Chapter 2
Neural Stem Cells in Response to Microenvironment Changes Inside and Outside of the Brain

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Contents

Abstract Neural stem cells/neural progenitor cells (NSCs/NPCs) residing in the neurogenic regions of the brain play a crucial role in brain development, brain plasticity, and brain repair. Recent progress in NSC/NPC research has advanced our understanding of the NSC/NPC niche and signaling networks controlling NSC/NPC proliferation and differentiation. However, the natural behavioral of NSCs/NPCs in response to physiological and pathological changes inside and outside the brain remains poorly understood. This chapter has summarized recent findings concerning NSC/NPC activity in the early stage of cortical brain ischemia and the hematopoietic stem cell growth factors in regulating NSC/NPC proliferation and differentiation. Moreover, future directions for NSC/NPC research are also discussed in this chapter.

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Abbreviations

Ara-C	Cytosine-β-d-Arabinofuranoside
bFGF	Basic fibroblast growth factor
bHLH	Basic helix-loop-helix
BDNF	Brain-derived neurotrophic factor
BrdU	Bromodeoxyuridine
EGF	Epidermal growth factor
EMVs	Exosomes and microvesicles
G-CSF	Granulocyte-colony stimulating factor
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
LTP	Long-term potentiation
Ngn1	Neurogenin 1
NSCs/NPCs	Neural stem cells/neural progenitor cells
OB	Olfactory bulb
RMS	Rostral migratory stream
RT-PCR	Reverse transcription polymerase chain reaction
SCF	Stem cell factor
SGZ	Subgranular zone
SHRs	Spontaneously hypertensive rats
SVZ	Subventricular zone
TuJ1	Neuron-specific class iii beta-tubulin
VEGF	Vascular endothelial growth factor

2.1 Introduction

Neural stem cells/neural progenitor cells (NSCs/NPCs) have the capacity for self-expansion (symmetrical division), self-renewal (asymmetrical dividing) and differentiation into neurons and glial cells. During embryonic brain development, NSCs/NPCs are located in the neuroepithelium lining the lumen of the lateral ventricle (the ventricular zone) to generate neurons and glial cells throughout the brain (Farkas and Huttner 2008; Egger et al. 2011). It has been widely accepted that there are two neurogenic regions in which NSCs/NPCs reside in the postnatal or adult mammalian brain: the subgranular zone (SGZ) in the dentate gyrus of the hippocampus and the subventricular zone (SVZ) in the forebrain (Gage 2000; Curtis et al. 2007). Convincing evidence has shown that the NSCs/NPCs in the postnatal brain or adult brain are involved in learning and memory (Kempermann et al. 2004a), and that they may also participate in brain repair in the settings of brain injury (Jin et al. 2001; Arvidsson et al. 2002).

Although much progress has been made towards understanding the NSC/NPC niche and signaling networks controlling NSC/NPC proliferation and differentiation, the natural behavioral of NSCs/NPCs in physiological and pathological conditions remains poorly understood. Greater knowledge also needs to be gained regarding the role of NSCs/NPCs in self-protection and brain protection in the settings of brain injury and the regulation of NSC/NPC proliferation and differentiation by the growth factors of other systems or organs. In this chapter, our recent findings concerning the NSC/NPC behavior in intact brain and in the brain of ischemic injury, the effects of NSCs/NPCs on brain protection, and the involvement of hematopoietic stem cell growth factors in directing NSC/NPC proliferation and differentiation will be reviewed, and future directions for NSC/NPC research will be discussed.

2.2 NSC/NPC Classification: Bridge Cells and Migrating Cells

A large body of evidence has supported the notion that NSCs/NPCs in the SGZ generate granular neurons locally in the hippocampus, whereas the NSCs/NPCs in the SVZ travel a relatively long distance along the rostral migratory stream (RMS) to the olfactory bulb (OB) where they give rise to the interneurons (Luskin 1993; Lois and Alvarez-Buylla 1994; Gage 2000; Lie et al. 2004) in the postnatal or adult brain. During the migration along the RMS, NSCs/NPCs form chains providing supportive substrates to help NSCs/NPCs move forward to their destination, the OB (Lois et al. 1996; Chazal et al. 2000).

