Neural stem cells: involvement in adult neurogenesis and CNS repair

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Recent advances in stem cell research, including the selective expansion of neural stem cells (NSCs) *in vitro*, the induction of particular neural cells from embryonic stem cells *in vitro*, the identification of NSCs or NSC-like cells in the adult brain and the detection of neurogenesis in the adult brain (adult neurogenesis), have laid the groundwork for the development of novel therapies aimed at inducing regeneration in the damaged central nervous system (CNS). There are two major strategies for inducing regeneration in the damaged CNS: (i) activation of the endogenous regenerative capacity and (ii) cell transplantation therapy. In this review, we summarize the recent findings from our group and others on NSCs, with respect to their role in insult-induced neurogenesis (activation of adult NSCs, proliferation of transit-amplifying cells, migration of neuroblasts and survival and maturation of the newborn neurons), and implications for therapeutic interventions, together with tactics for using cell transplantation therapy to treat the damaged CNS.

Keywords: neural stem cell; adult neurogenesis; niche; rostral migratory stream; reactive astrocytes; Musashi-1

1. INTRODUCTION

It has long been believed that the adult mammalian central nervous system (CNS) does not regenerate after injury (Ramón y Cajal 1928). However, recent progress in stem cell biology has provided the hope that regeneration of the injured CNS might be achieved. CNS ‘regeneration’ includes the (i) regeneration of neuronal axons, (ii) replenishment of lost neural cells, and (iii) recovery of neural functions (Okano 2003). The breakthrough of CNS regeneration will require the recapitulation of at least some aspects of the normal CNS developmental process, which is initiated by the induction of neural stem cell (NSCs) or NSC-like cells (Goodman & Doe 1993; Alvarez-Buylla *et al.* 2001; Okano 2006). This is the basic rationale behind the strategy of using NSCs for regeneration.

The NSCs are somatic stem cells present within the CNS that have both multilineage potency and self-renewal capacity (McKay 1997; Gage 2000; van der Kooy & Weiss 2000; Alvarez-Buylla *et al.* 2001; Okano 2002a,b). Recently, there has been rapid progress in the stem cell biology of the CNS, due to development of the basic technology for studying NSCs, including identification of their selective marker molecules (e.g. Musashi-1 (Sakakibara *et al.* 1996; Sakakibara & Okano 1997; Kaneko *et al.* 2000; Okano *et al.* 2002, 2005), nestin (Hockfield & McKay 1985; Lendahl *et al.* 1990) and Sox proteins (Pevny *et al.* 1998; Wood & Episkopou 1999)), development of selective culture methods (clonal (Qian *et al.* 2000) and high-density (Palmer *et al.* 1999) monolayer cultures and

neurosphere cultures (Reynolds & Weiss 1992)), and their prospective identification and isolation using cell-surface antigens (Uchida *et al.* 2000; Rietze *et al.* 2001) or green fluorescent protein (GFP) reporters (Roy *et al.* 2000a,b; Kawaguchi *et al.* 2001; Keyoung *et al.* 2001). Using these new techniques, we were able to identify and isolate NSCs or NSC-like cells prospectively from the adult human brain, in collaboration with Dr Steve Goldman’s group in the USA (Pincus *et al.* 1998; Roy *et al.* 2000a,b). The results indicated that the adult human brain contains NSCs or NSC-like cells in the periventricular area. Based on this finding, we have proposed two major strategies for inducing regeneration of the damaged CNS using NSCs: (i) activation of the endogenous regenerative capacity and (ii) cell replacement therapy, including the transplantation of NSCs (Okano 2006).

2. ADULT NEUROGENESIS

The first strategy for regenerating the damaged CNS, i.e. activation of the endogenous regenerative capacity, is tightly associated with a phenomenon called ‘adult neurogenesis’ (reviewed by Sohur *et al.* 2006). There is increasing evidence that neuronal cells are continuously produced in the adult mammalian brain under physiologic conditions, in the so-called adult neurogenic regions. The neurogenic regions in the adult mammalian brain are the (i) subventricular zone (SVZ) facing the lateral ventricles, which produces the olfactory interneurons and (ii) hippocampal dentate gyrus (DG; reviewed by Temple & Alvarez-Buylla 1999; Kempermann 2006). The adult neurogenesis that takes place in both these regions is likely to have various physiologically significant roles (Kempermann & Gage 1999; Kempermann 2006).

