma formation	Bjorklund et al. [2002]
Mouse ES cell line	Multistep protocol to generate TH+ neurons	TH+ neurons, astrocytes and oligodendrocytes n.a.	6-OHDA-lesioned adult rat striatum	Neurons (TH+, calbindin), astrocytes and oligodendrocytes n.a.	Kim et al. [2002]
Human ES cell line	Fibroblast feeder layer; later EGF, FGF	Neurons, oligodendrocytes, astrocytes	Neonatal mice, ventricle	Neurons (neurofilament+; mature neuronal marker n.a., astrocytes, oligodendrocytes	Reubinoff et al. [2001]
Human ES cell line	Fibroblast feeder layer; later FGF	Neurons, oligodendrocytes, astrocytes	Neonatal mice, ventricle	Neurons (Tuj1+, MAP2+), astrocytes, oligodendrocytes n.a.	Zhang et al. [2001]
Embryonic NSCs					
E12.5 rat ventral mesencephalon	FGF	Neurons, astrocytes	6-OHDA-lesioned adult rat striatum	Neurons (TH+), astrocytes and oligodendrocytes n.a.	Studer et al. [1998]
E12.5 rat ventral mesencephalon	EGF, FGF	n.a.	6-OHDA-lesioned adult rat striatum	Neurons (TH+), astrocytes, oligodendrocytes n.a.	Nishino et al. [2000]
E11–12 mouse/E13–14 rat ventral mesencephalon	FGF	Neurons, oligodendrocytes, astrocytes	6-OHDA-lesioned adult rat striatum	Neurons (TH+), astrocytes and oligodendrocytes n.a.	Sawamoto et al. [2001a]
E14.5 rat cortex	FGF	Neurons, oligodendrocytes, astrocytes	Adult rat: spinal cord lesion	Astrocytes, oligodendrocytes, no neurons	Cao et al. [2001]
E14.5 mouse ganglionic eminences, cortex, ventral mesencephalon	EGF	Neurons, oligodendrocytes, astrocytes	Embryonic rat, ventricle	Astrocytes, no neurons or oligodendrocytes	Winkler et al. [1998]
E14.5 rat cortex	FGF	n.a.	Embryonic rat hippocampus	Neurons (electrophysiology, NeuN+, Hu+), astrocytes, oligodendrocytes n.a.	Auerbach et al. [2000]
E14.5 rat spinal cord	EGF, FGF	Neurons, oligodendrocytes, astrocytes	Adult rat: spinal cord lesion	Astrocytes, oligodendrocytes, neurons only in BDNF-treated lesions	Chow et al. [2000]
E14.5 rat spinal cord	FGF	n.a.	Adult rat: spinal cord lesion	Neurons (Hu+), astrocytes and oligodendrocytes n.a.	Ogawa et al. [2002]
E16 rat striatum, mesencephalon	FGF	Neurons, astrocytes, oligodendrocytes	6-OHDA-lesioned adult rat	Neurons (TH+), astrocytes and oligodendrocytes n.a.	Svendsen et al. [1996]

Table 1 (continued)