In addition to the findings collected from the fixed brain tissue as stated above, a live cell imaging technique enabled us to extend our knowledge on the natural status of NSC/NPC migration. Using multiphoton microscopy, we recorded NSC/NPC migration in the RMS in the transgenic mice carrying a report gene, green fluorescent protein (GFP) driven by nestin promoter (nestin-GFP mice). The nestin-GFP mice were sacrificed at the age of 3–4 weeks in the conditions of intact or ischemic injury, and the sagittal slices of the brain were prepared for live imaging. We observed something very interesting: there were two different types of nestin-GFP-labeled NSCs/NPCs in the RMS: non-migrating cells and migrating cells. We named the non-migrating NSCs/NPCs as bridge NSCs/NPCs and the traveling NSCs/NPCs as migrating NSCs/NPCs (Zhao et al. 2007a). The bridge NSCs/NPCs act as a bridge to support migrating NSCs/NPCs for travel. The traveling directions of the migrating NSCs/NPCs are multiple: forward to the OB, backward to the SVZ, up or down in the RMS (Fig. 2.1 a). Interestingly, the bidirectional migration of NSCs/NPCs has also been found by other investigators in vitro (Wichterle et al. 1997) or in the neonatal rat brain (Suzuki and Goldman 2003). It remains uncertain, however, how NSCs/NPCs are divided into bridge cells and migrating cells, what molecules are involved in distinguishing these two subtypes of NSCs/NPCs and how they retain their unique biological function.

2.3 The Early Reaction of NSCs/NPCs in Response to Cortical Brain Ischemia is Self-Protective

Time-lapse images revealed that the early response to cortical brain ischemia for NSCs/NPCs was to escape away from the area of brain damage (Zhao et al. 2007a). Cortical brain ischemia was made by the permanent occlusion of the right common carotid artery and the right middle cerebral artery distal to the striatal branch. Brain slices were prepared 3 h after induction of cortical brain ischemia in the right hemisphere of nestin-GFP mice, live brain slice imaging was acquired with a multiphoton microscope, and time-lapse images were recorded for 15 h at 7 min intervals. Very interestingly, we observed that only bridge NSCs/NPCs remained in the RMS next to the infarct cortex, whereas there were no migrating NSCs/NPCs in the RMS adjacent to the infarct cortex (Fig. 2.1 b–f). The migrating NSCs/NPCs were seen in the RMS distal to the infarct area. The migrating NSCs/NPCs in this part of RMS continued touching each other for a while (Fig. 2.1 b); thereafter, some of the NSCs/NPCs immediately moved away from the lesion area and towards to the direction of OB, and some other NSCs/NPCs traveled toward to the direction of infarct area along the RMS (Fig. 2.1 c). However, once they touched the bridge NSCs/NPCs in the location closer to the infarct cortex (Fig. 2.1 d), these migrating NSCs/NPCs changed their direction 180° and started moving away from the infarct area (Fig. 2.1 e and f). This observation suggests that cell-cell communication between the NSCs/NPCs leads to changes of direction for the migrating NSCs/NPCs, and that the early reaction of the migrating NSCs/NPCs in response to ischemic damage in the cortex is to move away from the infarct area during the 3–18 h after the induction of cortical ischemia. The results of this study offer insights into the natural self-protective behavior of migrating NSCs/NPCs in response to brain ischemic injury in the early period of cortical ischemia–-escape from the infarct area, and this behavior is self-protection.

The open questions to be addressed in the future studies are: how the NSCs/NPCs sense the microenvironment changes, how the signals are transported/delivered among the NSCs/NPCs, and how NSCs/NPCs escape away from “danger”– the dead zone (infarct) induced by focal brain ischemia.

2.4 Focal Brain Ischemia-Induced NSC/NPC Proliferation is Required for Brain-Self Protective

It has been well documented that focal brain ischemia is a robust trigger for stimulating NSC/NPC proliferation in both SVZ and SGZ in the brain of adult rodents (Arvidsson et al. 2001; Jin et al. 2001; Arvidsson et al. 2002). It has been proposed that focal brain ischemia-induced NSC/NPC proliferation and neurogenesis may play a role in brain self-repair. However, more than 80 % of newly formed neurons have been found dead in the setting of focal brain ischemia, and the surviving