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Figure 1. Neurogenesis in the adult subventricular zone (SVZ). (a) The SVZ is located in the lateral wall of the lateral ventricle. (b) The SVZ contains endymal cells (dark grey), astrocytes (blue), transit-amplifying cells (light grey) and young neurons (red). (c) Young neurons generated in the SVZ migrate towards the olfactory bulb. (d) In the SVZ, GFAP-positive astrocytes (type B cells) act as neural stem cells that generate transit-amplifying cells (type C cells) and then differentiate into young neurons (type A cells). Modified from Alvarez-Buylla & Garcia-Verdugo (2002).

Doetsch *et al.* (1997) observed that there are specific cell types in the SVZ of the lateral ventricles that could be distinguished based on their morphology; they classified these cells as types A, B and C. Subsets of slowly dividing glial fibrillary acidic protein (GFAP)-positive cells that act as stem cells (Doetsch *et al.* 1999) were defined morphologically as type B cells. These stem cells (type B cells) give rise to rapidly proliferating precursor cells, called transit-amplifying cells, which are highly sensitive to AraC treatment and are defined morphologically as type C cells (Doetsch *et al.* 1997). Subsequently, the transit-amplifying cells generate migrating neuroblasts, which are defined morphologically as type A cells. Newly generated neuroblasts migrate from the SVZ of the lateral ventricle to the olfactory bulb (OB; figure 1a–d).

The second adult neurogenic site is the adult hippocampal DG, where the precursor cells reside in the subgranular zone (SGZ), the border between the granule cell layer and the hilus (or CA4; reviewed by Sohur *et al.* 2006). Maintenance of the neurogenic character of the hippocampal DG in adulthood is likely to involve more than a single class of niches, analogous to the fact that haematopoietic stem cells (HSCs) have osteoblastic and vascular niches within the adult bone marrow (reviewed by Suda *et al.* 2005). The SGZ of the hippocampal DG is highly vascularized and a proximal spatial relationship between the blood vessels and the dividing precursor cells has been noted (Palmer *et al.* 2000). Palmer *et al.* found that most newborn endothelial cells disappear over several weeks, suggesting that neurogenesis is intimately associated with active vascular recruitment and the subsequent remodelling. From these observations, this group proposed that adult neurogenesis occurs within a 'vascular niche' in the SGZ. Further study characterized the nature of the vascular niche (Shen *et al.* 2004). Shen *et al.* showed that the endothelial cells but not the vascular smooth muscle cells released soluble

factors that stimulated the self-renewal of NSCs, inhibited their differentiation and enhanced their neuron production, suggesting that endothelial cells are a critical component of the NSC niche.

In addition to the vascular niche, astrocytes in the hippocampus were shown to supply local environmental factors that regulate neurogenesis by instructing the stem cells to adopt a neuronal fate (Song *et al.* 2002); thus, there is also an 'astrocytic niche'. This conclusion was based on the experiments in which adult hippocampus-derived NSCs were co-cultured with hippocampal astrocytes as feeder cells; the results indicated that secreted or membrane factors derived from the hippocampal astrocytes were responsible for the observed niche activity. Interestingly, Song *et al.* (2002) showed that, in contrast to hippocampal astrocytes, astrocytes from the adult spinal cord, a non-neurogenic region, are ineffective in promoting neurogenesis from adult stem cells, suggesting that the hippocampal astrocytes specifically provide a unique niche for adult neurogenesis. Recently, Lie *et al.* (2005) showed that the Wnt3A/ β -catenin pathway participates in the induction of the neuronal differentiation of adult hippocampal NSCs by factors derived from hippocampal astrocytes. In the cellular architecture of the hippocampal SGZ, three different types of proliferative cells have been distinguished based on their morphologies, marker expression profiles and electrophysiological properties (Fukuda *et al.* 2003; Kempermann *et al.* 2004, reviewed by Kempermann 2006; Sohur *et al.* 2006): (i) 'type 1 cells' (radial glia-like stem cells), (ii) nestin-expressing 'type 2 cells' (transiently amplifying progenitor cells), and (iii) doublecortin (DCX)-positive, nestin-negative 'type 3 cells'. Cells that are rarely dividing and have a radial glia-like appearance (type 1 cells) have been identified as the predominant precursors in this region (Seri *et al.* 2001). Based on their morphology, these cells have also been termed as 'vertical astrocytes' and can be