Source	In vitro expansion/factors	In vitro differentiation	Host region and age	In vivo differentiation	Reference
Fetal human cortex	FGF	Neurons, glia	Adult rat striatum	Neurons (Tuj-1, NSE, MAP5), no astrocytes and oligodendrocytes	Sabate et al. [1995]
Fetal human mesencephalon	EGF	Neurons , astrocytes, oligodendrocytes	6-OHDA-lesioned adult rat striatum	Neurons (TH+), astrocytes and oligodendrocytes n.a.	Svendsen et al. [1996]
Fetal human brain	EGF, FGF	Neurons , astrocytes, oligodendrocytes	6-OHDA-lesioned adult rat striatum	Neurons (Tau+, TH+), astrocytes, oligodendrocytes n.a.	Svendsen et al. [1997]
Fetal human brain		Neurons, oligodendrocytes, astrocytes	Embryonic rats, telencephalon	Neurons (NF+), astrocytes and oligodendrocytes	Brustle et al. [1998]
Fetal human forebrain		Neurons, oligodendrocytes and astrocytes	Adult rat: Hippocampus SVZ/RMS Striatum	 Neurons (Hu, NeuN, Tuj1, calbindin) and astrocytes Neurons (Hu, NeuN, TH, GAD65, Tau) Neurons (Hu, GAD65, calbindin, DARPP32) and astrocytes	Fricker et al. [1999]
Fetal human diencephalon	EGF, FGF	Neurons, oligodendrocytes and astrocytes	6-OHDA-lesioned adult rat striatum	Neurons (NF+, Tuj1+), astrocytes, no oligodendrocytes	Vescovi et al. [1999]
Fetal human brain	EFG, FGF	Neurons , astrocytes, oligodendrocytes	Neonatal rat: Striatum Hippocampus RMS	 Neuronal morphologies, mature neuronal marker n.a., oligodendrocytes n.a. Neuronal morphologies, no overlap with mature neuronal marker, astrocytes, oligodendrocytes n.a. Neurons	Rosser et al. [2000]
Fetal human brain	EGF, FGF, LIF, NSF-1	Neurons, astrocytes, oligodendrocytes n.a.	Neonatal mice, ventricle SVZ Hippocampus Striatum	 Neurons (Tuj1+, NCAM+, few TH+), few astrocytes, oligodendrocytes n.a. Neurons (Tuj1+), astrocytes and oligodendrocytes n.a. Neurons and astrocytes	Uchida et al. [2000]
Fetal human mesencephalon and cortex		Neurons, astrocytes and oligodendrocytes n.a.	6-OHDA-lesioned adult rat striatum	Neurons (TH+), astrocytes and oligodendrocytes n.a.	Sanchez-Pernaute et al. [2001]
Fetal human brainstem cell line	EGF, FGF, LIF	Neurons, astrocytes, oligodendrocytes	Adult rat: Hippocampus Striatum SVZ RMS	 Tau+/DCX+ neurons (no overlap of mature neuronal markers); astrocytes Tau+/Thy-1+ neurons (no overlap of mature neuronal markers); astrocytes; oligodendrocytes n.a. DCX+ neurons Tau+, DCX+ neurons	Englund et al. [2002a]
Fetal human forebrain stem cells	EGF, FGF, LIF	Neurons, astrocytes, oligodendrocytes	Neonatal rat: Striatum Hippocampus SVZ	 Tau+ neurons, astrocytes, oligodendroglial morphology, but no colabel with NG2, CNPase or RIP Tau+, Thy-1 + neurons, astrocytes Tau+ neurons	Englund et al. [2002c]

Table 1 (continued)

Source	In vitro expansion/factors	In vitro differentiation	Host region and age	In vivo differentiation	Reference
Fetal human brain stem cell line	10% FBS	Neurons	Neonatal rat: Cortex Hippocampus	 Cortical pyramidal neurons, interneuron-like morphology, astrocytes, oligodendrocyte morphology Hippocampal pyramidal neurons, astrocytes, oligodendrocytes	Englund et al. [2002b]
Fetal human cerebral cortex	EGF, FGF	n.a.	Adult rat: Striatum Hippocampus	 Tau+ neurons with immature morphology, no overlap with mature neuronal marker, astrocytes, oligodendrocytes n.a. Tau+ neurons with immature morphology, no overlap with mature neuronal marker, astrocytes, oligodendrocytes n.a.	Jain et al. [2003]
Fetal human lateral ganglionic eminence	10% FCS, EGF, FGF	Neurons, astrocytes, oligodendrocytes	Neonatal rat: Striatum SVZ	 Tau+ neurons with immature morphology (no overlap with DARPP-32) DCX+ neurons, astrocytes	Parmar et al. [2003]
Adult NSCs					
Adult rat hippocampus	10% FCS, EGF, FGF	Neurons, astrocytes, oligodendrocytes	Adult rat hippocampus	Neurons (granule cell morphology), astrocytes	Gage et al. [1995a]
Adult rat hippocampus	10% FCS, EGF, FGF	Neurons, astrocytes, oligodendrocytes	Adult rat: Hippocampus RMS Cerebellum	 Neurons, astrocytes, oligodendrocytes n.a. Olfactory neurons, astrocytes, oligodendrocytes n.a. Astrocytes, no neurons, oligodendrocytes n.a.	Suhonen et al. [1996]
Adult rat spinal cord	FGF	Neurons, astrocytes, oligodendrocytes	Adult rat: Hippocampus Adult spinal cord	 Neurons (NeuN, calbindin), astrocytes, oligodendrocytes Astrocytes, oligodendrocytes, no neurons	Shihabuddin et al. [2000]
Adult rat spinal cord	FGF	Neurons, astrocytes, oligodendrocytes	Adult rat: spinal cord lesion	Astrocytes, oligodendrocytes, no neurons	Vroemen et al. [2003]