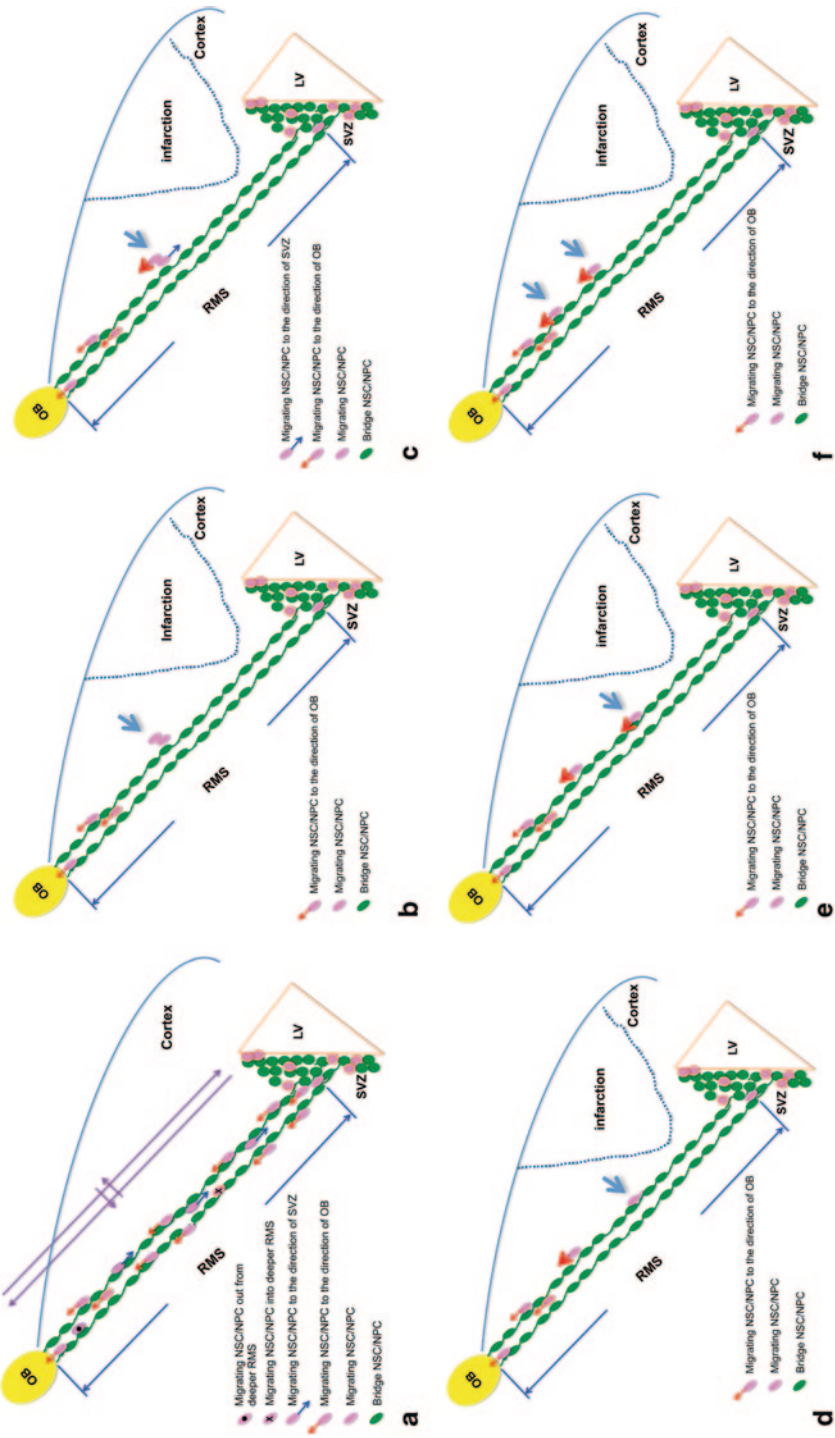

Fig. 2.1 Schematic diagram of NSC/NPC behavior in the RMS. **a** NSC/NPC behavior in the RMS of intact brain. Note that NSCs/NPCs in the RMS are divided into two sub-types: bridge NSCs/NPCs (*green*) and migrating NSCs/NPCs (*pink*). The bridge NSCs/NPCs are not traveling cells, and their biological function appears to generate a "bridge" between the olfactory bulb (OB) and subventricular zone (SVZ) for supporting NSC/NPC migration. The migrating

newborn neurons replace only 0.2 % of dead neurons by ischemic injury (Arvidsson et al. 2002). Therefore, the capability of brain-self repair by the endogenous NSCs/NPCs in the adult brain after ischemic injury is limited. Is brain ischemia-induced NSC/NPC proliferation per se actually involved in brain self-repair/protection? To address this question, we performed experiments in which NSC/NPC proliferation was blocked with a cell proliferating inhibitor, and we then determined whether NSC/NPC proliferation triggered by brain ischemia is vital for brain self-protection.

Cortical brain ischemia was produced by the permanent occlusion or ligation of the right common carotid artery and the middle cerebral artery distal to the striatal branch in male adult C57BL mice or spontaneously hypertensive rats (SHRs). Cytosine-β-D-Arabinofuranoside (Ara-C), a DNA synthesis inhibitor to prevent cell proliferation, was infused into the cerebral lateral ventricle ipsilateral to the ischemic hemisphere with a 7-day-infusion osmotic minipump. Ara-C was initially delivered one day before the induction of cortical brain ischemia or immediately after the induction of cortical brain ischemia, and the infusion of Ara-C was continued for 7 days. Bromodeoxyuridine (BrdU), a synthetic thymidine analog, can be incorporated into DNA during cell dividing. To identify proliferating NSCs/NPCs, BrdU was intraperitoneally injected daily during Ara-C infusion. We found that cortical brain ischemia-induced NSC/NPC proliferation in the bilateral SVZ and ipsilateral SGZ was completely inhibited by Ara-C (Li et al. 2010). In addition, slow dividing type −1 NSCs/NPCs in the bilateral SGZ were also significantly reduced by Ara-C infusion (Li et al. 2010). These findings indicate that cerebral lateral ventricle infusion of Ara-C is sufficient to prevent cortical brain ischemia-induced NSC/NPC proliferation in both SVZ and SGZ.