distinguished from astrocytes forming the astrocytic niche that are sometimes called 'horizontal astrocytes'. To explore the regulatory mechanisms of the proliferation and differentiation of each of these cell types at the hippocampal SGZ, Kempermann *et al.* (2006) took advantage of the fact that adult hippocampal neurogenesis is highly variable and heritable among laboratory strains of mice, and they performed a systematic quantitative analysis of adult hippocampal neurogenesis in two large genetic reference panels of recombinant inbred strains. They revealed a set of 190 genes with expression patterns that were highly variable among strains and that covaried with (i) the rate of cell proliferation, (ii) the rate of cell survival, (iii) the number of surviving new neurons, or (iv) the number of surviving astrocytes. Through these genome informatics analyses, they identified two candidate genes for controlling neurogenesis. These genes encode a neural RNA-binding protein, Musashi-1 (Okano *et al.* 2002, 2005), and a cell-surface antigen, *prominin1/CD133* (Kosodo *et al.* 2004), both of which play a major role in the control of stem cell maintenance (self renewal) and asymmetric stem cell divisions. Musashi-1 is expressed continuously in neural stem/precursor cells from the embryonic (Sakakibara *et al.* 1996) to adult stage (Sakakibara & Okano 1997) and belongs to a family of evolutionarily conserved RNA-binding proteins (reviewed by Okano *et al.* 2002, 2005). We found that Musashi-1 is required for the stem cell properties of NSCs probably or most likely by augmenting Notch signalling through the translational repression of *m-Numb* mRNA (Imai *et al.* 2001; Sakakibara *et al.* 2002). It would be interesting to examine whether a similar action of Musashi-1 is involved in the regulation of adult hippocampal neurogenesis. On the other hand, prominin-1/CD133 is a pentaspan membrane glycoprotein expressed on apical plasma membrane of many somatic stem cells including NSCs and is involved in defining the constituents of a specific plasma membrane microdomain. Through this mechanism, prominin-1/CD133 potentially contributes to the balance of symmetric versus asymmetric division of embryonic NSCs (Marzesco *et al.* 2005; Dubreuil *et al.* 2007).

3. INSULT-INDUCED NEUROGENESIS

NSCs or NSC-like cells have been identified in the adult mammalian CNS along with the entire neuroaxis from the forebrain to the spinal cord (Temple & Alvarez-Buylla 1999). The two sites described above are now well-known adult neurogenic regions that show continued neuronal production into adulthood. However, despite the presence of endogenous NSCs, most parts of the adult mammalian CNS are non-neurogenic under physiologic conditions (Temple & Alvarez-Buylla 1999; Okano 2006). For example, the striatum and cerebral cortex, which are major targets of brain ischaemia, do not show any detectable adult neurogenesis under physiologic conditions. However, recent studies have shown that some insults, including ischaemia, can induce neurogenesis in these non-neurogenic regions (Arvidsson *et al.* 2002; Nakatomi *et al.* 2002; Tonchev *et al.* 2003, 2005). Such

neurogenesis is induced by pathogenic conditions and is called 'insult-induced neurogenesis', indicating that some regenerative capacity is preserved in the adult mammalian brain, contradicting Cajal's dogma.

However, although insult-induced neurogenesis is detected, this regenerative capacity is very low. The potential problems associated with insult-induced neurogenesis as a therapeutic target are (Okano 2006) the following: (i) the efficiency is low, (ii) the newly generated neurons in response to CNS insult are mostly short-lived, possibly due to the absence of the formation of any functional synapses, (iii) in most cases, insult-induced neurogenesis *per se* is not sufficient for functional recovery of the neural deficits caused by the insults, and (iv) in parts of the adult CNS, even insult-induced neurogenesis is not always detectable.

How can we overcome these difficulties? Insult-induced neurogenesis follows a specific sequence of events: (i) the activation of adult NSCs, (ii) the proliferation of transit-amplifying cells and migration of neuroblasts, and (iii) the survival and maturation of the newborn neurons (Lindvall *et al.* 2004). These results are closely related to the normal process of CNS development and to the activities associated with the adult neurogenic sites. Thus, to improve the efficiency of insult-induced neurogenesis, it is essential to understand the mechanisms underlying the above-mentioned sequence of events. We have proposed several approaches to addressing these questions, some of which we introduce below.