n.a. = Not assessed; DCX = doublecortin; E12.5 = embryonic day 12.5; LIF = leukemia inhibitory factor; NF = neurofilament.

The use of ES cells as a source for NSCs has been more recently refined by using transgenic mice having a dominant endogenous reporter for neural progenitors. Making use of the expression of nestin as a marker for neural precursor cells, Andressen et al. [2001] used ES cells from mice expressing enhanced green fluorescent protein (EGFP) under the nestin promoter. Culturing the ES cells under conditions promoting neural precursor cells induced the expression of nestin-EGFP. Transplantation of these cells into the adult rat brain revealed that the cells could develop into neurons and astrocytes, but this study did not examine if the efficiency or repertoire of neuronal cell types generated from ES cells was expanded.

Recent efforts to direct the fate of ES cells to specific neural identities have begun to take a rational approach based on our understanding of the developmental events that lead to the generation of the nervous system. For instance, van der Kooy and colleagues have shown that blocking bone morphogenic protein (BMP) signaling can greatly increase the efficiency in which ES cells can be promoted to adopt NSC character [Tropepe et al., 2001]. Most impressively, work from the Jessell group has taken

this to the next logical step and directed ES cells to a motor neuron identity by the sequential application of the factors known to be responsible for this developmental progression in vivo. In dramatic fashion, these cells when transplanted into the chicken populate the spinal cord, project to the limb and form synapses with the muscles [Wichterle et al., 2002]. Given increasing data suggesting that NSC potential reflects their region of origin perhaps the bottom-up approach may yield greater promise as a wide-ranging therapeutic approach.

Using NSCs for Repairing the CNS

Mouse models of Parkinson's disease, which results in the specific loss of dopaminergic cells in the substantia nigra (SN), have proved a fertile area to explore the utility of NSCs for cell replacement therapies. In this regard, solid grafts of fetal midbrain into Parkinson's disease patients have in many cases resulted in a significant improvement in clinical performance. However, ethical issues, as well as the limited availability of fetal tissue have prompted calls for alternative approaches, most notably stem cell replacement therapies.

A number of studies have examined the potential of undifferentiated NSCs derived from the embryonic mesencephalon and subsequently back transplanted into the substantia nigra of mice used as an animal model of Parkinson's disease. These cells were observed to undergo glial and neuronal differentiation [Svendsen et al., 1996, 1997; Nishino et al., 2000], but only few expressed tyrosine hydroxylase (TH) and then only at low levels (TH is an enzyme required for the generation of dopamine). To improve on these results, some investigators have attempted to bias the NSCs to a dopaminergic phenotype through predifferentiation in vitro prior to transplantation. Although the methods to achieve this have been empirical rather than rational, these attempts have yielded more promising results. In these studies, a significant behavioral improvement of the symptoms was observed. Importantly, improvement only occurred when the SN was properly targeted and in cases where robust TH expression was observed in the grafted cells [Studer et al., 1998; Sanchez-Pernaute et al., 2001]. Thus, both deriving NSCs from the appropriate neuronal population as well as targeting the transplantation of these cells to the desired location in the CNS are key parameters to consider when implementing this approach [Studer et al., 1998; Sanchez-Pernaute et al., 2001; Sawamoto et al., 2001a, b].