Our research data also revealed that inhibiting cortical brain ischemia-induced NSC/NPC proliferation in both SVZ and SGZ exaggerated neuron loss in both the hippocampus and infarct cortex) (Li et al. 2010). There are two types of neuron loss in the setting of focal brain ischemia: primary neuron loss and secondary neuron loss. The primary neuron loss occurs in the ischemic core within several hours after focal brain ischemia, whereas the secondary neuron loss appears in the brain tissue remote to the infarct core days and even months after focal brain ischemia (Hara et al. 1993; Touzani et al. 2001). It is worth noting that focal brain ischemia-induced NSC/NPC

NSCs/NPCs are the traveling cells in the RMS, and their traveling directions are multiple (*purple arrows*). **b–f** NSC/NPC behavior in the RMS of the brain suffering from cortical ischemic injury during 3–18 h after the induction of cortical brain ischemia. Note that there are no migrating NSCs/NPCs in the RMS adjacent to the cortical infarct area; only bridge NSCs/NPCs remain in the RMS nearby the cortical infarction. **b** Two migrating NSCs/NPCs physically interact with each other (communication) in the RMS distal to the cortical infarction. **c** The result of communication: one of the migrating NSCs/NPCs moves forward in the direction of OB (escaping away from the dead zone—cortical infarction), and other migrating NSC/NPC travels towards the infarction area along the RMS. **d** The migrating NSC/NPC "communicates" with a bridge NSC/NPC in the RMS close to the zone of dying brain cells (the cortical infarction area). **e** and **f** The results of the communication shown in d. The migrating NSC/NPC flees away from the dead zone (cortical infarction). The schematic diagram summarizes the results of time-lapse images published elsewhere (Zhao and Nam 2007). *LV* lateral ventricle. *RMS* the rostral migratory stream

proliferation in the SVZ and SGZ is markedly increased during the period of 4 days to 2 weeks post-ischemia (Jin et al. 2001; Arvidsson et al. 2002) Therefore, focal brain ischemia-induced amplification of NSCs/NPCs in the SVZ and SGZ may contribute to protecting neurons from the ischemia-induced secondary death. The evidence supporting this hypothesis shows that inhibiting NSC/NPC proliferation in the SGZ causes significant neuron loss in the hippocampus 7 days after cortical brain ischemia (Li et al. 2010). In addition, the neuron loss in the hippocampus has been demonstrated as the secondary neuron loss through apoptosis (Li et al. 2010) because the stroke model used in this study only causes infarction in the ipsilateral cortex, whereas the hippocampus is spared from the infarct.

How focal brain ischemia-induced amplification of NSCs/NPCs in the SVZ and SGZ protects brain from ischemic damage remains poorly understood. One possibility may be due to releasing trophic factors as our findings have shown that adult brain-derived NSCs/NPCs can protect cortical neurons against excitotoxic damage through the NSC/NPC-released trophic factors, brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) (Li et al. 2010). In agreement with these observations, other investigators have also found that both BDNF and VEGF are produced by NSCs/NPCs (Leker et al. 2007; Mochizuki et al. 2008) and that administration of BDNF and VEGF in animal models of brain ischemia leads to reduction in infarction size (Schabitz et al. 1997; Ren and Finklestein 2005).

NSCs/NPCs may act as trophic factor providers to save neurons from the injury of focal brain ischemia. Several lines of evidence have shown that the NSCs/NPCs in the SVZ of adult brain can migrate long distances to the cortex adjacent to the infarct area. It has been demonstrated that the neuroblasts derived from proliferating NSCs/NPCs in the SVZ can migrate to the peri-infarct cortex through the white matter (Jin et al. 2003) and blood vessels (Ohab et al. 2006). In line with these findings, we also observed that SVZ-derived NSCs/NPCs migrated toward the cortical infarction along the corpus callosum at day 7 after induction of cortical brain ischemia (Li et al. 2010). Proliferating NSCs/NPCs in the SGZ may rescue hippocampal neurons through the delivery of BDNF via axonal projections between the dentate gyrus and hippocampal CA1 and CA3 subregions. It has been revealed that trkB, the receptor for BDNF, is expressed in CA1 and CA3 (Yan et al. 1997). BDNF has been shown to prevent glutamate-induced apoptotic cell death (Almeida et al. 2005).