4. ACTIVATION OF NEURAL STEM CELLS

A common stem cell property is cell-cycle quiescence (reviewed by Suda *et al.* 2005), which has critical biologic importance in protecting the stem cell compartment (Cheng *et al.* 2000). Adult NSCs in the SVZ at the lateral ventricle of the forebrain (Morshead *et al.* 1994; Doetsch *et al.* 1997, 1999) are—like other adult somatic stem cells, including HSCs (Arai *et al.* 2004; Suda *et al.* 2005), intestinal stem cells (Potten & Loeffler 1990; Potten *et al.* 2003), melanocyte stem cells (Osawa *et al.* 2005) and stem cells for the epidermis and hair in the skin (Taylor *et al.* 2000)—relatively quiescent. Various cellular stresses, including irradiation, oxidative stress and treatment with anti-cancer drugs, can induce these quiescent stem cells to enter the cell cycle and proliferate (Potten *et al.* 2003; Suda *et al.* 2005). In a similar way, various insults can stimulate the proliferation of endogenous stem cells and/or progenitors in the CNS, either in known neurogenic sites (Gould & Tanapat 1997; Liu *et al.* 1998; Yagita *et al.* 2001, 2002) or in non-neurogenic regions (Johansson *et al.* 1999; Magavi *et al.* 2000; Yamamoto *et al.* 2001; Nakatomi *et al.* 2002; Tonchev *et al.* 2003, 2005).

We have been investigating the mechanisms of activation of NSCs upon insult, using several strategies, including (i) *Drosophila* genetics to identify genes involved in the maintenance and proliferation of larval neuroblasts (Toriya *et al.* 2006), (ii) proteomics approaches to identify factors involved in the activation and proliferation of NSCs and (iii) candidate gene approaches to characterize the function of the neural

Figure 2. Expression pattern of EGFR in the SVZ after MCAO. (a) Anti-EGFR (green) and Hoechst (blue) staining of coronal sections of the SVZ. The number of EGFR-positive cells in the SVZ on day 7 after the induction of ischaemia (right) is larger than that in the intact brain (left). (b,c) Higher magnification images of the cells in the SVZ on day 7 after the induction of ischaemia. The cells were stained for (b) EGFR (green) and Mash1 (a marker for transit-amplifying cells; red), or (c) DCX (a marker for young neurons; red). Most of the EGFR-positive cells in the SVZ are Mash1-positive (arrowheads in (b)) but DCX-negative (c). Scale bar, 20 μ m. Modified from Ninomiya *et al.* (2006).

RNA-binding protein Musashi-1 (Okano *et al.* 2002, 2005; Okano 2006). Because the approaches using genetics have been well discussed in other reviews, here we will describe our proteomics-based studies.

To search for NSC-supporting/activating factors, we used mesenchymal stromal cell (MSC) lines that have a limited capacity for neuronal differentiation (Kohyama *et al.* 2001). This choice was prompted by the observation that the transplantation and/or mobilization of bone marrow-derived MSCs can often result in the functional recovery of damaged brain tissue, even though these cells do not exhibit robust neuronal differentiation (Kawada *et al.* 2006). Thus, MSCs may produce trophic factors that enhance the regenerative responses of the host CNS. We used a proteomics approach to identify such MSC-derived NSC-supporting factor(s) in MSC OP9 line, which has strong haematopoiesis-supporting activity (Nakano *et al.* 1994, 1996). Because various somatic stem cells share common regulatory mechanisms for self-renewal and differentiation (Reya *et al.* 2001), it was reasonable to use OP9 cells to look for NSC-supporting factor(s) (Sakaguchi *et al.* 2006).

First, to examine how OP9 cells affect the proliferation of NSCs, we cultured neurosphere cells (Reynolds & Weiss 1992) with or without conditioned medium from OP9 cells (OP9CM). At a density of one cell per well, neurospheres formed only in the presence of OP9CM. Next, to identify the OP9-derived molecules that promoted neurosphere formation, we used the protein chip system and mass spectrometry

(Fung *et al.* 2001) to detect molecules that were more abundant in the OP9CM than in the conditioned medium of inactivated OP9 (IA-OP9CM) obtained through repeated passage over six months. A signal at approximately 14.6 kDa showed a reproducible difference in peak height between the two CMs. This fraction was purified, analysed by tandem mass spectrometry and finally identified as Galectin-1, that had been reported as a carbohydrate-binding soluble protein. The addition of recombinant Galectin-1 protein to the culture medium enhanced the formation of neurospheres, indicating that Galectin-1 is one of the factors in the OP9CM that enhances neurosphere formation. Interestingly, we found that in the adult mouse brain, Galectin-1 was expressed in a population of slowly dividing GFAP-positive cells at the SVZ that includes NSCs.