While the results using mesencephalon-derived NSCs are encouraging, the constraint this places on the availability of appropriate precursors has prompted a number of researchers to use the bottom-up approach of beginning with ES cells. Using a variation of the rational methodologies described above [Wichterle et al., 2002], Lee et al. [2000] have successfully generated dopaminergic cells in vitro, using combinations of FGF and sonic hedgehog (Shh), which during normal development are thought to combinatorially mediate the induction of dopaminergic cells [Ye et al., 1998]. Upon grafting these cells into a Parkinson animal model, they observed that the electrophysiological properties of the grafted TH-positive neurons were similar to those of host mesencephalic dopaminergic neurons. More importantly they were able to demonstrate that the grafted cells formed functional synapses and animals receiving these grafts showed improved gait compared to mock-treated control animals [Kim et al., 2002]. Taken together, this study suggested great promise for the engineering of replacement neurons from ES cells.

NSCs have also shown considerable potential for the treatment of CNS injury. Traumatic spinal cord injuries resulting from mechanical damage show a characteristic progressive pathophysiology. Inflammation follows the primary insult, which in turn results in a secondary loss of nervous tissue. This is ultimately followed by the formation of a glial scar at the injury site, that in itself is thought to prevent the regeneration of axons [for a review, see Okano et al., 2003]. Several reports have shown that endogenous NSCs exist in the adult spinal cord, but after injury these appear to exclusively differentiate into astrocytes [Johansson et al., 1999; Namiki and Tator, 1999; Horner et al., 2000]. Transplantation studies of stem cells into the injured spinal cord have yielded varied results. Whereas in some studies the transplanted cells only differentiated into astrocytes [Chow et al., 2000; Shihabuddin et al., 2000; Cao et al., 2001], other studies have observed transplanted cells integrating into the injury site and becoming neurons [McDonald et al., 1999; Han et al., 2002; Ogawa et al., 2002; Vroemen et al., 2003].

Notably the therapeutic benefits derived from NSC transplants appear to be attributable more to their trophic influences than their direct contribution to the rewiring of the spinal cord. Furthermore, the timing of the NSC transplantation after injury seems to be the key to a successful outcome. If transplanted too early, during the inflammatory phase, only a small number of cells survive. If transplanted too late, the glial scar starts to form, inhibiting axonal regrowth. The optimal time point for the transplantation in the rat seems to be between 7–14 days

after the injury [McDonald et al., 1999; Ogawa et al., 2002; Okano et al., 2003]. In the cases of successful formation of neurons, behavioral tests also showed improved locomotor functions. Furthermore, this recovery appears to be attributable to remyelination of axons at the injury site, rather than de novo growth of projections across the region of injury [McDonald et al., 1999; Ogawa et al., 2002].

Future Perspectives

The implications of what has been learned from the study of NSCs are still being sorted out. Certainly many of the discoveries of recent years would not have been anticipated. In particular the observation that NSCs can in vitro be both expanded in number and directed to specific lineages would seem to hold great promise for the development of cell replacement therapies. That these cells upon transplantation into the developing and adult CNS can integrate and form function circuits is impressive. Moreover, the therapeutic benefits of NSCs for ameliorating damage to the CNS are far from fully explored. Whether the widely touted promise of NSCs will live up to reality is still uncertain.

Examination of the potential of NSCs and ES cells suggests that investigation along two distinct lines may be profitable. The fact that NSCs exist within the nervous system throughout life would seem to bode well for the notion that the adult CNS possesses the intrinsic ability for self-repair. Despite this the potential of these populations has been better demonstrated in vitro rather than in vivo. If the differentiation potential of NSCs seen in vitro can be replicated in vivo, we may discover that the ability of the CNS to correct damage resulting from disease or injury when properly stimulated is considerable. However, the notion that adult NSCs can be used generically for generating all neuronal cell types appears naïve, as these populations appear to have the same regional restrictions as the postmitotic cells they give rise to. If one's purpose is to generate specific neural populations in large numbers for cell replacement therapy, the rational approach of selectively directing the fate of ES cells would appear to be more hopeful. Notably the use of such populations appears to hold promise in equal measure for neuronal cell replacement and for the trophic benefits that NSCs appear to possess for reversing the damage done during acute or chronic insult to the nervous system.

Many questions remain concerning NSCs. Where exactly are NSCs located in the brain? What distinguishes a stem cell from the surrounding tissue and from more restricted progenitor populations? What are the factors that keep stem cells quiescent and protect them from differentiation within the brain? Do adult stem cells derive directly from their embryonic ancestors? Giving the excitement about NSCs and the promises of therapeutic success, we can hope to have answers to these questions in the near future.

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