Our knowledge and understanding of the precise role of focal brain ischemia-induced amplification of NSCs/NPCs in the SVZ and SGZ in adult brain is still far from complete. Is the focal brain ischemia-induced amplification of NSCs/NPCs part of a reaction from NSCs/NPCs to protect themselves in the condition of ischemic injury? It has been shown that a widespread hyperexcitability occurs in the intact brain regions including peri-infarct cortex and hippocampus, and that the hyperexcitability is most pronounced at 3–7 days and persists 1 month after cerebral cortical ischemia (Schiene et al. 1996; Qu et al. 1998; Redecker et al. 2002). NSCs/NPCs in the adult brain share many physiological features with astrocytes (Filippov et al. 2003; Kempermann et al. 2004b). Astrocytes can regulate inhibitory synapses formation in hippocampal neurons (Elmariah et al. 2005), manipulate glutamatergic

transmission and uptake glutamate (Newman 2003). Can focal brain ischemia-induced amplification of NSCs/NPCs in both SVZ and SGZ contribute to preventing the hyperexcitability-induced neuron damage?

Other interesting questions that remain elusive are: whether generating and releasing trophic factors from NSCs/NPCs are cell-status dependent (proliferation vs. quiescent), and whether there are other mechanisms involved in NSC/NPC -induced neuroprotection in addition to the trophic factors released by NSCs/NPCs. Recent studies have revealed that exosomes and microvesicles (EMVs) play a key role in cell-to-cell communication. EMVs contain membrane receptors, proteins, mRNAs, miroRNAs and organelles that offer genetic regulation of cell survival, phenotype, proliferation, differentiation or death to the target cells either nearby (paracrine) or at a distance (endocrine) (Camussi et al. 2010). Stem cells have been demonstrated to release abundant EMVs (Camussi et al. 2010; Aoki et al. 2014). Stem cell-derived EMVs have been shown to rescue cells from injury, reprogram terminally differentiated cells to re-enter the cycle, and promote tissue regeneration and repair (Camussi et al. 2010). However, the role of NSC/NPC-released EMVs in supporting brain tissue survival and regeneration in the setting of stroke remains an open question to be addressed in future.

Enhancing propagation of NSCs/NPCs has been shown to protect brain from ischemic injury in animal models. Systemic administration of stem cell factor (SCF) 3h after induction of cortical brain ischemia in SHRs dramatically increases NSC/NPC proliferation in the SVZ, reduces infarction size and eliminates neurological deficits (Zhao et al. 2007a). Sodium butyrate has been reported to enhance amplification of NSCs/NPCs, reduce infarction size and improves functional outcome when systemically administered for 7 days after focal brain ischemia in rats (Kim et al. 2007; Kim et al. 2009). Leker and co-workers (2007) (Leker et al. 2007) demonstrated that long-term delivery of fibroblast growth factor-2 (bFGF) immediately after induction of cortical brain ischemia in SHRs resulted in the enhancement of NSC/NPC proliferation in the SVZ, white matter (the corpus callosum) and peri-infarct cortex throughout 90 days after ischemia and the improvement of motor function. These findings provide insights into developing new therapeutic approaches for brain protection through increasing endogenous NSCs/NPCs.

2.5 Hematopoietic Growth Factors Govern NSC/NPC Lineage Differentiation

Stem cell factor (SCF) and granulocyte-colony stimulating factor (G-CSF) were initially discovered as the essential hematopoietic growth factors that regulate the mobilization, proliferation and differentiation of hematopoietic stem cells/hematopoietic progenitor cells (HSCs/HPCs) (Ashman 1999) (Ripa and Kastrup 2008). Accumulating evidence has revealed that these hematopoietic growth factors may also play roles in the central nervous system (CNS). SCF (Zhao et al. 2007a) and G-CSF (Schneider et al. 2005; Zhao et al. 2007a) have been proven to

pass through the blood-brain barrier in intact rodent brains. Systemic administration of SCF (Zhao et al. 2007b; Zhao et al. 2007a) and G-CSF (Schabitz et al. 2003; Six et al. 2003; Shyu et al. 2004; Schneider et al. 2005; Komine-Kobayashi et al. 2006; Zhao et al. 2007a) alone, or SCF in combination with G-CSF (Zhao et al. 2007b; Zhao et al. 2007a) has been shown to protect the brain against ischemic injury (Schabitz et al. 2003; Six et al. 2003; Shyu et al. 2004; Schneider et al. 2005; Komine-Kobayashi et al. 2006; Zhao et al. 2007a) and facilitate brain repair during chronic stroke (Zhao et al. 2007b). In addition, SCF and G-CSF appear to be involved in neuronal plasticity. Mice with mutations of SCF (Motro et al. 1996) or SCF receptor (Katafuchi et al. 2000) show impaired long-term potentiation (LTP) and spatial learning and memory. G-CSF deficient mice display cognitive impairment, LTP reduction, and poor neuronal networks in the hippocampus (Diederich et al. 2009). Interestingly, SCF receptor, ckit, and G-CSF receptor, GCSFR, have been found to be expressed in the NSCs/NPCs of the adult rodent brain (Schneider et al. 2005; Zhao et al. 2007a). However, the effects of SCF and G-CSF on the NSCs/NPCs remain largely unknown.