To address the function of Galectin-1, recombinant Galectin-1 protein was infused into the mouse lateral ventricle for 7 days, and the SVZ cells were isolated and cultured. We found that significantly more neurospheres were formed by SVZ cells isolated from the Galectin-1-infused adult brains than by SVZ cells from control brains. Furthermore, Galectin-1 infusion increased the population of slowly dividing cells in the SVZ, indicating the activation of relatively quiescent stem cells. These results showed that Galectin-1 is expressed in slowly dividing NSCs at the SVZ and plays an important role in the proliferation of adult neural precursor cells, including NSCs. We also demonstrated that extracellularly added Galectin-1 binds to the surface of NSCs, and the carbohydrate-binding activity of Galectin-1 is essential for its promotion of NSC proliferation. An attractive hypothesis is that Galectin-1 mediates the interaction of NSCs with the extracellular matrix at the SVZ, which constitutes the stem cell niche, through its carbohydrate-binding activity. Interestingly, we found that, after ischaemia, Galectin-1 expression was induced in reactive astrocytes in the ischaemic penumbra (Ishibashi *et al.* 2007), where it would be well positioned for involvement in the insult-induced proliferation of neural precursor cells. We are currently investigating the therapeutic effects of Galectin-1 for damaged CNS.

5. TRANSIT-AMPLIFYING CELL PROLIFERATION AND NEUROBLAST MIGRATION

Since transit-amplifying cells proliferate more rapidly than NSCs (Doetsch *et al.* 1997), regulation of the proliferation of transit-amplifying cells could be key in controlling the number of newborn neurons. Indeed, the stimulation of neural progenitors in the SVZ by various growth factors and/or neurotrophic factors has been shown to significantly increase their proliferation and migration into the intact striatum (Lichtenwalner & Parent 2006). For example, epidermal growth factor (EGF) infusion into the intact brain causes cell migration from the SVZ to the striatum. These cells differentiate into glia (Craig *et al.* 1996; Kuhn *et al.* 1997; Doetsch *et al.* 2002). In contrast, EGF infusion into the ischaemic brain increases the number of new neurons in the injured striatum several weeks after discontinuation of the infusion (Teramoto *et al.* 2003).

Figure 3. Neuroblasts migrate from the SVZ into the striatum after cerebral ischaemia. Cre-encoding plasmid was injected into the lateral ventricles of transgenic mice carrying the floxed GFP gene. (a,b) In the normal brain, GFP-positive cells (green) are observed only in the SVZ on (a) day 0 and (b) day 18 after the induction of ischaemia. (c) In the ischaemic brain, many SVZ-derived GFP-positive cells (green) are observed in the peri-infarct striatum on day 18 after the induction of ischaemia. The numbers and thin dotted lines indicate the distance (in micrometres) from the SVZ. (d) High-power confocal image of a GFP/DCX-expressing cell exhibiting morphological features typical of migrating neuroblasts. Scale bar, 200 μm (a–c) and 10 μm (d). Modified from Yamashita *et al.* (2006).

Figure 4. Migration and maturation of SVZ-derived neurons in the ischaemic striatum. (a) Double immunohistochemistry of PECAM-1 (a marker for endothelial cells; red) and DCX (a marker for young neurons; green) on day 18 after the induction of ischaemia. Migrating neuroblasts formed spherical (arrowhead) or elongated aggregates (arrows) associated with blood vessels in the ischaemic striatum. Scale bar, 20 μm . (b) The SVZ-derived cells were genetically labelled with GFP and traced for 90 days after the induction of ischaemia. An electron micrograph showing a GFP-labelled axon (asterisk) containing presynaptic vesicles in the striatum. Scale bar, 0.5 μm . (c) High-power magnification view of the region indicated by the boxed area in (b). The postsynaptic density is indicated by arrowheads. Modified from Yamashita *et al.* (2006).

To examine this issue further, we determined the expression pattern of EGF receptors (EGFRs) in the SVZ after middle cerebral artery occlusion (MCAO) in mice (Ninomiya *et al.* 2006). We also examined the effects of EGF infusion on the number of transit-amplifying cells and neuroblasts in the ischaemic brain (Ninomiya *et al.* 2006; figure 2). Our results indicated that in this model the presence or lack of EGF affects the SVZ of ischaemic brains during at least two stages of recovery from ischaemia: (i) its presence supports the expansion of transit-amplifying cells in the SVZ and (ii) its discontinuation promotes the differentiation of these expanded cells into neuroblasts.

Our results obtained in the mouse MCAO model raised additional issues. It is important to resolve whether such EGF-induced overproliferation of the SVZ cells could lead to the subsequent formation of brain tumours (Sanai *et al.* 2005) and whether human SVZ cells, like those of rodents, could regenerate striatal neurons after cerebral ischaemia. Clearly, more

detailed studies on the human SVZ are needed before any consideration of the clinical applications of EGF can be made. Cerebral ischaemia stimulates neurogenesis in the SVZ and striatum of monkeys (Tonchev *et al.* 2005) as well as rodents. Recent studies have suggested that the adult human SVZ also contains NSCs and/or NSC-like cells (Pincus *et al.* 1998; Roy *et al.* 2000b; Sanai *et al.* 2004) and EGFR-positive cells similar to the transit-amplifying cells found in rodents (Weickert *et al.* 2000), suggesting that the adult human SVZ might also be responsive to EGF infusion. Thus, growth factor-induced expansion of transit-amplifying cells may be a promising strategy for promoting neuronal regeneration after cerebral ischaemia. Nonetheless, it remains possible that the mechanisms outside the EGFR pathway contribute to the proliferation of transit-amplifying cells in the SVZ.

In the SVZ, transit-amplifying cells give rise to neuroblasts, which form an extensive network of chains as they migrate anteriorly (Doetsch & Alvarez-Buylla

1996; Lois *et al.* 1996). Most of the neuroblasts migrate through the rostral migratory stream (RMS) into the OB, where they differentiate into interneurons (Lois & Alvarez-Buylla 1994). From the perspective of a migrating neuron, possibly the most challenging part of the journey, in terms of navigation decisions, is the initial phase when cells migrate within the SVZ of the lateral ventricle. Here, chains of neuroblasts form a complex, two-dimensional mesh extending across most of the lateral wall of the lateral ventricle, presumably in response to migratory cues. Chemorepulsive guidance activities have been found in the caudal septum (Hu & Rutishauser 1996; Wu *et al.* 1999) and the choroid plexus (CP; Hu 1999). Slit proteins expressed in these regions have been proposed to be the relevant chemorepulsive factors (Hu 1999; Wu *et al.* 1999; Nguyen-Ba-Charvet *et al.* 2004). Both the caudal septum and CP are separated from the SVZ by the lateral ventricle, which is filled with cerebrospinal fluid (CSF), and by the epithelial lining of the lateral ventricle, the ependyma. This physical separation raises the issues of how Slit factors are transported to the migrating neuroblasts to exert their chemorepulsive activity, and how they can establish a guidance gradient over such a long and complex migratory route.

The explanation for this important question is becoming clear. A recent report clearly demonstrated that the direction of CSF flow closely matches the orientation of the chains in the SVZ and the direction of the neuroblast migration (Sawamoto *et al.* 2006). Normal CSF flow, which is determined by the planar polarity of ependymal cells and beating of ependymal cilia, is required for normal SVZ cell migration. *In vitro* and *in vivo* experiments showed that chemorepulsive factors, including Slit, are secreted from the CP and form a concentration gradient in the SVZ. The transplantation of the CP to an ectopic position close to the RMS leads to disorganization in the network of SVZ chains and prevents neuroblast migration into the OB. Therefore, controlled flow of the CSF in the lateral ventricle is an important mediator of the directional migration of the SVZ neuroblasts in the intact adult brain.

Upon insult, on the other hand, newly generated neurons are likely to migrate towards the damaged sites in directions different from those observed in the intact brain (reviewed by Lindvall *et al.* 2004). In fact, we recently performed SVZ-specific labelling experiments using a Cre-*loxP* system and obtained direct evidence that a subpopulation of SVZ neuroblasts, which migrate anteriorly in the normal brain, are redirected laterally to the injured striatum after MCAO (Yamashita *et al.* 2006; figure 3). There are two possible mechanisms for the ischaemia-induced change in the direction of neuroblast migration. First, ischaemia may disrupt the mechanisms that sequester neuroblasts within the SVZ. Second, ischaemia may promote the lateral migration of neuroblasts by altering the expression of guidance molecules, such as Slit proteins, which act as repellents to determine the direction of neuroblast migration in the SVZ (Wu *et al.* 1999; Sawamoto *et al.* 2006). Chemotactic cytokines, such as stromal cell-derived factor 1 α , that are expressed in the injured striatum, may also be involved in this process (Robin *et al.* 2006;

Thored *et al.* 2006). Our results demonstrated that SVZ-derived neuroblasts migrate towards the infarcted region in chain-like structures that run parallel to blood vessels. Based on these findings, we propose that blood vessels in the damaged striatum play a crucial role in the migration and/or survival of these neuroblasts, perhaps by acting as a physical scaffold for migration and by releasing diffusible factors, such as brain-derived neurotrophic factor (BDNF) (Louissaint *et al.* 2002). It is interesting that vascular systems could have various roles in adult neurogenesis by (i) providing a niche for the continuous production of new neurons (Palmer *et al.* 2000) and (ii) supporting the migration of newly generated neurons after an ischaemic insult (Yamashita *et al.* 2006).