Using the approaches of cellular biology and molecular biology, we have determined the regulatory effects of SCF and G-CSF on lineage commitment of NSCs/NPCs. First of all, we observed that the receptors for SCF and G-CSF were expressed on the NSCs/NPCs in the ventricular zone of the developing rat brain at embryonic day 18 (Piao et al. 2012). The NSCs/NPCs were then isolated from the ventricular zone of the embryonic rat brain at E18, a time period in which both neurogenesis and gliogenesis take place. The NSCs/NPCs were then grown in cell culture dishes to form neurospheres. After forming the secondary neurospheres, NSCs/NPCs were disassociated into single cells and were then allowed to differentiate in a differentiation medium. SCF and G-CSF alone or in combination were added at the beginning of the differentiation stage of NSCs/NPCs. We found that SCF and G-CSF alone or in combination increased the number of TuJ1-positive neuronal cells and reduced GFAP-positive astrocytes (Piao et al. 2012), suggesting a regulative role of SCF and G-CSF on lineage switch of NSCs/NPCs. In line with our observation, other investigators have revealed that SCF (Jin et al. 2002) and G-CSF (Schneider et al. 2005), promote neurogenesis in vivo.

Very interestingly, SCF and G-CSF can promote neuronal fate determination of NSCs/NPCs at the proliferation stage. SCF and G-CSF alone or in combination were added to the dividing NSCs/NPCs in the secondary neurospheres for 3–4 days. Thereafter, the mitogens (bFGF and EGF) and the hematopoietic growth factors were then withdrawn, and the NSCs/NPCs were allowed to differentiate. We observed that only SCF in combination with G-CSF (SCF+G-CSF) treatment showed a significant increase in neurogenesis and reduction in gliogenesis (Piao et al. 2012). This observation led us to hypothesize that SCF+G-CSF promotes neuronal lineage commitment of NSCs/NPCs through the regulation of the cell cycle. To test this hypothesis, we examined the effects of SCF+G-CSF on the cell cycle and NSC/NPC propagation. The results of a cell cycle assay showed that SCF+G-CSF increased the population of NSCs/NPCs at the G1/G0 phase and decreased the population of NSCs/NPCs at the S phase (Piao et al. 2012), suggesting that

SCF+G-CSF causes the cell cycle arrest of NSCs/NPCs. To further confirm this result, BrdU was added into the NSCs/NPCs during the proliferating stage. We chose BrdU for labeling the dividing NSCs/NPCs at the S phase because BrdU is a thymidine analogue and can be incorporated into the newly synthesized DNA in S-phase cells. We found that SCF+G-CSF treatment during NSC/NPC proliferation resulted in a significant reduction of BrdU positive NSCs/NPCs, indicating that the NSC/NPC dividing is inhibited by SCF+G-CSF (Piao et al. 2012). Furthermore, when we examined NSC/NPC growth curve, we observed that NSC/NPC propagation was reduced by SCF+G-CSF (Piao et al. 2012). Together, these data suggest that SCF+G-CSF leads to cell cycle exit of NSCs/NPCs and inhibits NSC/NPC proliferation. Inducing cell cycle arrest of NSCs/NPCs is the critical and initial step to promote neuronal lineage differentiation of NSCs/NPCs. This notion is supported by the findings that pro-differentiation molecule-induced neuronal differentiation is preceded by cell cycle withdrawal (Henrique et al. 1997; Farah et al. 2000; Politis et al. 2008) and that promoting the cell cycle leads to inhibition of neuronal differentiation (Ohnuma et al. 2001).

How does SCF+G-CSF induce neuronal fate determination and commitment of NSCs/NPCs? To address this question, we examined the effects of SCF+G-CSF on the regulation of proneural bHLH transcription factors and Notch signaling pathway. Convincing evidence has shown that neurogenin 1 (Ngn1), one of the proneural bHLH transcription factors, promotes neuronal differentiation of NSCs/NPCs. Ngn1 has been found to be expressed in the ventricular zone, and Ngn1 only appears during cortical neurogenesis (Gradwohl et al. 1996; Sommer et al. 1996; Ma et al. 1997). In fact, Ngn1 acts as an activator of neuronal transcription factor that promotes neurogenesis and inhibits glial differentiation (Sun et al. 2001). Erythropoietin, one of the hematopoietic growth factors, has been shown to promote neuronal differentiation of NSCs/NPCs through Ngn1 mediation (Wang et al. 2006). By contrast, Notch1/Hes1 signaling contributes to maintaining NSCs/NPCs in an undifferentiated stage (Artavanis-Tsakonas et al. 1999; Mizutani and Saito 2005), enhances astroglial differentiation (Schmid et al. 2003; Anthony et al. 2005), and suppresses neuronal lineage commitment of NSCs/NPCs (Kageyama and Ohtsuka 1999). Based on these findings, we hypothesized that SCF+G-CSF promotes neuronal differentiation of NSCs/NPCs through acting the Ngn1 transcription and inhibiting the Notch1/Hes1 signaling. To test this hypothesis, we quantified gene expression of NSCs/NPCs with real-time RT-PCR after SCF+G-CSF treatment. The gene expression data showed that Ngn1 gene expression was significantly upregulated by SCF+G-CSF. In addition, both Notch1 and Hes1 genes were significantly down-regulated by SCF+G-CSF (Piao et al. 2012). Moreover, when Ngn1 transcription was silenced by the siRNAs against Ngn1, SCF+G-CSF-induced enhancement of neuronal lineage commitment and inhibition of astroglial differentiation was significantly prevented (Piao et al. 2012). These findings suggest that Ngn1 is required for SCF+G-CSF-induced lineage determination and neuronal fate commitment of NSCs/NPCs. A putative model of the effects of SCF+G-CSF on cell cycle withdrawal and cell fate switch in NSCs/NPCs is presented in Fig. 2.2.