6. SURVIVAL AND MATURATION OF NEWBORN NEURONS

After their long journey, most of the neuroblasts differentiate into interneurons in the OB, where they integrate with the existing circuitry and contribute functionally to olfaction in the intact adult mouse brain (Gheusi *et al.* 2000). Previous studies have indicated that approximately half of the newborn neurons die before reaching maturity in the DG and OB (Petreanu & Alvarez-Buylla 2002; Dayer *et al.* 2003), but the mechanisms regulating the apoptosis of these neurons are largely unknown.

An interesting question is whether neurons that are newly generated after ischaemia can survive to become mature functioning cells. To address this issue, the phenotype of SVZ-derived cells in the ischaemic striatum was examined by light and electron microscopy after 90 days (Yamashita *et al.* 2006; figure 4). The labelled cells possessed long processes, expressed Neuronal Nuclei (NeuN) and formed synaptic structures in the damaged striatum 90 days after the ischaemic event. These results strongly suggested that SVZ cells are able to generate functional mature neurons that survive in the damaged striatum for considerable periods. However, it remains unclear what types of neurons are generated. In addition, the number of newborn neurons is too small to enable the recovery of neurological functions after MCAO (Arvidsson *et al.* 2002). Thus, it will be necessary to develop methods for enhancing the survival, and/or neuronal maturation of SVZ cells and their progeny.

We recently reported that cholinergic fibres innervate both the OB and the DG, where neuronal progenitor cells and immature neurons express various subtypes of acetylcholine receptors (AChRs; Kaneko *et al.* 2006). These data suggested that the progenitors and immature neurons are directly controlled by cholinergic input. To study the effect of enhanced cholinergic neurotransmission on neurogenesis, we used donepezil, a potent acetylcholinesterase inhibitor (ChEI), which is widely used clinically to ameliorate the cognitive impairment and memory disturbance of Alzheimer's disease (Rogers & Friedhoff 1996). Cholinergic stimulation promoted the survival of newborn neurons in the adult DG and OB. Therefore, increasing the ACh level using ChEI is a promising strategy for reversing the decrease in neurogenesis resulting from various insults, including

chronic stress (Kaneko *et al.* 2006). Regarding the mechanism for how ChEI supports the survival of newly generated neurons, a recent report indicated that donepezil treatment enhances the survival of newborn cells in the DG via cAMP response element-binding protein signalling without affecting neural progenitor cell proliferation or neuronal differentiation (Kotani *et al.* 2006). If the increased survival of newborn neurons results in functional recovery, then ChEI treatment may be useful for increasing neuronal plasticity and facilitating neuronal regeneration in injury/ageing and under normal conditions. On the other hand, although ChEI is one candidate treatment, to elicit the functional maturation of newly generated neurons many new interventions, including treatment with other small chemical compounds, are likely to be needed.

7. ROLES OF REACTIVE ASTROCYTES IN CNS REPAIR

The above-mentioned findings indicate that the mechanisms of insult-induced neurogenesis are beginning to be elucidated at the molecular level, revealing feasible targets for drug discovery. However, neurogenesis is not the only regenerative response of the damaged adult CNS; reactive astrocytes also play a role in CNS repair. Upon damage to the CNS, such as spinal cord injury (SCI), 'reactive astrocytes' showing a strong expression of intermediate filaments, such as GFAP and nestin, appear by one week after the injury. In the injured CNS, reactive astrocytes form a glial scar and are considered to be detrimental for axonal regeneration (we propose this idea as 'bad guy' hypothesis). However, recently we showed that reactive astrocytes play a crucial role in wound healing and functional recovery at the subacute phase of SCI, before completion of the glial scar (Okada *et al.* 2006). At the subacute phase (one to two weeks after injury), astrocytes migrate to compact the lesion, presumably secluding the inflammatory cells to prevent them from spreading into the parenchyma of the spinal cord (we propose this idea as 'good guy' hypothesis).