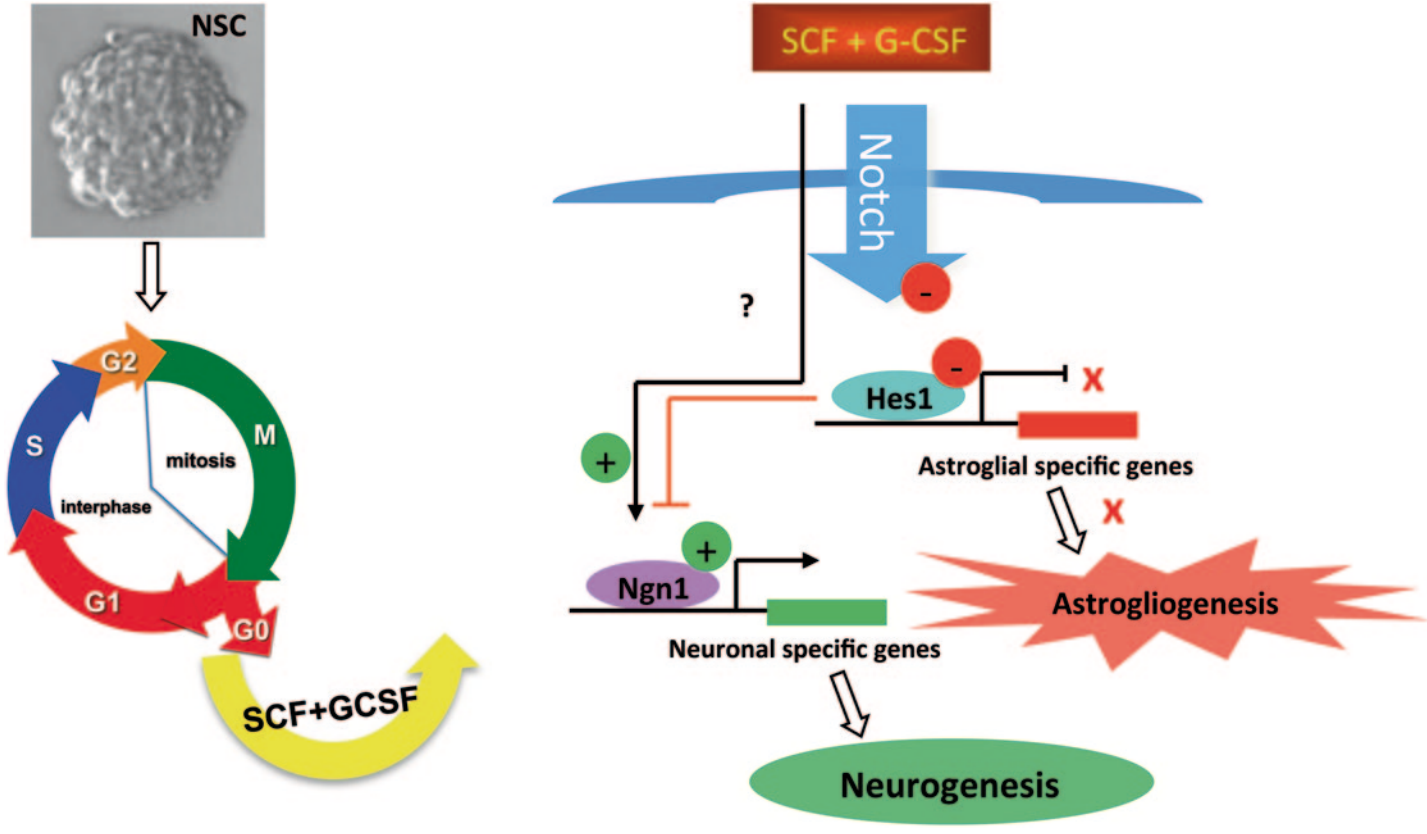

Fig. 2.2 A model for regulation of neuronal fate commitment of NSCs/NPCs by SCF+G-CSF during NSC/NPC dividing. SCF+G-CSF treatment during NSC/NPC proliferation (mitotic phase) coordinately arrests NSC/NPC cycle, suppresses astrocyte lineage commitment, and enhances neuronal fate differentiation through inhibiting Notch1/Hes 1 signaling and activating Ngn1. The dual effects of SCF+G-CSF on regulation of both NSC/NPC cycle and NSC/NPC lineage