To address the regulatory mechanisms behind the reactive response of astrocytes, we investigated the role of Stat3, which is the principal mediator in a variety of biological processes (Sano *et al.* 1999; Hirano *et al.* 2000), such as cancer progression, wound healing and cell movement—including that in gastrulation. In the injured spinal cord, Stat3 activation (phosphorylation and nuclear localization) was observed in reactive astrocytes surrounding the lesion. To examine the role of Stat3 in the migratory behaviour of reactive astrocytes, we generated reactive astrocyte-selective conditional Stat3-deficient mice (*nestin-Cre; Stat3^{loxP}/-* mice). The SCI model of the conditional Stat3-deficient mice showed impaired compaction of the lesion centre by reactive astrocytes and delayed functional recovery, associated with widespread CD11b-positive inflammatory cell invasion and demyelination. Thus, Stat3 is likely to be a key molecule for the migratory function of reactive astrocytes, which may be deeply involved in tissue repair and functional recovery after SCI. We are currently investigating the downstream mechanisms of Stat3's action in this context.

8. CELL TRANSPLANTATION THERAPY

In addition to the activation of endogenous regenerative capacity, another approach for repairing the damaged CNS is cell transplantation therapy. The rationales for cell transplantation therapy are to (i) replace cells that are lost due to injury or disease with graft-derived cells (cell replacement therapy) and (ii) induce trophic actions, such as the production of extracellular matrix and diffusible factors, that enhance the regenerative responses of the host CNS (Bjorklund & Lindvall 2000; Kempermann 2006). Although most studies aimed at the realization of cell transplantation therapies are still at the basic research stage, researchers worldwide may soon begin focusing more on clinical applications. Obviously, it is important to approach this goal in a step-by-step manner, using progressively modified technology in animal experiments followed by studies in small numbers of patients, applying well-validated assessment protocols (Okano 2002b). Numerous preclinical studies have been reported; their results indicate that cell transplantation can have therapeutic effects in animal models of SCI (McDonald *et al.* 1999; Ogawa *et al.* 2002; Cummings *et al.* 2005; Iwanami *et al.* 2005b; Keirstead *et al.* 2005), stroke (Ishibashi *et al.* 2004; Kelly *et al.* 2004), lysosomal storage disorders (Snyder *et al.* 1995; Fukuhara *et al.* 2006), Parkinson's disease (Studer *et al.* 1998; Sawamoto *et al.* 2001; Bjorklund *et al.* 2002; Yoshizaki *et al.* 2004; Takagi *et al.* 2005; Brederlau *et al.* 2006) and demyelinating disorders (Brustle *et al.* 1999; Pluchino *et al.* 2003). In these studies, tissue- or embryonic stem cell-derived neural stem/precursor cells were transplanted and led to cell replacement and functional recovery. Nevertheless, we believe that the following points still need to be addressed at the preclinical level before clinical trials can be begun: (i) the types of cells that should be transplanted (Ogawa *et al.* 2002; Okano 2002a,b; Okano *et al.* 2003; Hofstetter *et al.* 2005; Keirstead *et al.* 2005), (ii) the number of cells that should be transplanted, (iii) the location of the transplanted cells, (iv) the therapeutic time window (Ogawa *et al.* 2002; Okano 2002a,b; Okano *et al.* 2003; Okada *et al.* 2005), (v) the mechanism of functional recovery (Cummings *et al.* 2005), (vi) the tracing of transplanted cells by *in vivo* imaging (Okada *et al.* 2005; Lepore *et al.* 2006; Zhu *et al.* 2006), (vii) the combination of cell transplant therapy with tissue engineering and drugs (Teng *et al.* 2002; Ikegami *et al.* 2005), (viii) evaluation of the therapeutic efficacy using primate models (Iwanami *et al.* 2005a,b; Takagi *et al.* 2005), and (ix) safety issues, including the possibility of tumorigenesis. Efforts in all these areas will be necessary before stem cell therapy can be developed as an effective method for regenerating the damaged CNS.

9. CONCLUSION AND PERSPECTIVES

As mentioned above, cell transplantation therapies have been developed experimentally for some CNS disorders. It also came to be realized that adult mammalian CNS has some regenerative capacity. Thus, regeneration of the damaged CNS is becoming feasible from the clinical aspect, although it still remains

primitive. Based on the above-mentioned facts and discussions, it is obvious that both (i) activation of the endogenous regenerative capacity and (ii) cell transplantation therapy are very important strategies for CNS repair. Elucidation of the molecular and cellular mechanisms of the stem cell regulation and normal CNS developmental process in combination with molecular-targeted drug discovery would be essential for the future development of innovative therapeutic interventions of various CNS damages including SCI, stroke and neurodegenerative disorders.

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