Taken together, our research data support that hematopoietic growth factors, SCF+G-CSF, can govern NSC/NPC proliferation and differentiation and control lineage switch of NSCs/NPCs. SCF+G-CSF appears to display the dual effects on promoting the exit of proliferative NSCs/NPCs from the cell cycle and directing neuronal fate differentiation of NSCs/NPCs. It remains, however, to be further determined why and how SCF in combination with G-CSF but not SCF or G-CSF alone effectively stops the cell cycle for NSCs/NPCs and directs NSC/NPC differentiation into the neuronal fate, whether SCF+G-CSF facilitates survival and maturation of newborn neurons, and whether SCF+G-CSF-induced neurogenesis play a functional role in brain repair after brain injury. These mechanistic studies would advance our understanding of hematopoietic growth factors on neurogenesis and would help in developing a unique pharmaceutical therapy to reinforce endogenous NSCs/NPCs for repairing the brain in the condition of brain injury.

Other interesting questions to be addressed in future include whether SCF and G-CSF play a role in maintaining neurons in a differentiated stage and assuring neurons in a healthy and functioning status, and whether SCF and G-CSF are involved in pathogenesis of age-related neurodegenerative diseases. Addressing these open questions is important because a large body of evidence has shown that cell cycle re-entry of mature neurons leads to neuronal degeneration in Alzheimer's disease (Raina et al. 2004; McShea et al. 2007).

It is worth noting that the biological function of SCF and G-CSF is not restricted on regulation of the lineage commitment of NSCs/NPCs. Receptors for SCF and G-CSF have been found to be expressed not only on the NSCs/NPCs but also on the neurons (Schneider et al. 2005; Zhao et al. 2007a). In addition to the effects of SCF and G-CSF on neuroprotection as stated earlier in this chapter, our research group has recently demonstrated that SCF+G-CSF synergistically promotes neurite outgrowth (Su et al. 2013). Moreover, systemic administration of SCF+G-CSF in the phase of chronic stroke increases neuronal network formation in the cortex adjacent to the infarct cavities (Cui et al. 2013). These findings support a novel view concerning the role of SCF and G-CSF in the CNS that SCF+G-CSF has the capability to cease NSC/NPC dividing, guide NSCs/NPCs for neuronal lineage differentiation, promote neurite extension, enhance neuronal network formation, and protect neurons from injury. These research data suggest that SCF+G-CSF may have much broader biological function involved in brain development, brain plasticity, and brain repair after injury.

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switch may be accomplished through the following processes: **a** Repress NSC/NPC proliferation and drive NSCs/NPCs out of cycle by inhibiting Notch1/Hes1 signaling and enhancing Ngn1; **b** Inhibit Notch1/Hes1 signaling and release the repressive effect of Hes1 on Ngn1 to repress astrogliogenesis and to trigger neuronal fate commitment; and **c** Directly activate Ngn1 to promote the differentiation of NSCs/NPCs into neurons. This schematic diagram summarizes the data of SCF+G-CSF on NSC/NPC differentiation published elsewhere. (Piao et al 2012)

Concluding Remarks

NSCs/NPCs do not reside in an "isolated" microenvironment in the brain, the factors or molecules produced by other organs or systems may also regulate the proliferation and differentiation of NSCs/NPCs. Hematopoietic growth factors, SCF and G-CSF, are produced by bone marrow stromal cells and fibroblasts (Heinrich et al. 1993; Watari et al. 1994) to govern bone marrow stem cell survival, proliferation and differentiation. The findings provided in this chapter supports that SCF and G-CSF are also involved in cell fate determination and commitment of NSCs/NPCs. This observation leads to the insight that the physiological and pathological changes in other systems may also affect NSC/NPC proliferation and differentiation in the CNS. In fact, the precise role of NSCs/NPCs in the setting of brain injury remains poorly understood. Our research data suggest that the early reaction of NSCs/NPCs in the condition of cortical brain ischemia is self-protective: escaping away from the area suffering from ischemic injury. Cell-cell interaction and communication play a key role in directing the migrating NSCs/NPCs to flee. In addition, focal brain ischemia-induced the increase in proliferation of NSCs/NPCs during the 1st week after brain ischemia appears to be critical for neuroprotection. Further clarification of the contribution of brain ischemia-induced NSC/NPC amplification in brain protection is critically important because it will provide crucial evidence needed to assist in developing new therapeutic strategies for the treatment of stroke.

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